STRATEGIES FOR MITIGATING THE ENVIRONMENTAL IMPACTS OF PIG AND POULTRY PRODUCTION

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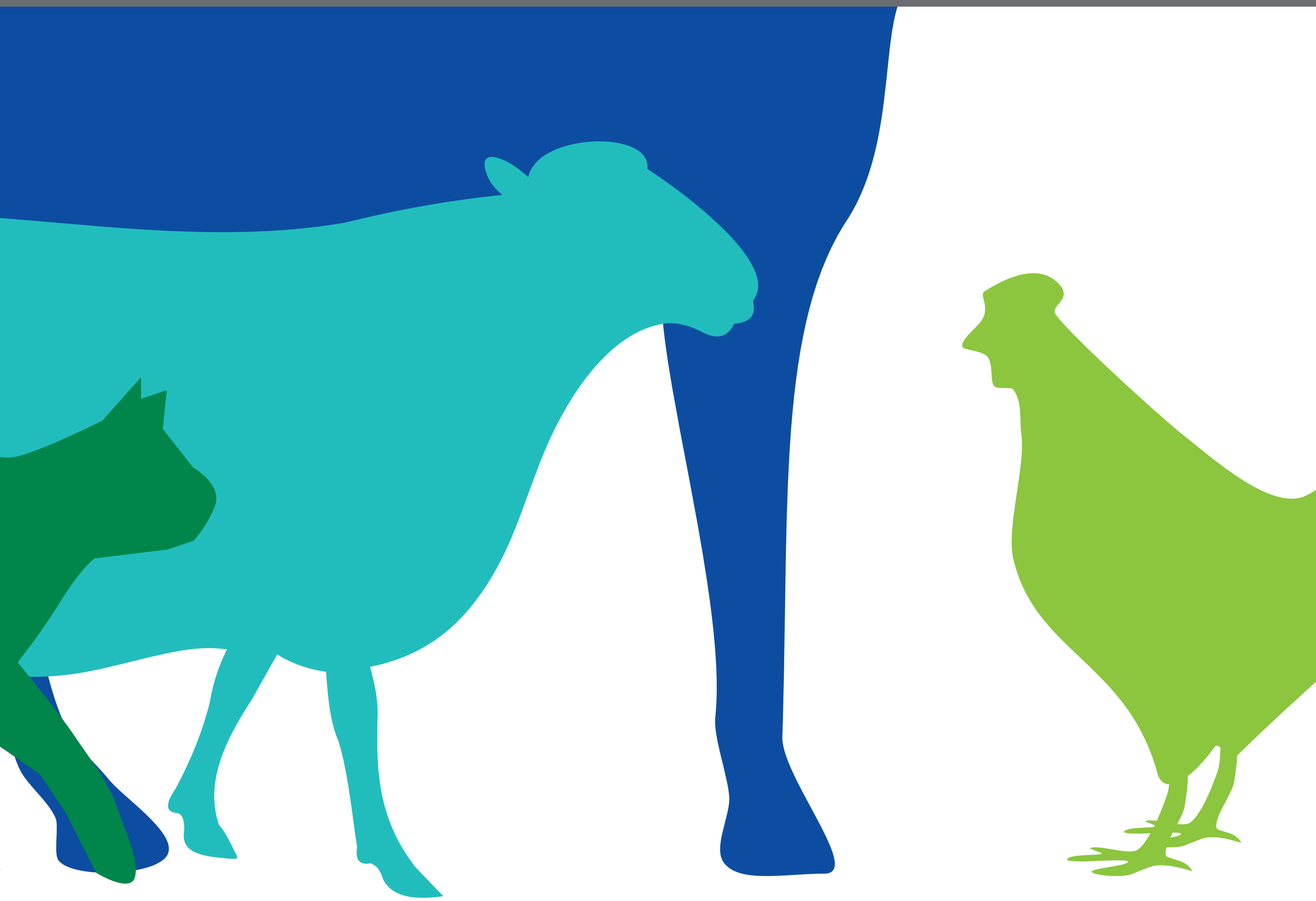

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STRATEGIES FOR MITIGATING THE ENVIRONMENTAL IMPACTS OF PIG AND POULTRY PRODUCTION

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EDITORIAL
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Editorial: Strategies for Mitigating the Environmental Impacts of Pig and Poultry Production

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Editorial on the Research Topic

Strategies for Mitigating the Environmental Impacts of Pig and Poultry Production

Pig and poultry production are crucial agriculture-based industries in many countries. These production systems are frontlines in the fight against food insecurity. However, these livestock sectors also contribute to the excretion of potential pollutant substances to the environment, like nitrogen and phosphorus. Furthermore, cereals grains used in poultry and pig feeding are also related to land use and many emissions from the cultivation to the processing steps.

In recent years, the poultry and pig industries have advanced in reducing their impacts on the environment. However, there is still much room for improvement, and scientists can contribute to greener animal production by proposing and testing solutions that make these production systems more environmental-friendly. This Research Topic presented scientific evidence that is possible and viable to mitigate the environmental impact of poultry and pig production systems using currently available nutritional tools.

The feed has a major contribution to the environmental impacts of pig and broiler production. However, it should be noted that animal nutrition research has a critical role in attenuating this impact. The use of nutritional tools is one of the main insights provided by Andretta et al. after performing a systematic review of original studies that estimated the environmental impacts associated with both pig (55 studies) and poultry productions (30 studies). The study's conclusion supports the hypothesis that novel feeding techniques may be necessary to mitigate the environmental footprint of both production chains.

One of the most promising options to mitigate environmental impacts is to optimize nutrient-use efficiency by applying precision feeding techniques, as highlighted by Pomar et al.. The potential of novel diet formulation strategies as tools to mitigate the carbon footprint was also described in this paper, as well as the several limitations of standard formulation methods in the context of conventional and precision feeding systems. In agreement with this premise, innovative formulation methodologies that incorporate the environmental impacts of feed ingredients were described and validated by de Quelen et al. as efficient ways to reduce the environmental impacts of pig production without compromising animal performance.

In addition to this innovative formulation method, consistent information on the animal nutrient requirements and the feedstuff characteristics are non-negotiable items when proposing a precision feeding program. The methods available to accurately evaluate the nutritional values of feed ingredients and to assess phosphorus requirements were reviewed by Lautrou et al.. Undoubtedly, a better understanding of the nutritional

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requirements is of utmost importance for mitigating pollutant excretion. To accomplish this, an alternative modeling framework that incorporated uncertain traits of individual pigs in a precision feeding modeling framework was presented by Misiura et al.. This data-driven approach can improve the estimation of individual nutrient requirements and, therefore, the economic and environmental sustainability of pig production systems.

Still focusing on mitigation options related to the feed formulas, the beneficial effects of supplementing feed-grade amino acids on the environmental impacts of broiler and pig production were reviewed by Cappelaere et al.. The current knowledge on using low-crude protein diets was summarized, and factual research information was provided to quantify direct (feed production) and indirect (emissions from manure) impacts. In addition, Hickmann et al. provided several results indicating the potential of using feed additives as eco-friendly strategies during formulation. The study was developed focusing on β-mannanase supplementation, which reduced the amount of soybean oil used in feed formulas and, consequently, mitigated the environmental impacts of pig and poultry feeding programs. Both papers highlighted the importance of choosing feed ingredients considering not only performance or economic criteria but also the environmental standpoint.

In fact, growth performance and environmental aspects cannot be separated. The relationships between different performance selection traits and environmental impacts were evaluated by Monteiro et al. in individual growing pigs. This study concluded that genetic selection to improve feed conversion ratio is the best option to benefit both performance and environmental impacts. The authors also suggested using a similar approach on actual data (e.g., information collected by genetic companies). Another important insight from this study is that improving performance can be a way to improve environmental sustainability too. Indeed, the findings presented by Chen et al. revealed a significant correlation between sow gut microbiota and litter size, providing another piece of evidence that several responses are connected in animal science and that an overall assessment should be preferred instead of focusing on isolated impacts.

Evaluating the overall aspects of a given system is not an easy task, but protocols or assessment routines can help. Thus, a protocol to assess the energy performance of broiler facilities was developed and presented by Baxevanou et al.. After applying the protocol in production units with different technology levels (study-case), the authors also proposed energy-saving measures that can mitigate the environmental footprint of broiler farms, which included proper insulation. In addition, efficient climatization is vital to save energy and prevent physiological and metabolic implications of heat stress on broilers. These effects were reviewed by Nawaz et al., who also suggested strategies to improve broiler production in a warming world.

All the studies presented in this Research Topic can be seen as attempts to improve the way pigs and poultry are raised nowadays. Facing challenges of modernization, farmers and food producers have become more efficient over the last decades; however, the systems' environmental sustainability still needs improvements. We do believe the more the production chains are studied, the more effective the actions will be to move toward a more resource-efficient and sustainable food production system. This aspect is particularly crucial because the growing demand for food worldwide must be met at an affordable cost without compromising environmental integrity. Access to food is a fundamental human right. This Research Topic demonstrated that scientists are working worldwide to make food available with as little impact as possible to meet current demands and ensure the same right for the next generations.

AUTHOR CONTRIBUTIONS

IA and MK wrote the first draft of the manuscript. All authors contributed to manuscript revision and approved the submitted version.

Conflict of Interest: AR was employed by Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada. MK was employed by Elanco Animal Health, São Paulo, Brazil.

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BRIEF RESEARCH REPORT
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Environmental Impacts and Their Association With Performance and Excretion Traits in Growing Pigs

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The selection of pigs for improved production traits has been, for a long time, the major driver of pig breeding. More recently, because of the increasing concern with the environment, new selection criteria have been explored, such as nitrogen (N) excretion. However, many studies indicate that life cycle assessment (LCA) provides much better indicators of environmental impacts than excretion. Therefore, the objective of this study was to investigate, using a modeling approach, the relationships between production traits and LCA impacts of individual growing pigs calculated at the farm gate for 1 kg of body weight gain. Performances of pigs were simulated for 2-phase (2P) and precision feeding (PR), using the InraPorc population model (on 1,000 pigs). Nitrogen excretion was positively correlated with feed conversion ratio (FCR; $r = +0.96$), climate change (CC; $r = +0.96$), acidification potential (AC; $r = +0.97$), eutrophication potential (EU; $r = +0.97$), and land occupation (LO; $r = +0.96$), whatever the feeding program. However, FCR appeared to be a better indicator of LCA impacts, with very high and positive correlations ($r > +0.99$) with CC, AC, EU, and LO for both feeding programs. The CC, AC, and EU impacts of pig production for PR feeding were 1.3, 10, and 7.5% lower than for 2P, respectively, but the correlations within each outcome were very similar among feeding programs. It was concluded that the use of FCR as a selection criterion in pig breeding seems to be a promising approach to associate improved performance and low environmental impact of pig fattening.

Keywords: feed efficiency, environmental impacts, life cycle assessment, growing pig, modeling

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INTRODUCTION

The selection of animals for improved production traits has been, for a long time, the major driver of pig breeding (1, 2). More recently, because of the increasing concern with environment, new selection criteria have been explored, such as nitrogen (N) or phosphorus (P) excretion, which are related to both feed efficiency and environmental impact (3). Residual feed intake (RFI) was also proposed as a possible selection criterion to simultaneously improve feed efficiency and reduce N and P excretion (1).

However, the pig supply chain involves a complex system, which requires production of fertilizers and pesticides; production of feed ingredients; feed processing; animal raising; transportation of animals and feed; water use for drinking and cleaning; energy use for light, heat, and ventilation; and waste management (4). Therefore, the environmental degradation is not the consequence of only one process (e.g., the raising of pigs) or one element (e.g., N excretion) and, as reviewed by McAuliffe et al. (4), impacts are better evaluated through integrated methodologies such as life cycle assessment (LCA).

Recently, a comparative LCA showed that pigs selected for low RFI have, on average, 6% lower environmental impacts on climate change (CC), acidification (AC), eutrophication (EU), land occupation (LO), and water depletion than these selected for high RFI (5). However, in this study, RFI did not appear to be the optimum measure for efficient environmentally friendly selection, since it was rather poorly correlated to environmental impacts ($r = 0.73$ for CC in the low RFI line).

The objective of the present study was thus to investigate, using a modeling approach, the relationships between different performance selection traits and LCA environmental impacts evaluated in individual growing pigs.

MATERIALS AND METHODS

Feeding Strategies and Animal Performance

This study considered a conventional growing–finishing pig unit located in West France, as described in detail by Monteiro et al. (6). Two feeds were formulated on the basis of net energy (NE, 9.6 MJ/kg), standardized ileal digestible (SID) amino acids, and digestible phosphorus (P): feed A to achieve 110% the mean population nutrient requirements at the beginning of the growing period (9.84 g/g SID lysine, 3.01 g/kg digestible P), and feed B to achieve 90% the mean population nutrient requirements at the end of the finishing period (4.55 g/kg SID lysine, 1.68 g/kg digestible P). The two feeds were blended according to two feeding programs: 2-phase feeding (2P) corresponding to the strategy used in French central test stations or precision feeding (PR). The 2P pigs were fed with feed A from 30 to 70 kg BW, and then with a blend of 50% of each feed until the end of fattening, to achieve 110% the mean population SID-lysine requirement at the start of the finishing period. For PR pigs, the blend of the two feeds was calculated according to a factorial approach in order that each pig received each day the exact amount of SID lysine required to achieve its potential of protein deposition, which was defined according to a Gompertz function, as described by van Milgen et al. (7).

Simulations for a virtual population of 1,000 female pigs were performed individually, from 30 to 115 kg of BW, for each feeding program to determine individual animal performance, nutrient balance, and excretion according to InraPorc population model (8). This virtual population was generated according to the method described by Brossard et al. (8), from a variance–covariance matrix with two parameters describing individual pig feed intake (the net energy intake at 50 and 100 kg BW: 20.2 ± 2.0 and 25.0 ± 2.9 MJ NE/day, respectively) and three parameters describing the Gompertz function of potential protein deposition (the BW at 70 days: 30.0 ± 2.9 kg, the mean protein deposition rate between 70 days of age and 110 kg BW: 142.8 ± 15.2 g, and the precocity b-value of the Gompertz function: 0.0169 ± 0.0103).

The simulated performance and excretion data were then used to calculate gaseous emissions from animals and manure, according to Rigolot et al. (9). The pig production system considered was a conventional growing–finishing pig farm located in Brittany (West France) with indoor raising of animals on complete slatted floor, in a building with mechanical ventilation and collection and storage of manure as liquid slurry (6).

Life Cycle Assessment

The LCA was performed for each pig, considering all the impacts associated with feed production, animal housing, and manure management (as described by 6). We based our analysis on the CML 2001 (baseline) method version 3.02 as implemented in SimaPro software version 8.05 (PRé Consultants) and added the category land occupation from CML 2001 (all categories) version 2.04. Thus, we considered the potential impacts of pig production on CC (kg CO_2-eq), EU (g PO_4-eq), AC (g SO_2-eq), and LO ($m^2 \cdot$ year). The CC was calculated according to the 100-year global warming potential factors in kilograms CO_2-eq. Impacts were calculated at the farm gate, and the functional unit considered was 1 kg of BW gain over the fattening period.

Statistical Analysis

The LCA calculation model was implemented using SAS software (SAS Inst. Inc., Cary, NC). Performance and environmental impacts were subjected to variance analysis using GLM procedure with feeding strategy as main effect. Pearson correlations for each feeding strategy were calculated between performance and environmental impacts data using CORR procedure, and pigs were ranked according to their CC impact, considering the feeding strategy and using the RANK procedure. All analyses were conducted using SAS software version 9.1 (SAS Inst. Inc., Cary, NC).

All the data used in the statistical analysis are available in the INRAE data repository (10).

RESULTS AND DISCUSSION

Feeding Strategies, Animal Performance, and Environmental Impacts

Feeding strategies affected most of the parameters evaluated (**Table 1**); effects were more accentuated for N excretion and N retention efficiency, and for CC, EU, and AC environmental impacts, which are highly dependent on dietary crude protein (CP) content, which was on average lower for PR (144 g/kg) than for 2P (167 g/kg). Compared to 2P, with PR, ADG was slightly improved (by 1.3%), efficiency of N retention was increased (40.5 vs. 36.2%), N excretion was reduced (by 16%), and environmental impacts were decreased (CC, AC, EU, and LO impacts 1.3, 10.0, 7.5, and 0.8% lower than for 2P, respectively). These results are in agreement with previous studies indicating that PR feeding strategy allows the improvement of the performance of pigs, compared to phase feeding, by providing sufficient amount of amino acids even to the animals with the highest potential of protein retention, which may not be the case with phase feeding, especially at the beginning of each phase. In

TABLE 1 | Effect of feeding strategy on pig performance, nitrogen excretion, and environmental impacts measured by life cycle assessment ($n = 1,000$ pigs).

Item	Two-Phase feeding	Precision feeding	*P*-value[a]
Performance			
ADFI, g/day	2,310 ± 259	2,316 ± 261	ns
ADG, g/day	864 ± 112	876 ± 116	*
FCR, kg/kg	2.69 ± 0.29	2.67 ± 0.32	*t*
SID lysine intake, g/day	1.89 ± 0.20	1.47 ± 0.11	***
Protein intake, g/day	375 ± 42.2	340 ± 32.5	***
Protein retained, g/day	135 ± 18.4	138 ± 20.1	*
N retention efficiency, %	36.2 ± 4.8	40.5 ± 4.8	***
Environmental impacts			
N excreted, kg/pig	3.83 ± 0.69	3.20 ± 0.56	***
CC, kg CO_2-eq/kg BW gain	2.34 ± 0.25	2.31 ± 0.28	*
EU, g PO_4-eq/kg BW gain	17.4 ± 2.34	16.1 ± 2.22	***
AC, g SO_2-eq/kg BW gain	48.1 ± 7.29	43.3 ± 6.60	***
LO, m^2 year/kg BW gain	3.77 ± 0.40	3.74 ± 0.45	*t*

ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; CC, Climate Change; EU, Eutrophication potential; AC, Acidification Potential; LO, Land Occupation. Two-phases feeding = pigs received a "growing" diet from 30 to 70 kg of BW and a "finishing" diet from 70 to 115 kg of BW; Precision feeding = individual pigs were fed daily with a diet providing the exact amount of digestible amino acids they required.

[a] *t: $P < 0.10$; *$P < 0.05$; ***$P < 0.001$.*

TABLE 2 | Correlations[a] between performance traits, nitrogen excretion, and environmental impacts, for the precision (PR) and the two-phase (2P, in italic) feeding strategies.

		ADFI	ADG	FCR	NR	NEff	NE	CC	EU	AC
ADG	PR	0.683								
	2P	*0.576*								
FCR	PR	0.181	−0.583							
	2P	*0.246*	*−0.636*							
NR	PR	−0.692	−0.002	−0.643						
	2P	*−0.675*	*−0.112*	*−0.593*						
Neff	PR	−0.361	0.543	−0.986	0.683					
	2P	*−0.385*	*0.400*	*−0.959*	*0.669*					
NE	PR	0.187	−0.552	0.963	−0.428	−0.940				
	2P	*0.125*	*−0.682*	*0.956*	*−0.450*	*−0.953*				
CC	PR	0.188	−0.577	0.999	−0.647	−0.987	0.963			
	2P	*0.249*	*−0.633*	*0.999*	*−0.599*	*−0.960*	*0.955*			
EU	PR	0.225	−0.546	0.998	−0.625	−0.983	0.971	0.998		
	2P	*0.225*	*−0.645*	*0.997*	*−0.610*	*−0.973*	*0.966*	*0.997*		
AC	PR	0.241	−0.532	0.996	−0.621	−0.981	0.972	0.996	0.999	
	2P	*0.224*	*−0.643*	*0.996*	*−0.617*	*−0.977*	*0.968*	*0.996*	*0.997*	
LO	PR	0.181	−0.583	0.999	−0.643	−0.986	0.963	0.999	0.998	0.996
	2P	*0.245*	*−0.637*	*0.999*	*−0.593*	*−0.959*	*0.956*	*0.999*	*0.997*	*0.996*

ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; NE, nitrogen excretion; CC, Climate Change; EU, Eutrophication potential; AC, Acidification Potential; LO, Land Occupation.

[a] *All correlations were significantly different from 0 ($P < 0.001$).*

PR compared to 2P feeding strategy, protein and SID lysine intakes were reduced by 9.3 and 22.2%, respectively. Combined with the slightly improved protein retention in PR pigs, this resulted in a significant increase of N retention efficiency (from 36.2 to 40.5%) and a reduction of nutrient load in excreta, contributing to the lower CC, EU, and AC impacts with precision feeding, as already shown by Monteiro et al. (6) and Andretta et al. (11).

Correlation Between Performance, Excretion, and Environmental Impacts

Correlations between performance, excretion and environmental impacts are shown in **Table 2**. The correlation values obtained for 2P and PR strategies were very close. Nitrogen excretion was highly and positively correlated with CC ($r = +0.96$, **Figure 1A**), AC ($r = +0.97$), EU ($r = +0.97$), and LO ($r = +0.96$). Correlations between environmental impacts and NR were much

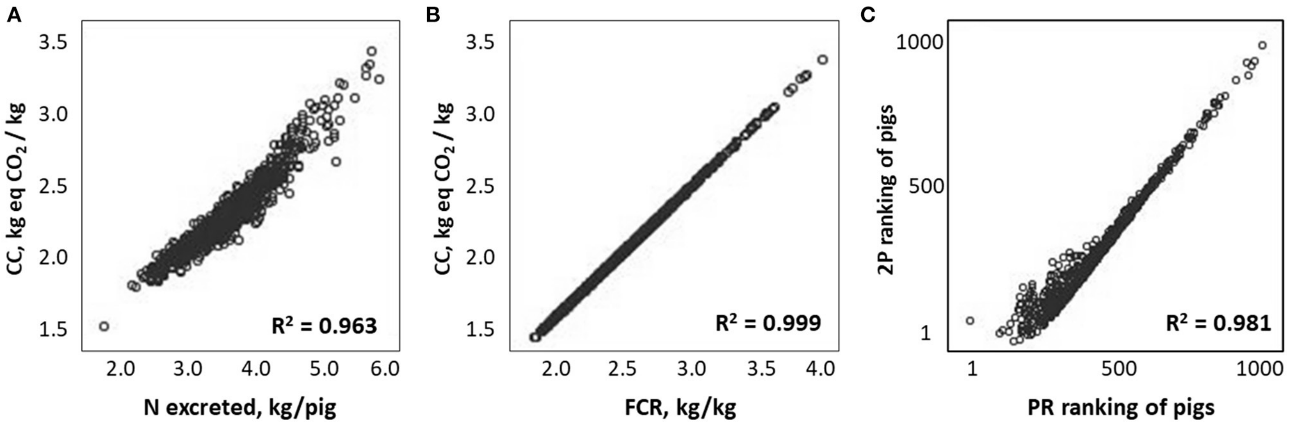

FIGURE 1 | Relationships between climate change (CC) impact and nitrogen excreted **(A)** or feed conversion ratio (FCR, **B**), and effect of feeding program on ranking of pigs according to CC impact **(C)** ($N = 1,000$ pigs).

lower than with NE, with *r* values ranging between 0.42 and 0.64, depending on the category. Correlations between environmental impacts and N retention efficiency were similar to these obtained with N excretion.

Average daily feed intake (ADFI) presented much lower correlation with all the impact categories ($r = +0.21$ on average). The weak correlation between ADFI and environmental impacts corroborated the 0.25–0.30 values obtained by Soleimani and Gilbert (5).

Feed conversion ratio appeared the best indicator of LCA impacts, with very high and positive correlations (**Table 2**, $r > +0.99$) with CC (**Figure 1B**), AC, EU, and LO for both feeding programs. This is consistent with the major contribution of feed intake to most environmental impacts (more than 70% for CC, EU, and LO, and about 30% for AC; 6), as well as to FCR. Moreover, efficient pigs, with lower FCR, ingest less energy and protein per kilogram of gain, which results in reduced enteric and manure methane production, and reduced organic matter, N, and P excretion. Gaseous emissions of N compounds from excreta have an important contribution to CC (due to N_2O emission) and to AC and EU (due to NH_3 emission). Moreover, NO_3^- and PO_4^- leaching after manure spreading also contributes to EU. This contributes to explain the close correlation between N retention efficiency and environmental impacts (*r* ranging from 0.96 to 0.98 depending on the impact category). These reductions in enteric emissions and emissions from excreta and manure from more efficient pigs (with low FCR) also contribute to explain the close relationship obtained between FCR and environmental impacts, both expressed per kilogram of body weight gain.

Despite the lower CC, AC, EU, and LO of pig production in the PR program, the correlations within each outcome were very similar among feeding programs.

Between-Animal Variability

It has already been shown that precision feeding strategy removes a constraint on reaching maximum growth potential and allows all animals to express their maximum growth potential, whereas with phase-feeding strategy, the performance of the highest potential animals may be limited due to insufficient amino acid supplies (8, 12). This explains that the variability of performance and environmental impacts may differ according to the feeding strategy. For instance, the coefficient of variation of CC impact was higher with PR than with 2P feeding strategy (12.1 and 10.7%, respectively). This affects the pigs' ranking, as illustrated in **Figure 1C**, which shows the correlation between the ranking of pigs according to CC impact with the two feeding strategies. Similar results were obtained for FCR.

CONCLUSIONS

The results of this study indicate that FCR is better correlated with environmental impacts evaluated using LCA than nitrogen excretion or other performance criteria. This offers interesting perspectives for the improvement of both feed efficiency and environmental impacts. However, further studies are still required before implementing LCA environmental impacts (or FCR as a proxy of these impacts) in selection programs. The same approach as the one used in this study with simulated data could be carried out on real data collected from selection programs. This would allow the assessment of the genetic parameters of the different LCA impacts and would allow taking better account of all the biological phenomena influencing growth performance, nutrient excretion, and enteric emission, which are probably not completely represented in the growth simulation model. Moreover, the correlated effects on other important criteria, such as carcass lean percentage, meat quality, or animal health and behavior, should also be evaluated.

DATA AVAILABILITY STATEMENT

All the data used in the statistical analysis are available in the INRAE data repository (10).

AUTHOR CONTRIBUTIONS

AM and J-YD contributed to conception and design of the study. AM, LB, and J-YD organized the database. AM, LB, HG, and J-YD wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Saintilan R, Mérour I, Brossard L, Tribout T, Dourmad, JY, Sellier P, et al. Genetics of residual feed intake in growing pigs: relationships with production traits, and nitrogen and phosphorus excretion traits. *J Anim Sci.* (2013) 91:2542–54. doi: 10.2527/jas.2012-5687
2. Niemi JK, Sevón-Aimonen ML, Stygar AH, Partanen K. The economic and environmental value of genetic improvements in fattening pigs: an integrated dynamic model approach. *J Anim Sci.* (2015) 93:4161–71. doi: 10.2527/jas.2015-9011
3. Shirali M, Duthie CA, Doeschl-Wilson A, Knap PW, Kanis E, van Arendonk JAM, et al. Novel insight into the genomic architecture of feed and nitrogen efficiency measured by residual energy intake and nitrogen excretion in growing pigs. *BMC Genomics*. (2013) 14:1–12. doi: 10.1186/1471-2156-14-121
4. McAuliffe GA, Chapman DV, Sage CL. A thematic review of life cycle assessment (LCA) applied to pig production. *Environ Impact Assess Rev.* (2016) 56:12–22. doi: 10.1016/j.eiar.2015.08.008
5. Soleimani Jevinani F, Gilbert H. Evaluating environmental impacts of selection for residual feed intake in pigs. *Animal.* (2020) 14:2598–608. doi: 10.1017/S175173112000138X
6. Monteiro ANTR, Garcia-Launay F, Brossard L, Wilfart A, Dourmad JY. Effect of feeding strategy on environmental impacts of pig fattening in different contexts of production: evaluation through life cycle assessment. *J Anim Sci.* (2016) 94:4832–47. doi: 10.2527/jas.2016-0529
7. van Milgen J, Valancogne A, Dubois S, Dourmad JY, Sève B, Noblet J. InraPorc: a model and decision support tool for the nutrition of growing pigs. *Anim Feed Sci Technol*. (2008) 143:387–405. doi: 10.1016/j.anifeedsci.2007.05.020
8. Brossard L, Vautier B, van Milgen J, Salaün Y, Quiniou N. Comparison of *in vivo* and *in silico* growth performance and variability in pigs when applying a feeding strategy designed by simulation to control the variability of slaughter weight. *Anim Prod Sci.* (2014) 54:1939–45. doi: 10.1071/AN14521
9. Rigolot C, Espagnol S, Pomar C, Dourmad JY. Modelling of manure production by pigs and NH3, N2O and CH4 emissions. Part I: animal excretion and enteric CH4, effect of feeding and performance. *Animal.* (2010) 4:1401–12. doi: 10.1017/S1751731110000492
10. Dourmad JY. Article Data - Environmental impacts and their association with performance and excretion traits in growing pigs. *Front Vet Sci.* (2021). doi: 10.15454/IRDMYU
11. Andretta I, Hauschild L, Kipper M, Pires PGS, Pomar C. Environmental impacts of precision feeding programs applied in pig production. *Animal.* (2018) 12:1990–8. doi: 10.1017/S1751731117003159
12. Andretta I, Pomar C, Rivest J, Pomar J, Lovatto PA, Radünz Neto J. The impact of feeding growing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition. *J Anim Sci.* (2014) 92:3925–36. doi: 10.2527/jas.2014-7643

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Eco-Friendly Feed Formulation and On-Farm Feed Production as Ways to Reduce the Environmental Impacts of Pig Production Without Consequences on Animal Performance

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Animal feeding has a major contribution to the environmental impacts of pig production. One potential way to mitigate such effects is to incorporate an assessment of these impacts in the feed formulation process. The objective of this study was to test the ability of innovative formulation methodologies to reduce the impacts of pig production while also taking into account possible effects on growth performance. We compared three different formulation methodologies: least-cost formulation, in accordance with standard practices on commercial farms; multiobjective (MO) formulation, which considered feed cost and environmental impacts as calculated by life cycle assessment (LCA); and MO formulation, which prioritized locally produced feed ingredients to reduce the impact of transport. Ninety-six pigs were distributed between three experimental groups, with pigs individually weighted and fed using an automatic feeding system from 40 to 115 kg body weight. Based on the experimental results, six categories of impacts were evaluated: climate change (CC), demand in non-renewable energy (NRE), acidification (AC), eutrophication (EU), land occupation (LO), and phosphorus demand (PD), at both feed plant gate and farm gate, with 1 kg of feed and 1 kg of live pig as functional units, respectively. At feed level, MO formulations reduced CC, NRE, AC, and PD impacts but sometimes increased LO and EU impacts. These formulations reduced the proportion of cereals and oil meals into feeds (feed ingredients with high impacts), while the proportion of alternative protein sources, like peas, faba beans, or high-protein agricultural coproducts increased (feed ingredients with low impacts). Overall, animal performance was not affected by the dietary treatment; because of this, the general pattern of results obtained with either MO formulation at farm gate was similar to that obtained at feed level. Thus, MO diet formulation represents an efficient way to reduce the environmental impacts of pig production without compromising animal performance.

Keywords: life cycle assesment, multiobjective formulation, local feed ingredients, low-impact feed, pig fattening

INTRODUCTION

Livestock production is a significant contributor to global environmental change. The associated greenhouse gas emissions, water pollution, acidification (AC), and primary energy consumption can have serious environmental impacts, in particular in territories with high concentrations of livestock (1, 2). For pig farming, such impacts are the consequences of feed production, direct farm energy use (electricity, gas, and oil consumption), and emissions from housing and manure management systems (3, 4). In particular, depending on the production system in question, animal feed accounts for 55–75% of the effects of climate change (CC), 70–90% of energy use, and 85–100% of land occupation (LO) associated with production (5). This is due, in part, to crop production processes for feed ingredients that are reliant on mineral fertilizers and pesticides, contribute to LO and transformation, consume significant amounts of energy, and use large-scale transportation networks (6). The challenge, then, is to reduce emissions and increase the efficient use of resources. Feed ingredients can vary dramatically in their environmental impacts; certain ingredients, like imported soybean meal, are resource intensive compared with alternative protein sources that can be locally produced (peas, faba beans, or high-protein agricultural coproducts) (6). Several studies have investigated the possibility of reducing the environmental impacts of pig production by modifying the composition of the diet. For example, Eriksson et al. (7) substituted soybean meal with peas and rapeseed meal in growing–finishing pig diets and observed reductions of 10% in energy use, 7% in global warming potential (GWP), and 17% in eutrophication (EU). Similarly, van Zanten et al. (8) showed that replacing soybean meal with rapeseed meal in the diets of finishing pigs reduced GWP as well as LO and energy use. A study of pig diets that substituted coproducts of wheat for corn and soybean meal also reported decreases in the potential for AC and EU, non-renewable energy (NRE) use, and GWP (9). Therefore, there is a possibility to reduce environmental impacts by selecting feed ingredients with relatively low impacts like alternative protein sources (peas, faba beans, or high-protein agricultural coproducts) or by using ingredients locally produced in order to reduce the impact of transport (6).

The traditional approach to feed formulation is based only on cost and makes no consideration of environmental factors. To reduce the overall impacts of pig production, new methods have been proposed that incorporate the environmental impacts of feed ingredients in the feed formulation process. For example, Garcia-Launay et al. (10) developed a multiobjective (MO) formulation method based on the environmental impacts of feed ingredients as calculated by life cycle assessment (LCA). Previous studies that have included environmental objectives in the calculation of feed formulations (9–11) have generated diets with lower proportions of cereals and oil meals and higher proportions of alternative protein sources (peas, faba beans, or high-protein agricultural coproducts). However, these studies were all based on models that assumed animal performance would be unaffected by these dietary changes. In general, feed formulations that are designed to minimize environmental impacts contain a higher proportion of protein-rich crops and coproducts, which may have potentially undesirable consequences with respect to the nutritional composition of feed and/or variability in energy, fiber, or protein content (12, 13). Indeed, using an experimental approach, Shaw et al. (14) reported a negative effect on pig growth of the incorporation of wheat middlings in the diet. Similarly, the replacement of soybean meal with rapeseed meal in the diet may also decrease pig performance (15). An increased incorporation of coproducts associated with the MO formulation could therefore adversely affect the pig performance and, consequently, reduce the improvement obtained at feed level. The objective of this study was then to test the effectiveness of innovative formulation methodologies in fattening pigs to reduce the environmental impacts of pig production, while taking into account their possible effects on animal performance. The global approach adopted was (i) to formulate diets based on these innovative feed formulation methodologies combining economy and environment, (ii) to test these diets experimentally on growing–finishing pigs, and (iii) to use the results of the experiment to assess the associated environmental impacts using LCA.

MATERIALS AND METHODS

Three different formulation methodologies were compared:

- least-cost formulation (Control-diet), in accordance with standard practices on commercial farms;
- MO formulation (Eco-diet) that simultaneously optimized feed cost and environmental impacts as calculated by LCA; and
- MO formulation using locally produced feed ingredients (Local-diet) to reduce the impact of feed transport.

Feed Formulation

Information on the nutritional composition of feed ingredients was obtained from the French nutritional table INRA-CIRAD-AFZ (16). Diets were formulated to meet the nutritional requirements of an average growing [40–65 kg body weight (BW)] or finishing (65–115 kg BW) pig. Minimum contents for standardized ileal digestible amino acids were set according to expected performance using the InraPorc® model (17) and also took into consideration French regulatory guidelines on maximum feed protein content (18). Minimum and maximum values of net energy content were defined in accordance with NRC 2012. The ingredients used in this study were analyzed before the diets were created in order to adjust diet composition according to the real nutritional values of ingredients (dry matter, organic matter, crude protein, and gross energy). Consequently, the incorporation rates of wheat, corn, soybean meal, and sugar beet pulp were slightly modified because their energy and protein contents were slightly different from the ones in the tables. Ingredient prices and availability were provided by IFIP (Didier Gaudré, personal communication). Ingredient prices of October 2018 (price of wheat: 203€/t; price of soybean meal: 351€/t) were used.

The environmental impacts of ingredients were taken from the ECOALIM dataset (version 7, October 1, 2019, https://

www6.inrae.fr/ecoalim/) of the AGRIBALYSE database (6). They included International Reference Life Cycle Data System (ILCD) metrics of AC potential (expressed in molc H^+-eq/kg) and CC, which included land use change (CC, expressed in kg CO_2-eq/kg). They also included Center for Environmental Studies (CML) EU potential (expressed in kg PO_4^{3-}-eq/kg from the Center for Environmental Studies); cumulative energy demand 1.8 (CED v1.8) as NRE demand (expressed in MJ/kg); and CML LO (expressed in m^2year/kg) and phosphorus (P) demand (6) (PD; expressed in kg P/kg). For crops, data used for the life cycle inventories (crop management practices; yields; and amounts of fertilizers, pesticides, and seeds) were obtained from French agricultural data and represented the national averages. All impacts from the ECOALIM dataset were considered to be those at the storage agency gate for the least-cost formulation (Control-diet) and the Eco-diet formulation, and to be those at the farm gate for the Local-diet formulation (except for rapeseed meal and for amino acids, premix, and phytase, which are not assumed to be produced on farm). An economic allocation approach was used to partition environmental impacts between a product and its c-product (**Supplementary Table 1**) as described in Wilfart et al. (6, 19) and advised by the Food and Agriculture Organization (20) and the French guideline to perform environmental assessment of agricultural product (21). Furthermore, Ardente and Cellular (22) recommended the use of the economic allocation concerning production of a main product with high economic value where coproducts are only a side effect of production.

The MO method developed by Garcia-Launay et al. (10) was used to formulate the experimental diets. This method considers animals' nutritional requirements, the cost of feed, and various environmental impacts. A detailed description of the method can be found in the original publication (10). As developed, the MO formulation method uses linear programming (Simplex algorithm) in the Python programming language (http://www.python.org). For the least-cost formulation, only feed cost was minimized. For the MO formulation, the objective function (Equation 1) included global environmental impacts calculated through the LCA, i.e., CC, NRE, LO, and PD, under a varying constraint ϵ of maximum feed cost (Equation 2) (10). Constraints were added on the environmental impacts of the formulated feed to ensure that the MO formulation did not increase any impact by more than 5% relative to the environmental impacts of the reference least-cost feed (Equation 3). Constraints were also applied to nutritional composition (**Supplementary Table 2**) and the incorporation rates of feed ingredients (Equation 4) (**Supplementary Tables 3–5**).

$$f(x) = \sum_{i \in I} coef_i \frac{Impact_i{}^t x - Min_i}{Ref_{impact_i} - Min_i} \quad (1)$$

$$c^t x \leq \epsilon \quad \epsilon = \left\{Ref_{price}, \ldots, Max_{price}\right\} \quad (2)$$

$$Impact_i{}^t x \leq 1.05 \times Ref_{impact_i} \quad (3)$$

$$\begin{pmatrix} q_{\min} \\ n_{\min} \\ 1 \end{pmatrix} \leq \begin{pmatrix} Q \\ N \\ 1^t \end{pmatrix} x \leq \begin{pmatrix} q_{\max} \\ n_{\max} \\ 1 \end{pmatrix} \quad (4)$$

$$i = \{CC,\ NRE,\ LO,\ AC,\ EU\}$$

$Impact_i{}^t$: vector of impact i of feed ingredients; c: matrix of feed ingredient prices; Max_{price}: price of feed when formulating without constraint ϵ; Min_i: level of impact i when formulated at lowest impact i; x: matrix of incorporation rates of feed ingredients (decision variables); Ref_{impact_i} and Ref_{price}: impact i and price of least-cost feed formulation; $coef_i$: weighting factor of impact I, with coefCC being double that of the other impacts. q_{min} and q_{max} are the minimum and maximum incorporation constraints on feed ingredients, respectively. n_{min} and n_{max} are the lower and upper bounds, respectively, for the nutritional constraints applied to the feed. The objective function weighted the environmental impacts of CC by 2, and those of NRE, LO and PD by 1.

The best feed formula is that for which the marginal decrease in the environmental index ($\frac{Impact_i^t x}{Ref_{impact_i}}$) is less than the marginal increase in the cost index $\frac{c^t x}{Ref_{price}}$.

The Local-diet was also formulated with the MO approach but with locally produced ingredients (cereals and protein-rich crops like peas and faba beans) as well as rapeseed meal.

The composition and the environmental impacts of the three growing diets and the three finishing diets are given in **Tables 1**, **2**, respectively.

Animal Study

The experiment was conducted in accordance with French legislation on animal experimentation and approved by the Regional Ethics Committee (authorization: 2019041815163846).

A total of 96 Pietrain × (Large White × Landrace) pigs were raised in a single experimental room; each pig was weighed and fed individually using an automatic weighing and feeding system. The experiment was conducted at the INRAE Pig Physiology and Phenotyping Experimental Facility (UE3P) located in Saint Gilles, France (https://doi.org/10.15454/1.5573932732039927E12). Pigs were distributed among three experimental groups: Control-diet, Eco-diet, and Local-diet (**Tables 1**, **2**). Pigs were assigned to the experimental treatments according to sex and litter origin according to a randomized complete block design. Therefore, each experimental group had an equal number of entire males and females ($n = 16$ per group per sex). Pigs in the experiment started at 40 kg average BW and ended at 115 kg average BW. Based on BW, pigs received experimental diets that met the requirements for growing (40–65 kg) or finishing (65–115 kg). Prior to entering the experimental room, pigs were tagged in the right ear with a serial number and an RFID chip for identification in the sorter (which also served as the weighing machine) and at the automated feeders. A detailed description of the feeding system used in this experiment was provided by Pomar et al. (23). The experimental room had two feeding zones that the pigs accessed by passing

TABLE 1 | Composition of experimental growing diets[a].

Diets	Control-diet	Eco-diet	Local-diet
Ingredients, %			
Corn	19.20	31.00	10.70
Wheat	36.00	15.22	29.50
Triticale	10.00		10.00
Barley	5.50		12.25
Wheat middlings	5.10	17.80	
Peas	10.00	20.00	20.00
Faba bean		5.00	10.00
Rapeseed oil		1.50	
Sunflower meal	2.00		
Rapeseed meal	1.10	7.00	5.00
Soybean meal	8.44		
L-lysine HCl	0.33	0.26	0.25
DL-methionine	0.04	0.05	0.09
L-threonine	0.09	0.09	0.10
L-tryptophan	0.01	0.03	0.03
Sodium chloride	0.45	0.45	0.45
Monocalcium	0.19		0.01
Calcium carbonate	1.05	1.10	1.12
Trace elements and mineral premix[b]	0.50	0.50	0.50
Phytase G5000	0.02	0.01	0.01
Chemical composition, g/kg			
Dry matter[c]	886	884	885
Organic matter[d]	838	833	839
Crude protein[c]	148	151	147
Crude fat[c]	21.3	40.5	18.4
Crude fiber[c]	31.2	41.3	34.3
Ca[d]	6.67	6.74	6.67
P[d]	4.35	4.67	3.86
P digestible[d]	2.35	2.33	2.36
Na[d]	1.75	1.74	1.75
K[d]	6.62	6.862	6.17
GE, MJ/kg[c]	15.89	16.34	15.84
NE, MJ/kg[d]	9.82	9.82	9.83
Environmental impacts of diets, per kg of feed[e]			
CC (g CO_2-eq)	518	378	338
NRE (MJ)	5.13	4.58	3.11
AC (molc H^+-eq)	0.0093	0.0082	0.0075
EU (g PO_4^{3-}-eq)	4.08	3.50	3.95
LO (m²year)	1.43	1.39	1.61
PD (g P)	4.09	2.53	2.83

[a]*Diet fed in pellet form.*

[b]*Provided per kilogram of complete diet: vitamin A, 1,000,000 IU; vitamin D, 3,200,000 IU; vitamin E, 4,000 mg; vitamin B1, 400 mg; vitamin B2, 800 mg; calcium pantothenate, 2,170 mg; niacin, 3,000 mg; vitamin B12, 4 mg; vitamin B6, 200 mg; vitamin K3, 400 mg; folic acid, 200 mg; biotin, 40 mg; choline chloride, 100,000 mg; iron (sulfate), 11,200 mg; iron (carbonate), 4,800 mg; copper (sulfate), 2,000 mg; zinc (oxide), 20,000 mg; manganese (oxide), 8,000 mg; iodine (iodate), 40 mg; cobalt (carbonate), 20 mg; and selenium (selenite), 30 mg.*

[c]*Analyzed values.*

[d]*Calculated values.*

[e]*CC, climate change; NRE, non-renewable and fossil energy demand; AC, acidification; EU, eutrophication; LO, land occupation; PD, P demand.*

TABLE 2 | Composition of experimental finishing diets[a].

Diets	Control-diet	Eco-diet	Local-diet
Ingredients, %			
Corn	25.20	37.40	2.45
Wheat	30.20		21.70
Triticale	10.00	14.60	10.00
Barley	7.00		34.50
Wheat middlings	5.00	19.50	
Peas	10.00	26.04	27.48
Faba bean			1.40
Sugar beet pulp	2.60		
Sunflower meal	2.00		
Rapeseed meal	1.00		
Soybean meal	4.60		
L-lysine HCl	0.31	0.22	0.22
DL-methionine	0.03	0.06	0.08
L-threonine	0.08	0.08	0.09
L-tryptophan	0.01	0.04	0.03
Sodium chloride	0.45	0.45	0.45
Monocalcium	0.11		0.05
Calcium carbonate	0.90	1.10	1.05
Trace elements and mineral premix[b]	0.50	0.50	0.50
Phytase G5000	0.01	0.01	0.01
Chemical composition, g/kg			
Dry matter[c]	887	880	884
Organic matter[d]	843	834	840
Crude protein[c]	132	136	135
Crude fat[c]	22.2	27.9	17.1
Crude fiber[c]	34.1	34.8	33.9
Ca[d]	6.16	6.20	6.11
P[d]	3.96	4.22	3.55
P digestible[d]	2.14	2.14	2.14
Na[d]	1.82	1.72	1.75
K[d]	5.93	6.59	5.93
GE, MJ/kg[c]	16.03	16.02	15.83
NE, MJ/kg[d]	9.85	9.85	9.87
Environmental impacts of diets, per kg of feed[e]			
CC (g CO_2-eq)	479	364	339
NRE (MJ)	5.06	4.55	3.06
AC (molc H^+-eq)	0.0094	0.0077	0.0074
EU (g PO_4^{3-}-eq)	3.98	3.60	4.06
LO (m²year)	1.41	1.40	1.68
PD (g P)	3.37	2.22	2.87

[a]*Diet fed in pellet form.*

[b]*Provided per kilogram of complete diet: vitamin A, 1,000,000 IU; vitamin D, 3,200,000 IU; vitamin E, 4,000 mg; vitamin B1, 400 mg; vitamin B2, 800 mg; calcium pantothenate, 2,170 mg; niacin, 3,000 mg; vitamin B12, 4 mg; vitamin B6, 200 mg; vitamin K3, 400 mg; folic acid, 200 mg; biotin, 40 mg; choline chloride, 100,000 mg; iron (sulfate), 11,200 mg; iron (carbonate), 4,800 mg; copper (sulfate), 2,000 mg; zinc (oxide), 20,000 mg; manganese (oxide), 8,000 mg; iodine (iodate), 40 mg; cobalt (carbonate), 20 mg; and selenium (selenite), 30 mg.*

[c]*Analyzed values.*

[d]*Calculated values.*

[e]*CC, climate change; NRE, nonrenewable and fossil energy demand; AC, acidification; EU, eutrophication; LO, land occupation; PD, P demand.*

through an automatic sorter. Each feeding zone was equipped with four automatic feeders. The sorter was programmed in random order so pigs could access either zone at random. Feed and water were provided *ad libitum*. Six pigs were removed from the experiment as a result of bodily injuries (4) or death (2) from causes unrelated to the experimental diets.

Live weight was measured automatically when the pigs passed through the automatic sorter. For each pig, average daily BW was calculated as the average of all of the BW recordings taken each day. Individual daily feed intake was calculated based on the recordings of the automatic feeding system according to the number of feed servings (in theory, one serving = 25 g) and a calibration factor. Calibration measurements were performed weekly on all feeders to adjust for the actual amount of feed delivered per serving. From these measurements, the calibration factor was calculated as the ratio of the actual amount delivered to the theoretical value.

All pigs fasted 24 h before slaughter; BW at slaughter was the final measurement taken as the pigs passed through the automatic sorter upon their departure for the slaughterhouse. Carcass characteristics, including carcass weight, lean meat percentage, and carcass yield, were measured at the slaughterhouse.

Life Cycle Assessment

Goal and Definition of Scope

The potential environmental impacts were calculated for each of the three experimental treatments using LCA; this approach evaluated the whole process of pig production, farrow to finish, in this case as carried out in Brittany, northwest France. The pig production system considered was a conventional growing–finishing pig farm in which animals are raised indoors on a slatted floor and manure is collected and stored externally as liquid slurry in an uncovered pit. To investigate the specific mechanisms by which each of the feed strategies modified the environmental impacts of pig production, three different system boundaries were considered (**Figure 1**):

- For the feed production process, the system boundaries (SB1) included the production and transport of feed ingredients and feed production, either at the feed factory (for the Control-diet and Eco-diet) or on-farm (Local-diet). In this case, the functional unit considered was 1 kg of feed leaving the feed factory (or, for the Local-diet, leaving the farm feed unit).
- For the fattening process, the system boundaries (SB2) were derived from Monteiro et al. (24) and included the production and transport of feed ingredients to the feed factory, the production process for growing and finishing feeds, transport of the feed to the farm (for the Control-diet and Eco-diet), growing to finishing pig production, and manure storage. For these system boundaries, the functional unit was 1 kg of live weight gain during fattening.
- For the entire farrow-to-finish production process, the system boundaries (SB3) were derived from Dourmad et al. (5) and included the production of piglets (farrowing unit) as well as the postweaning and growing–finishing periods, the production and transport of feed ingredients to the feed factory, the production of feed on-farm or at the feed factory, and emissions from animals and manure storage. The associated functional unit was 1 kg of live weight at the farm gate, including fattening pigs and culled sows.

For the fattening and farrow-to-finish analyses, the environmental impacts were calculated individually for each pig according to its individual performance. To this end, the impacts associated with piglet production and the postweaning period were accounted for in each pig using the following equation:

$$Impact_{ij}^{SB3} = \frac{Impact_{ij}^{Fattening} \times NbW + \left(Impact_i^{PW} \times NbP\right) + Impact_i^{FU}}{LW_j^{Slaughter} \times NbS + \left(LW^{CulledSow} - LW^{Gilt}\right) \times CullingRate} \quad (5)$$

with $Impact_{ij}^{SB3}$: the impact i of pig j per kilogram of live weight at the farm gate, NbW: the number of weaners produced per sow per year, NbP: the number of weaned piglets per sow per year, NbS: the number of slaughtered pigs per sow per year, $Impact_{ij}^{Fattening}$: the total impact i of fattening pig j during the fattening period, $Impact_i^{PW}$: the total impact i of one pig during the postweaning period, $Impact_i^{FU}$: the total impact i of one sow over 1 year, $LW_j^{Slaughter}$: the live weight of pig j at the farm gate, $LW^{CulledSow}$: the live weight of the culled sow at the farm gate, LW^{Gilt}: the live weight of the gilt at first mating, and *CullingRate*: the replacement rate of sows on the farm.

Life Cycle Inventories

The environmental impacts of the feed ingredients that were incorporated in the growing and finishing feeds came from the ECOALIM dataset (version 7, October 1, 2019, https://www6.inrae.fr/ecoalim/) (6). Hypothesized impacts of the transport of feed ingredients from field to feed factory and of the transport of feeds from feed factory to pig farm came from Méda et al. (25). The impacts of feeds for sows and postweaning piglets came from Méda et al. (25). Estimates of energy consumption in buildings were obtained from Dourmad et al. (5) for sows, postweaning piglets, and fattening pigs. The impact of processing in the feed factory was included in the life cycle inventories of Control-diet and Eco-diet feeds, with the assumptions that grinding and pelleting required 41 kWh of electricity and 20.5 kWh of natural gas per ton of feed produced (26). For on-farm feed production, grinding and mixing were estimated to require 18 kWh of electricity per ton of feed produced (27). The construction of buildings and manure storage units, as well as veterinary and cleaning products, were not included in the life cycle inventories. Background data for energy and transport came from ecoinvent v3.5 (28) included in the Agribalyse v3 database available in SimaPro®.

Nitrogen (N), phosphorus (P), and potassium excretions of sows, postweaning piglets, and fattening pigs were calculated using the mass balance approach of BRSPorc (29). Excretion of total ammoniacal N was calculated for fattening pigs as urinary N, resulting from the difference between the intake of digestible N and its retention in the body. For sows and piglets, excretion of ammoniacal N was calculated

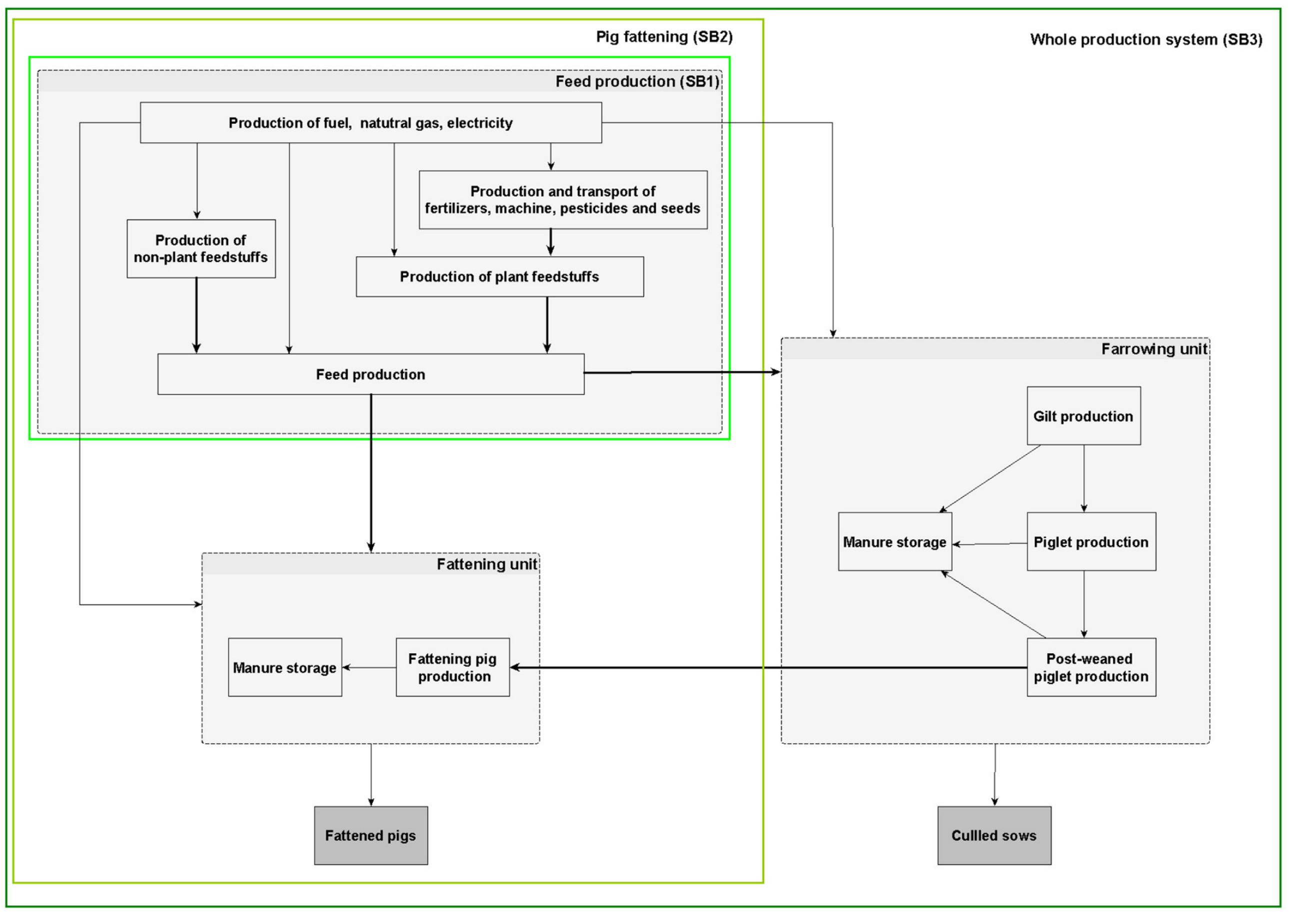

FIGURE 1 | Description of the three system boundaries (SB) considered in this study.

as a fixed proportion of N excretion, established by expert knowledge (Sandrine Espagnol, personal communication). Gaseous losses of nitrogen from manure in buildings and during manure storage were calculated in one of two ways: for NH_3, NO_x, and N_2 emissions, conversion factors from the European Monitoring and Evaluation Programme (EMEP) (2016) emission guidebook were applied to excreted ammoniacal N, and for N_2O, conversion factors were applied to total N excreted as per IPCC (2006). Excretion of organic matter was determined as a function of feed composition; emissions of CH4 from enteric fermentation and from manure storage were calculated using methods from the Intergovernmental Panel on Climate Change (IPCC) (2006) and Rigolot et al. (30, 31).

Life Cycle Impact Assessment

Six categories of impacts were calculated: CC, NRE, AC, EU, LO, and PD. The indicator result for each category was determined by multiplying the aggregated resources used and the aggregated emissions of each individual substance by a characterization factor unique to each applicable category. For CC (kg CO_2-eq) and AC (molc H^+-eq), impacts were estimated according to the International Reference Life Cycle Data (ILCD) System (32). EU (kg PO_4^{3-}-eq) and LO (m^2year) were calculated using the approach of the CML, and NRE (MJ) was predicted according to CED v1.08 (implemented in SimaPro® v. 8.0.5.13). All calculations were made with a publicly available software developed in Python 3.7 (https://doi.org/10.15454/PIJXCR) and extracted from the model developed by Cadéro et al. (33), which contains all equations and inputs for LCA described in this manuscript.

Statistical Analysis

Since all pigs were raised in a single experimental room, the statistical unit was the pig. Animal performance and farm-gate environmental impacts were subjected to an analysis of variance that tested the effects of gender (G), sire (S), and experimental diet (D) while taking into account the random effect of sire; the pig was the statistical unit considered. For this, the LME (linear mixed-effects) function from the NLME package of R software [version 3.5.1, (34)] was used, and results were considered significant for p-values lower than 0.05.

RESULTS

Experimental Diets

Diet Composition

The mean composition of each experimental diet with respect to ingredient (%) and nutritional content (g/kg) is provided in **Tables 1**, **2**. Compared with the Control-diet, the MO formulations contained a smaller proportion of cereals and oil meals and a larger proportion of protein-rich crops and coproducts. Specifically, the Control-diet contained an average of 71% cereals, 10% protein-rich crops (peas), 9.5% oil meals, 5% wheat middlings, 2.6% sugar beet pulp (only finishing), and 2.7% (growing) or 2.4% (finishing) additives (amino acids, vitamins, trace elements, and phytase). For the Eco-diet, the growing feed contained 46.2% cereals, 25% protein-rich crops (peas and faba beans), 17.8% wheat middlings, 7% rapeseed meal, 1.5% rapeseed oil, and 2.5% additives (amino acids, vitamins, trace elements, and phytase), while the finishing feed contained 52% cereals, 26% protein-rich crops (peas and faba beans), 19.5% wheat middlings, and 2.5% additives (amino acids, vitamins, trace elements, and phytase). The Local-diet contained 62.5% (growing) or 68.6% (finishing) cereals, 30% (growing) or 28.9% (finishing) protein-rich crops, 5% oil meals (only growing), and 2.5% additives (amino acids, vitamins, trace elements, and phytase).

LCA Impacts of the Diets (Per Kilogram of Feed)

The detailed LCA impacts of the experimental growing and finishing diets (expressed per kilogram of feed) are provided

TABLE 3 | Effect of diets on the growth performance of pigs.

	Control-diet	Eco-diet	Local-diet	RSD	Statistics
Animals, *n*	31	29	30		
Initial BW, kg	40.8	40.5	40.9	0.11	
Growing BW, kg	61.4	61.1	60.6	0.09	
Final BW, kg	113	113	113	0.08	G**
Growing period					
Initial BW, kg	40.8	40.5	40.9	0.10	
Growing BW, kg	61.4	61.1	60.6	0.09	
Duration, d	23	23	23		
Total feed intake, kg/pig	47.3	45.9	48.0	0.15	
ADG, g/d	896	898	854	0.15	G**
ADFI, kg/pig/d	2.06	1.99	2.09	0.15	G***
FCR	2.32^{ab}	2.24^{b}	2.48^{a}	0.11	G*, S**,D**
Daily water consumption, L/pig/d	4.47	4.47	4.84	0.31	G*, S*
Finishing period					
Initial BW, kg	61.4	61.1	60.6	0.09	
Final BW, kg	113	113	113	0.08	G**
Duration, d	55	55	55		
Total feed intake, kg/pig	142.5	144.3	149.9	0.11	
ADG, g/d	938	940	963	0.10	G**
ADFI, kg/pig/d	2.69	2.72	2.83	0.11	S***
FCR	2.65	2.69	2.72	0.09	G***,S**
Daily water consumption, L/pig/d	5.26	5.52	5.99	0.31	S***
Growing–finishing period					
Duration, d	78	78	78		
Total feed intake, kg/pig	189.8	190.2	198.0	0.11	
ADG, g/d	926	927	931	0.10	G***
ADFI, kg/pig/d	2.50	2.50	2.60	0.11	S***
FCR	2.64	2.64	2.74	0.11	G***, S***
Daily water consumption, L/pig/d	5.02	5.20	5.64	0.30	S**
Carcass yield, %	78.2	78.3	78.4	0.01	G**, S*
Lean meat, %	61.0	61.3	60.7	0.03	G*
Carcass weight, kg	88.4	88.3	89.0	0.08	G*

*ADG, average daily gain (g/d); ADFI, average daily feed intake (kg/pig/d); FCR, feed conversion ratio (ADFI/ADG); G, gender; S, sire; RSD, Residual standard deviation; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ^{a,b}Means with different superscripts (a, b) are significantly different between the experimental diet ($p < 0.05$).*

TABLE 4 | Environmental impacts at farm gate (per kilogram of body weight gain in fattening unit and per kilogram of pig live weight at farrow-to-finish farm gate).

Impacts at fattening unit gate (per kilogram of body weight gain)					
	Control-diet	**Eco-diet**	**Local-diet**	**RSD**	**Statistics**
CC (kg CO_2-eq)	2.40^{a}	2.04^{b}	1.95^{b}	0.14	G**, S***, D***
NRE (MJ)	19.06^{a}	17.30^{b}	12.82^{c}	0.19	G***, S***, D***
AC (molc H^{+}-eq)	0.112^{a}	0.104^{b}	0.108^{a}	0.11	G**, S***, D**
EU (g PO_4^{3-}-eq)	226^{a}	208^{b}	234^{a}	0.11	G**, S***, D***
LO (m²year)	4.65^{b}	4.58^{b}	5.68^{a}	0.14	G***, S***, D***
PD (g P)	115^{a}	74^{c}	97^{b}	0.20	G*, S***, D***
Impacts at farm gate (per kilogram of body weight)					
CC (kg CO_2-eq)	2.40^{a}	2.17^{b}	2.11^{b}	0.08	G***, S***, D***
NRE (MJ)	22.37^{a}	21.22^{b}	18.30^{c}	0.10	G***, S**, D***
AC (molc H^{+}-eq)	0.085^{a}	0.080^{b}	0.083^{a}	0.08	G*, S***, D**
EU (g PO_4^{3-}-eq)	198^{a}	186^{b}	203^{a}	0.08	G**, S***, D***
LO (m²year)	4.33^{b}	4.28^{b}	4.99^{a}	0.1	G**, S***, D***
PD (g P)	122^{a}	95^{c}	110^{b}	0.12	G***, S***, D***

CC, climate change; NRE, non-renewable and fossil energy demand; AC, acidification; EU, eutrophication; LO, land occupation; PD, P demand; G, gender; S, sire; D, diet; RSD, residual standard deviation; $^{**}p < 0.01$, $^{***}p < 0.001$; ^{a,b,c}Means with different superscripts (a, b, c) are significantly different between the experimental diet ($p < 0.05$).

in **Tables 1**, **2**. Compared with the Control-diet, the growing and finishing Eco-diets reduced the impact of CC by 27.0 and 24.0%, NRE by 10.8 and 9.9%, AC by 11.8 and 18.2%, EU by 14.2 and 9.6%, LO by 3.4 and 0.5%, and PD by 38.1 and 34.1%, respectively. Again compared with controls, the growing and finishing Local-diets reduced the impact of CC by 34.7 and 29.2%, NRE by 39.4 and 39.5%, AC by 20.1 and 21.6%, EU by 3.2% (only for the growing diet), and PD by 30.8 and 34.1%, respectively. However, the impact of EU increased by 2.0% with the finishing Local-diet, and the impact of LO increased with both Local-diets: 12.2% with the growing diet and 19.6% with the finishing diet. When we compared the Eco-diet and Local-diet, we found very similar patterns regardless of the growth stage targeted: the Eco-diet had higher CC impacts (10.6% for growing and 6.9% for finishing), higher NRE impacts (32.1 and 32.8%, respectively), higher AC impacts (9.5 and 4.2%, respectively), lower EU impacts (12.9 and 12.8%, respectively), lower LO impacts (16.1 and 20.2%, respectively), and lower PD impacts (11.9 and 29.3% respectively) than the Local-diet.

Animal Performance

Indicators of pig performance are presented in **Table 3**. Measurements of initial, growing, and final BW did not differ between the experimental groups (40.7, 61.0, and 113.0 kg on average; $p = 0.915$, $p = 0.852$, and $p = 0.943$, respectively). During the growing period, average daily gain (ADG) (883 g/d), average daily feed intake (ADFI) (2.05 kg/d), and total water consumption per pig per day (4.59 L) were similar among the experimental groups ($p = 0.336$, $p = 0.442$, and $p = 0.486$, respectively). Pigs fed the Local-diet had the highest feed conversion ratio (FCR), while those fed the Eco-diet had the lowest (2.48 vs. 2.24 kg/kg; $p < 0.01$); the FCR for the Control-diet group was intermediate in value (2.32 kg/kg). During the finishing period, we did not observe any significant differences between the experimental groups with respect to ADG (947 g/d), ADFI (2.75 kg/d), FCR (2.69 kg/kg), and daily water consumption per pig (5.59 L) ($p = 0.505$, $p = 0.108$, $p = 0.569$, and $p = 0.195$, respectively).

When we examined the performance of pigs over the total experimental period, we detected no significant differences in ADG (928 g/d), ADFI (2.53 kg/d), FCR (2.67 kg/kg), and total water consumption per pig per day (5.29 L) among the three experimental groups ($p = 0.976$, $p = 0.188$, $p = 0.139$, and $p = 0.238$, respectively). Values of carcass yield (78.3%), lean meat percentage (61%), and carcass weight (88.6 kg) were also similar in the three groups ($p = 0.819$, $p = 0.362$, and $p = 0.919$, respectively).

Globally, we observed significant ($p < 0.01$) differences between females and entire males with respect to final BW (110.4 vs. 116.0 kg), ADG (895 vs. 964 g/d), FCR (2.76 vs. 2.57 g/g), carcass yield (78.59 vs. 78.02%), lean meat percentage (60.6 vs. 61.4%), and carcass weight (86.8 vs. 90.5 kg).

Environmental Impacts at Farm Gate

LCA Impacts of Pig per Kilogram of BW Gain

The environmental impacts of growing–finishing pigs are reported in **Table 4**, per kilogram of BW gain (BWG). Compared with the Control-diet, the Eco-diet significantly reduced CC by 15.0%, NRE by 9.2%, AC by 7.5%, EU by 7.9%, and PD by 35.3% ($p < 0.01$). The LO impact was similar between the Control-diet and the Eco-diet (4.65 and 4.58 m²year, respectively; $p = 0.808$). The Local-diet, in comparison with the Control-diet, significantly reduced CC by 18.6%, NRE by 32.7%, and PD by 15.5% ($p < 0.01$). No modification of AC (0.112 and 0.104 molc H^{+}-eq, respectively) and EU (226 and 208 g PO_4^{3-}-eq, respectively) impacts was observed between the Control-diet and the Local-diet ($p = 0.316$ and $p = 0.221$, respectively). However, relatively to the Control-diet, the Local-diet significantly increased LO by 22.1% ($p < 0.01$). The Eco-diet and the Local-diet had similar CC impact (2.04 and 1.95 kg CO_2-eq, respectively; $p = 0.180$). The Eco-diet had higher NRE impact than the Local-diet (increased by 25.9%), but lower AC (decreased by 4.6%), EU (decreased by 12.6%), LO (decreased by 24.0%), and PD (decreased by 30.7%) impacts than the Local-diet ($p < 0.01$).

LCA Impacts of Pig per Kilogram of BW at Farm Gate

The details of LCA impacts of pig production at farm gate per kilogram of BW are also presented in **Table 4**. In comparison with the Control-diet, the Eco-diet significantly decreased the CC impact by 9.7%, the NRE impact by 5.1%, the AC impact by 6.2%, the EU impact by 5.8%, and the PD impact by 21.9% ($p < 0.01$). No difference in LO impact was observed between the Control-diet and the Eco-diet (4.33 and 4.28 m^2year, respectively; $p = 0.808$). The Local-diet significantly decreased CC impact by 12.2%, NRE impact by 18.2%, and PD impact by 9.8% in comparison with the Control-diet ($p < 0.01$). No modification of AC (0.085 and 0.080 molc H^+-eq, respectively) and EU (198 and 186 g PO_4^{3-}-eq, respectively) impacts was observed between the Control-diet and the Local-diet ($p = 0.227$ and $p = 0.174$, respectively). However, relatively to the Control-diet, the Local-diet significantly increased the LO impact by 15.4% ($p < 0.01$). The CC impact per kilogram of BW was similar between the Eco-diet and the Local-diet (2.17 and 2.11 kg CO_2-eq, respectively; $p = 0.129$). In comparison with the Local-diet, the Eco-diet had higher NRE impact (increased by 13.8%) and lower AC (decreased by 3.6%), EU (decreased by 9.0%), LO (decreased by 16.5%), and PD (decreased by 15.4%) impacts ($p < 0.01$).

DISCUSSION

Effectiveness of MO Formulation Approach on the Environmental Impacts of Feeds

In the least-cost formulated Control-diet, the main ingredients were cereals (71%), supplemented with protein-rich crops (10%), oil meals (9.5%), and coproducts of wheat (5%) (**Table 1**). The feeds obtained with the MO formulation approach differed from this in important ways: the Eco-diet was characterized by a lower proportion of cereals, while both the Eco-diet and Local-diet contained higher proportions of alternative protein sources and smaller proportions of oil meals, especially soybean meal, which was substituted with rapeseed meal, peas, or faba beans (**Tables 1**, **2**). These changes were consistent with those reported from previous efforts to include environmental objectives in the calculation of feed formulations (9, 10). Here, the composition of the diets obtained with the MO formulation was close to that formulated by Garcia-Launay et al. (10). Similarly, Mackenzie et al. (9) reported that diets formulated with environmental objectives in mind included a smaller proportion of cereals and a higher proportion of coproducts than diets formulated with economic objectives only (6).

With MO formulations, the relative incorporation rates of feed ingredients are shaped by trade-offs between the nutritional value, cost, and environmental impacts of each ingredient. Compared with cereals, protein-rich ingredients obtained from legume seeds, like peas and faba beans, are characterized by lower CC and NRE impacts because, unlike cereals, they do not require mineral nitrogen fertilization (6); this was the reason for their relatively high contributions to the Eco-diet and Local-diet. However, because of lower production yields, locally produced protein-rich crops have a higher LO impact than cereals (6). The production and utilization of crops on farm—and thus a reduced reliance on transport—decreased the average CC impact of ingredients by 8%, NRE by 14%, AC by 4.5%, and EU, LO, and PD by 2.5%. The environmental impacts of coproducts were relatively low, partly due to the economic allocation of impacts. Moreover, the industrial processes associated with their production are not input intensive (6). For example, the CC impact of wheat middlings was 75% lower than that of wheat, even though its crude protein content is about 50% higher; similar patterns were observed for other impacts as well. Among all the feed ingredients used in this study, wheat middlings had the lowest value for all impact categories (except for the AC impact, for which it was the second lowest). Similarly, the LCA impacts of rapeseed meal are about 60% lower than those of rapeseed grain. On the other end of the spectrum is soybean meal, which is associated with serious environmental impacts: most soybean meal in France is imported from South America, where agriculture-associated deforestation remains widespread (35). This means that soybean meal has a CC impact four times higher and an NRE impact three times higher than rapeseed meal, while its price and protein content are only about 30–40% higher.

In the MO formulation approach, the objective function weighted the environmental impacts of CC more heavily (×2) than those of NRE, LO, and PD (×1) because the mitigation of CC is considered to be a priority [Paris Agreement, 2015; (10)]. This was the underlying reason for the higher proportions of protein-rich crops and wheat coproducts and the reduced proportions of cereals and imported soybean meal in diets formulated with both environmental and economic objectives. Specifying weighting factors to the various environmental impacts is still a matter of debate in the literature. As recommended by Garcia-Launay et al. (10), in this study, we chose a pragmatic approach that consists in providing the same factors to all global impacts (NRE, PD, and LO) and a higher factor to CC. Performing feed formulation while accounting for various environmental impacts requests weighting the various impacts in the objective function. Indeed, formulating while minimizing a single impact leads to pollution transfer to other impacts or the increased use of limited resources (9). Using constraints on the various environmental impacts requires a step-by-step approach to find the adequate constraints for each single impact. Other approaches include basing weighting factors on monetary valuation, public opinion, or the state of the receiving environment (36). Although relevant for comparing the LCA of various scenarios, using these approaches for optimization may increase greatly impacts that are associated with lowest weighting factors.

When we compared the MO-formulated feeds to the least-cost Control-diet, we found that the environmental impacts of the Eco-diet were universally smaller. The Local-diet, instead, had smaller impacts with respect to CC, NRE, AC, and PD; no change for EU; and an increased impact on LO. In the least-cost formulation, imported soybean meal accounted for 19.5% of the CC impact and 12.9% of the NRE impact. Our results are consistent with those of Eriksson et al. (7) and van Zanten et al. (8), who reported a similar reduction in environmental impacts after replacing soybean meal with peas or rapeseed meal. Specifically, Eriksson et al. (7) substituted soybean meal with peas and rapeseed meal in growing–finishing pig diets and observed a reduction of 7% in the CC impact and 10% in the NRE impact; van Zanten et al. (8) showed that replacing soybean meal with rapeseed meal in a finishing pig diet reduced the CC impact by 10%. Here, the AC impact of the least-cost formulation (0.0094 molc H^+-eq) was a little higher than that of the Eco-diet (0.0078 molc H^+-eq) and the Local-diet (0.0074 molc H^+-eq) (**Supplementary Table 6**). This was the result of higher proportions of high-AC cereals in the least-cost diet and a shift to higher proportions of low-AC protein-rich crops and wheat coproducts in the MO formulations. In addition, a higher proportion of wheat middlings in the Eco-diet resulted in a reduced EU impact (3.6 g PO_4^{3-}-eq) relative to that of the least-cost formulation (4.0 g PO_4^{3-}-eq) and the Local-diet (4.0 g PO_4^{3-}-eq) (**Supplementary Table 6**). However, the reductions in AC and EU impacts with the MO formulation were minor compared with those observed for other impacts, mainly due to the fact that the objective function did not include either AC or EU. Patterns of PD among the diets (3.5 g P with least-cost formulation compared to 2.3 and 2.9 g P with Eco-diet and Local-diet, respectively) (**Supplementary Table 6**) could also be traced back in large part to the Control-diet's reliance on soybean meal, which has a P demand three times higher than that of other ingredients; the inclusion of wheat middlings in the Eco-diet also played a role, as the P demand of wheat middlings is five times lower than that of other ingredients. Overall, then, the MO formulation approach appeared to be quite effective in reducing the environmental impacts of pig fed. However, in one case, the MO approach significantly increased one environmental impact: the Local-diet had an LO impact that was about 18% higher (1.66 m^2year) than that of the least-cost formulation (1.41 m^2year) or the Eco-diet (1.40 m^2year) (**Supplementary Table 6**). Because of their generally lower yields, protein-rich crops need more land than cereals or coproducts to produce the same quantities (6–8). Moreover, for some crops, like soybean meal grown in South America, more than one crop can be harvested per year, which results in reduced values for LO. The Local-diet contained a similar proportion of cereals as the Control-diet but a higher proportion of protein-rich crops, and these two ingredient families have strong impacts on LO.

In agreement with the literature, we confirm that substituting cereals and soybean meal with alternative protein sources (rapeseed meal, peas, faba beans, or wheat middlings) is an efficient means of reducing the environmental impacts of pig feed; the overall balance of impacts can be further mediated by factors associated with ingredient production (i.e., locally produced vs. imported). The incorporation of ingredients that are produced locally further decreases the CC and NRE impacts of feed because of a reduction in transportation requirements; however, this strategy also increases the impact of LO because it relies more heavily on lower-yielding crops.

Animal Performance

Previous studies that have included environmental objectives in the calculation of feed formulations (9, 11) based their models of environmental impacts on the assumption that animal performance would be unaffected by feed composition. However, feeding strategies that minimize environmental impacts typically contain higher proportions of protein-rich crops and coproducts that may vary in their nutritional composition and energy, fiber, or protein content (12, 13). Such variability may have consequences on feed intake or digestibility, with potential repercussions for animal performance (14, 15). With an experimental approach, Shaw et al. (14) showed that the incorporation of wheat middlings in pig feed had a negative effect on growth. Similarly, the substitution of soybean meal with rapeseed meal may also decrease pig performance (15). Therefore, one potential concern with MO-formulated diets is that the environmental improvements obtained for the feed might be offset, partially or in total, by losses in pig performance. Here, we observed a slightly lower ADG and a slightly higher ADFI in the Local-diet group during the growing period, which resulted in an FCR that was significantly higher than that of the two other groups. We do not fully understand this difference in animal performance, as the estimated net energy and lysine concentrations in the three diets were formulated to be equal and based on the real nutritional values of ingredients (dry matter, organic matter, crude protein, and gross energy). This response might be due to interactions among ingredients that then affect digestibility, but in any event, it deserves further study. During the finishing period, animal performance was unaffected by the feeding strategies. The three groups all demonstrated similar carcass characteristics, including carcass weight, lean meat percentage, and carcass yield, resulting in similar carcass value. Over the fattening period as a whole, the MO formulations had no effect on animal performance, indicating that this innovative formulation method is effective in reducing environmental impacts without compromising performance or carcass quality.

Overall, unlike previous studies, we found no evidence of impaired performance due to the inclusion of alternative protein sources (rapeseed meal, protein-rich crops, or cereal coproducts) in animal diets. This difference may be related to the relatively higher levels of variability in the nutritional value of these ingredients. Instead of designing diets based on published average nutritional values, our study analyzed the real nutritional value of ingredients to determine the composition of diets. In addition, diets were carefully formulated to ensure they met the minimum nutritional content for pigs, in order to meet the requirements for net energy and standardized amino acid content established by the performance objective.

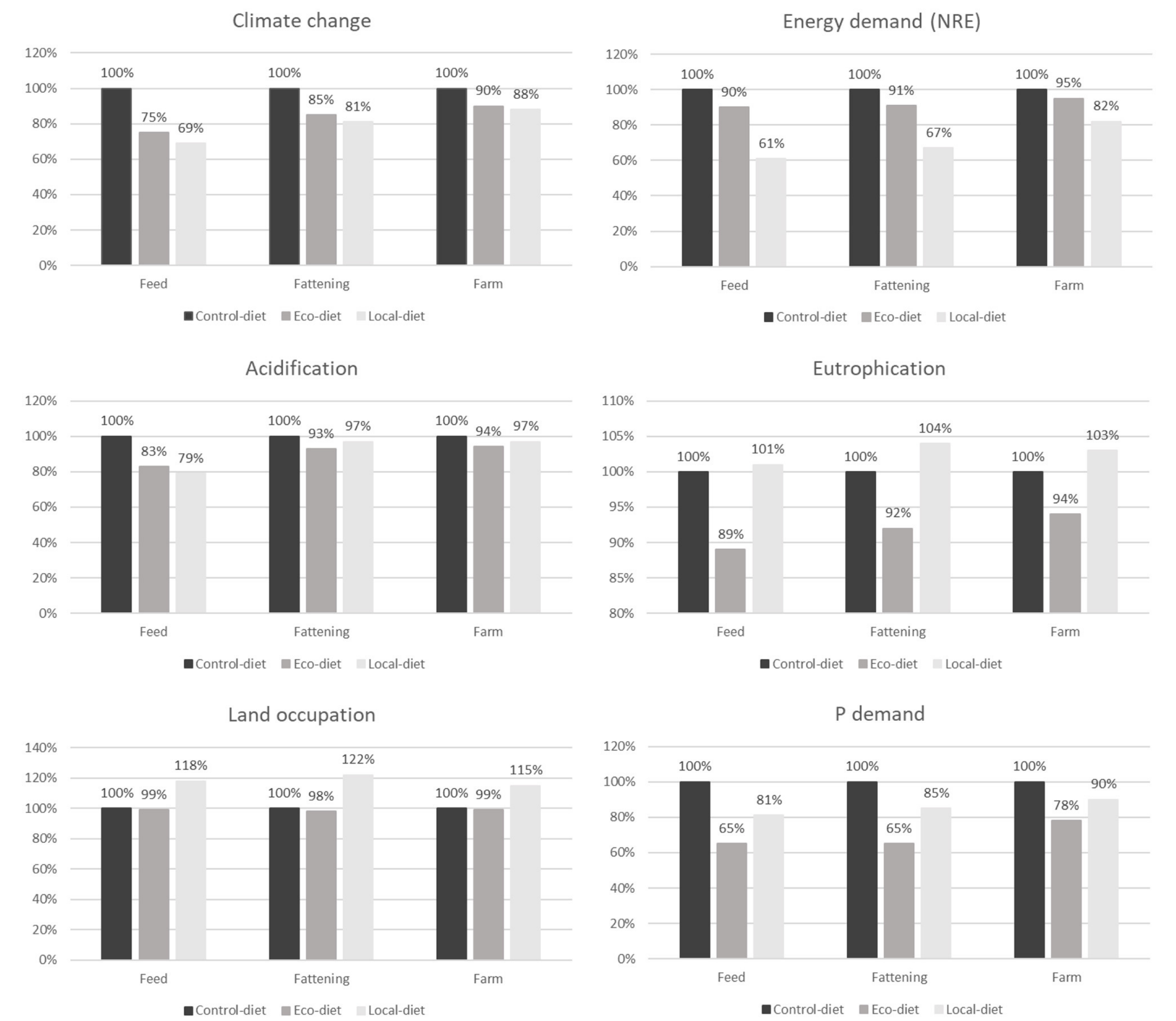

FIGURE 2 | Effect of the strategy for diet formulation on environmental impacts of the average feed and of pig production at fattening unit gate or at farm gate. Values are expressed as a percentage of the value obtained for the Control-diet strategy.

Environmental Impacts of Fattening Unit and Farrow-To-Finish Production

In our study, we calculated the environmental impacts of each diet strategy in three contexts: with respect to the feed only (i.e., impacts arising from feed ingredients and feed production processes), in the context of a fattening unit (i.e., the cumulative impacts required to raise an animal that is ready to be transported to the slaughterhouse), and in the context of an entire farrow-to-finish production farm (i.e., the cumulative impacts related to breeding, growing, and finishing). Since animal performance was similar among the three feeding strategies tested, the effects of the different feed formulations on the environmental impacts of fattening units followed the same general pattern as those obtained for the feed only (**Figure 2**). In comparison with the least-cost formulation, the Eco-diet significantly reduced all impacts except LO. The extent of the reduction was the same for the feed and the fattening unit with respect to NRE (−10%), LO (−1%), and PD (−35%) (**Figure 2**). However, for CC, AC, and, to a lesser extent, EU, the reduction in the fattening unit was smaller in magnitude than that observed for the feed (−15 vs. −25% for CC, −8 vs. −17% for AC, and −8 vs. −11% for EU, respectively; **Figure 2**). This might be explained by the fact that, for NRE and LO, the feed production process made a higher relative contribution to the total impact (>90%) than it did for CC, AC, and EU (30, 50, and 60%, respectively) (24). With respect to AC and EU, the relatively small degree of improvement seen with the use of the Eco-diet compared to the Control-diet can probably be explained by the fact that emissions from housing and manure were similar between the two feeding strategies.

In the same way, the effect of the Local-diet on the overall environmental impact of a fattening unit was the same as the effect of the diet alone (relative to the Control-diet) for three categories: NRE (−39% for Local-diet compared with Control-diet), LO (+20%), and PD (+20%). Instead, the fattening-unit effect was lower in magnitude for CC, AC, and EU (−31% for feed-only vs. −20% for the fattening unit with respect to CC, −21 vs. −3% for AC, and +1 vs. +4% for EU; **Figure 2**). In the context of the fattening unit, then, use of the Local-diet still reduced the impacts of CC, NRE, and PD compared with the Control-diet but was not significantly different from the Control-diet with respect to the impacts on AC and EU. At the level of both feed and the fattening unit, the Local-diet significantly increased LO over control values to a similar extent (+18 and +22%, respectively).

When applied to a fattening unit, the Eco-diet was more effective in reducing the impacts of AC, EU, LO, and PD than the Local-diet; however, the impact on CC was similar between the two strategies. Furthermore, the Local-diet was more efficient in reducing the impact of NRE per kilogram of BWG (−40%) than the Eco-diet. Garcia-Launay et al. (10), Wilfart et al. (11), and Méda et al. (25) all obtained similar results based on models of animal performance.

For CC and NRE, the differences among the feeding strategies were more muted when examined in the context of a farrow-to-finish production farm than in a fattening unit, while for AC, EU, LO, and PD, the relative differences between strategies remained generally similar. Specifically, implementation of the Local-diet and Eco-diet reduced the CC impact of a production farm by only 10% compared with the Control-diet strategy (the corresponding reduction for the Local-diet and Eco-diet in fattening units being 15 and 19%, and in the feed-only analysis, 25 and 31%, respectively; **Figure 2**). Similarly, use of the Local-diet reduced NRE on the production farm by only 5% compared to a 39% reduction for feed only and a 33% reduction for the fattening unit. These differences are mainly related to the contributions of the farrowing and postweaning units, which consume a significant amount of energy for heating (5). Furthermore, the farrow-to-finish LCA was carried out based on the assumption that sows and piglets were given conventional (least-cost formulated) diets, and it is likely that this also contributed to the reduction (or dilution) in the apparent effects of the different fattening feeds. If MO formulations had also been applied for the phases of gestation, lactation, and weaning, it is probable that the difference between feeding strategies would have been more marked.

CONCLUSION

MO formulation is a useful strategy for reducing the environmental impacts of pig production. Using this approach, we were able to select feed ingredients with lower environmental impacts, such as protein-rich crops or agricultural coproducts, and thus efficiently reduce the impacts of pig production without adverse consequences on animal performance or carcass quality. Before such diets can be applied, however, it is important to first analyze the nutritional composition of the ingredients in order to adjust the composition of the diet according to their real nutritional values. Another potential challenge could arise regarding the availability of ingredients: wide-scale incorporation of these ingredients in ecofriendly diets could result in scarcity, as protein-rich crops currently represent only 2% of cultivated land in France. Moreover, increasing demand for coproducts could affect feed prices and, consequently, the economic allocation of environmental impacts. Such potential constraints must be taken into consideration by future efforts to implement these innovative formulation methodologies at a large scale.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: https://data.inrae.fr/dataset.xhtml?persistentId=10.15454/PIJXCR10.15454/PIJXCR.

ETHICS STATEMENT

The animal study was reviewed and approved by Regional Ethics Committee of Brittany (authorization: 2019041815163846).

AUTHOR CONTRIBUTIONS

FQ, LB, AW, J-YD, and FG-L contributed to the conception and design of this study. AW and FQ contributed to funding acquisition. FQ and LB were responsible for the animal experiment. FG-L provided the development of the LCA model and the following evaluation and assessment of this model. FQ wrote the first draft of the manuscript. J-YD and FG-L wrote sections of the manuscript. All authors contributed to manuscript revision and read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Steinfield H, Gerber P, Wassenaar T, Castel V, Rosales M, Haan C. *Livestock's Long Shadow - Environmental Issues and Options.* Rome: United Nations Food and Agriculture Organization (2006).
2. Gerber PJ, Steinfield H, Henderson B, Mottet A, Opio C, Dijkman J, et al. *Tackling Climate Change Through Livestock - A Global Assessment of Emissions and Mitigation Opportunities*. Rome: Food and Agriculture Organization of the United Nations (FAO) (2013).
3. Basset-Mens C, Van Der Werf HMG. Scenario-based environmental assessment of farming systems: the case of pig production in France. *Agric Ecosyst Environ.* (2005) 105:127–44. doi: 10.1016/j.agee.2004.05.007
4. Reckmann K, Traulsen I, Krieter J. Environmental impact assessment - methodology with special emphasis on European pork production. *J Environ Manag.* (2012) 107:2561–71. doi: 10.1016/j.jenvman.2012.04.015
5. Dourmad JY, Ryschawy J, Trousson T, Bonneau M, Gonzàlez J, Houwers HWJ, et al. Evaluating environmental impacts of contrasting pig farming systems with life cycle assessment. *Animal.* (2014) 8:2027–37. doi: 10.1017/S1751731114002134
6. Wilfart A, Espagnol S, Dauguet S, Tailleur A, Gac A, Garcia-Launay F. Ecoalim: a dataset of environmental impacts of feed ingredients used in french animal production. *PLoS ONE.* (2016) 11:e0167343. doi: 10.1371/journal.pone.0167343
7. Eriksson IS, Elmquist H, Stern S, Nybrant T. Environmental systems analysis of pig production - the impact of feed choice. *Int J Life Cycle Assess.* (2005) 10:143–54. doi: 10.1065/lca2004.06.160
8. Van Zanten HHE, Mollenhorst H, de Vries JW, van Middelaar CE, van Kernebeek HRJ, de Boer IJM. Assessing environmental consequences of using co-products in animal feed. *Int J Life Cycle Assess.* (2014) 19:79–88. doi: 10.1007/s11367-013-0633-x
9. Mackenzie SG, Leinonen I, Ferguson N, Kyriazakis I. Can the environmental impact of pig systems be reduced by utilising co-products as feed? *J Clean Prod.* (2016) 115 :172–81. doi: 10.1016/j.jclepro.2015.12.074
10. Garcia-Launay F, Dusart L, Espagnol S, Laisse-Redoux S, Gaudré D, Méda B, et al. Multiobjective formulation is an effective method to reduce environmental impacts of livestock feeds. *Br J Nutr.* (2018) 120 :1298–309. doi: 10.1017/S0007114518002672
11. Wilfart A, Dusart L, Méda B, Gac A, Espagnol S, Morin L, et al. Réduire les impacts environnementaux des aliments pour les animaux d'élevage. *INRA Prod Anim.* (2018) 31:289–306. doi: 10.20870/productions-animales.2018.31.2.2285
12. Cozannet P, Primot Y, Gady C, Métayer JP, Lessire M, Skiba F, et al. Energy value of wheat distillers grains with solubles for growing pigs and adult sows. *J Anim Sci.* (2010) 88:2382–92C. doi: 10.2527/jas.2009-2510
13. Zijlstra RT, Beltranena E. Swine convert co-products from food and biofuel industries into animal protein for food. *Anim Front.* (2013) 3:48–53. doi: 10.2527/af.2013-0014
14. Shaw DT, Rozeboom DW, Hill GM, Booren AM, Link JE. Impact of vitamin and mineral supplement withdrawal and wheat middling inclusion on finishing pig growth performance, fecal mineral concentration, carcass characteristics, and the nutrient content and oxidative stability of pork. *J Anim Sci.* (2002) 80:2920–30. doi: 10.2527/2002.80112920x
15. Hulshof TG, Van der Poel AFB, Hendriks WH, Bikker P. Processing of soybean meal and 00-rapeseed meal reduces protein digestibility and pig growth performance but does not affect nitrogen solubilization along the small intestine. *J Anim Sci.* (2016) 94:2403–14. doi: 10.2527/jas.2015-0114
16. Sauvant D, Perez JM, Tran G. A*ssociation Française de Zootechnie. Tables de composition et de valeur nutritive des matiéres premiéres destinées aux animaux d'élevage (Porcs, volailles, ovins, caprins, lapins, chevaux, poissons). INRA Editions*, 2[éme] édition. Paris (2004). p. 301.
17. Van Milgen J, Valancogne A, Dubois S, Dourmad JY, Sève B, Noblet J, et al. InraPorc: a model and decision support tool for the nutrition of growing pigs. *Anim Feed SciTechnol.* (2008) 143:387–405. doi: 10.1016/j.anifeedsci.2007.05.020
18. CORPEN. *Estimation des rejets d'azote-phosphore-potassium-cuivre et zinc des porcs. Influence de la conduite alimentaire et dumode de logement des animaux sur la nature et la gestion des déjections produites. Paris: Comité d'Orientation pour des Pratiques Agricoles Respectueuses de l'Environnement; Ministére de l'écologie*. (2003). p. 41.
19. Wilfart A, Gac A, Salaün Y, Aubin J, Espagnol S. Allocation in the LCA of meat products: is agreement possible? *Cleaner Environ Syst.* (2021) 2:100028. doi: 10.1016/j.cesys.2021.100028
20. Food and Agriculture Organization. *Environmental Performance of Animal Feeds Supply Chains. Livestock Environmental Assessment and Performance Partnership.* Rome: FAO (2014).
21. Koch P, Salou T. AGRIBALYSE(R): Methodological Report - Version 1.2. Angers: ADEME (2015). p. 385.
22. Ardente F, Cellura M. Economic allocation in life cycle assessment. the state of the art and discussion of examples. *J Ind Ecol.* (2012) 16:387–98. doi: 10.1111/j.1530-9290.2011.00434.x
23. Pomar J, López V, Pomar C. Agent-based simulation framework for virtual prototyping of advanced livestock precision feeding systems. *Comput Electron Agric.* (2011) 78:88–97. doi: 10.1016/j.compag.2011.06.004
24. Monteiro A, Garcia-Launay F, Brossard L, Wilfart A, Dourmad JY. Effect of feeding strategy on environmental impacts of pig fattening in different contexts of production: evaluation through life cycle assessment. *J Anim Sci.* (2016) 94:4832–47. doi: 10.2527/jas.2016-0529
25. Méda B, Garcia-Launay F, Dusart L, Ponchant P, Espagnol S, Wilfart A. Reducing environmental impacts of feed using multiobjective formulation: what benefits at the farm gate for pig and broiler production? *Animal.* (2021) 15:100024. doi: 10.1016/j.animal.2020.100024
26. Garcia-Launay F, van der Werf HMG, Nguyen TTH, Le Tutour L, Dourmad JY. Evaluation of the environmental implications of the incorporation of feed-use amino acids in pig production using life cycle assessment. *Livest Sci.* (2014) 161:158–75. doi: 10.1016/j.livsci.2013.11.027
27. Badouard B, Roy H. Bien calculer le coût de fabrication à la ferme des aliments pour porcs. *Tech Porc*. (2011). p. 3.
28. Wernet G, Bauer C, Steubing B, Reinhard J, Moreno-Ruiz E, Weidema BP. The ecoinvent database version 3 (part I): overview and methodology. *Int J Life Cycle Assess.* (2016) 21:1218–30. doi: 10.1007/s11367-016-1087-8
29. RMT Elevages & Environnement. *Evaluation des rejets d'azote, phosphore, potassium, cuivre et zinc des porcs. Influence de l'alimentation, du mode de logement et de la gestion des effluents*, Paris, (2016).
30. Rigolot C, Espagnol S, Pomar C, Dourmad JY. Modelling of manure production by pigs and NH3, N2O and CH4 emissions. Part I: animal excretion and enteric CH4, effect of feeding and performance. *Animal.* (2010) 4:1401–12. doi: 10.1017/S1751731110000492
31. Rigolot C, Espagnol S, Robin P, Hassouna M, Beline F, Paillat JM, et al. Modelling of manure production by pigs and NH3, N2O and CH4 emissions. Part II: effect of animal housing, manure storage and treatment practices. *Animal.* (2010) 4:1413–24. doi: 10.1017/S1751731110000509
32. Joint Research Centre. *Characterisation Factors of the ILCD Recommanded Life Cycle impact asessment methods. Database and Supporting Information.*. 1st ed. Luxembourg: European Commission, JRC, Institute for Environment and Sustainability (2012).
33. Cadéro A, Aubry A, Brossard L, Dourmad JY, Salaün Y, Garcia-Launay F. Modelling interactions between farmer practices and fattening pig performances with an individual-based model. *Animal.* (2018) 12:1277–86. doi: 10.1017/S1751731117002920

34. Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RC. *NLME: Linear and Nonlinear Mixed Effects Models. R package version 3*. Vienna: R Foundation (2018). p. 1–137.
35. Van der Werf HMG, Petit J, Sanders J. The environmental impacts of the production of concentrated feed: the case of pig feed in Bretagne. *Agric Syst.* (2005) 83:153–77. doi: 10.1016/j.agsy.2004.03.005
36. Soares SR, Toffoletto L, Deschenes L. Development of weighting factors in the context of LCIA. *J Clean Prod.* (2006) 14:649–60. doi: 10.1016/j.jclepro.2005.07.018

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REVIEW
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Poultry Response to Heat Stress: Its Physiological, Metabolic, and Genetic Implications on Meat Production and Quality Including Strategies to Improve Broiler Production in a Warming World

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The continuous increase in poultry production over the last decades to meet the high growing demand and provide food security has attracted much concern due to the recent negative impacts of the most challenging environmental stressor, heat stress (HS), on birds. The poultry industry has responded by adopting different environmental strategies such as the use of environmentally controlled sheds and modern ventilation systems. However, such strategies are not long-term solutions and it cost so much for farmers to practice. The detrimental effects of HS include the reduction in growth, deterioration of meat quality as it reduces water-holding capacity, pH and increases drip loss in meat consequently changing the normal color, taste and texture of chicken meat. HS causes poor meat quality by impairing protein synthesis and augmenting undesirable fat in meat. Studies previously conducted show that HS negatively affects the skeletal muscle growth and development by changing its effects on myogenic regulatory factors, insulin growth factor-1, and heat-shock proteins. The focus of this article is in 3-fold: (1) to identify the mechanism of heat stress that causes meat production and quality loss in chicken; (2) to discuss the physiological, metabolic and genetic changes triggered by HS causing setback to the world poultry industry; (3) to identify the research gaps to be addressed in future studies.

Keywords: heat stress, poultry, meat production, meat quality, muscle development

INTRODUCTION

The increasing world population demands a more efficient food production system since the global food shortage issue keeps on rising. The poultry sector is noted to make a considerable contribution to global nutrition and food security, which helps in the provision of cheap protein, essential micronutrients, and energy to humans (1). Poultry, owing to their short production cycles and having the potential of converting wide ranges of agricultural food waste and by-products into eggs and meat edible for humans. Poultry meat production have been reported to increase from 120.5 MMT (million metric tons) in 2017 to 122.5 MMT in 2018 (2). FAO (3) also estimated its production to reach 137 MMT in

2020, with growth being anticipated in China, Britain, the EU, Mexico, and Brazil, suggesting the poultry industry's hidden potentials.

Recently, there has been a remarkable escalation in global environmental temperature, which poses serious implications to the farming sector in both tropical and subtropical regions of the world. A gradual increase in ambient temperature affects all living organisms (4, 5). In living organisms, if the temperature exceeds the normal range (thermo-neutral zone), it disturbs the normal physiological functioning and induces cell injury. Usually, high ambient temperature leads to stress associated problems such as production losses, metabolic changes, growth depression, and poor efficiency (6, 7). In temperate regions of world the high ambient temperature during the summer season often proves disastrous for poultry farming as thermal stress induced by extremely high temperatures is responsible for massive economic losses to poultry industry. According to a report, the U.S. livestock production industry suffers a severe loss of $1.69 to $2.36 billion because of high environmental temperature; out of which poultry industry accounts the loss of $128 to $165 million (8). Heat stress (HS) is widely classified into acute heat stress (AHS), which is the intense environmental temperature for a brief period and chronic heat stress (CHS) characterized by high temperature for a longer duration. Unluckily, both AHS and CHS challenge the genetic, nutritional, pharmaceutical, and management developments made by the animal farming industries that cause a considerable drop in production, proving to be one of the major hurdles to achieve efficient livestock farming in many regions of the world (9, 10). Chronic heat stress has permanent damaging effect on the broiler chicken, if heat stress persists for longer period of time it increases fat content and damages the muscle portion of chicken unlike acute heat stress. Apart from duration of heat stress, the extent of production damage is also dependent on the intensity of heat stress (11). Harmful consequences of heat exhaustion (temperature exceeds beyond thermo-neutral zone and animal no more able to regulate body temperature) in animal farming would become more challenging as temperature keeps rising due to global warming. Climate change due to global warming is becoming more relevant these days, especially for the chicken meat industry (12, 13). The broiler industry faces the challenge of HS, which increases production cost and severely damages the meat quality due to poultry's susceptibility to heat because of their rapid metabolic rate and high growth. Metabolic changes occur in chickens, specifically, broilers, reared in a HS environment, causing a considerable decrease in breast muscle size of the broiler chicken. HS is also responsible for the reduction in the protein content of muscles (14). Both AHS and CHS could cause a sharp decline in the metabolism of birds, which in turn will induce serious complications regarding the growth and performance of the broilers, such as a change in color, the decline in muscle pH, water-holding capacity (WHC), and juiciness of chicken meat (15, 16). Many studies have revealed that high ambient temperature causes oxidative stress by producing reactive oxygen species (ROS). ROS has severe implications on skeletal muscle development, as they are responsible for lipid peroxidation in muscles (17, 18). Thus, understanding the mechanisms underlying, the causes, and effects of HS and the strategies that can be put in place to curb or control such global menace, can be beneficial in solving the global food insecurity issues. This review dealt deep in analyzing the available information surrounding HS impact and the strategies to limit the unwanted implications of this threat. **Figure 1** illustrates the physiological, metabolic and genetic changes amid HS and its relation to meat production and quality in chicken.

HEAT STRESS IN BROILERS; HOW DOES IT PROCEED?

Any foreign stimuli, which alters the normal biological and physiological mechanisms within living cells and threatens the living organism's survival, is referred as stress (18, 19). In broiler production, environmental stress is often caused by numerous factors, including ambient temperature, which severely compromises birds' normal physiology, leading to poor production efficiency and food safety (20). In animals, stress often manifests in three stages. Firstly, the recognition of external stress by the body is known as a state of alarm. Secondly, stress induces the immune mechanism in living cells; thus, if stress persists, the body tries to adapt to that new environment. Despite all resistance, if the body still fails to cope with that stress, it leads to the exhaustion stage (21). Every living organism responds to HS accordingly, depending on the intensity and duration of stress. Numerous studies reported a substantial reduction in feeding and walking duration (discrete values) of birds kept under HS conditions as heat-stressed birds spend most of the time in acclimatizing activities such as panting, drinking more water, and resting to cope with the HS (22).

The neuroendocrine system plays a very significant role in HS response by inducing the autonomic nervous system (ANS) that often regulates fight and flight situations in living organisms (23). In response to HS, the ANS takes charge and triggers tachycardia (increased heartbeat), increases respiration rate and enhances the blood flow toward the body peripheries (skin) for maximum heat loss to maintain body temperature (23, 24). It also promotes the breakdown of glycogen into glucose in muscles and reduces their capacity to store energy (6, 13). Activation of the neuroendocrine system positively regulates the release of catecholamine. Catecholamine acts on beta androgenic receptors of skeletal muscles and initiates a series of reactions, disturbing the normal enzymatic activity in skeletal muscles as it inhibits the enzyme glycogen phosphorylase and activates the muscle glycogenolysis (25). HS also activates the hypothalamic-pituitary-adrenal axis (HPA) along with the sympathetic-adrenal-medullar axis (SAM), which promotes the release of glucocorticoids, vasodilation, lipolysis, and proteolysis in muscles (26, 27). Glucocorticoids enhance glucose synthesis to confirm the survival of animals under such critical conditions as HS. The substantial release of glucocorticoids characterizes AHS as compared to CHS. Glucocorticoids encourage proteolysis by damaging myofibrils in skeletal muscles facilitated through major proteolytic mechanisms (ca^{+2} dependent, ubiquitin-proteasome system) (28, 29).

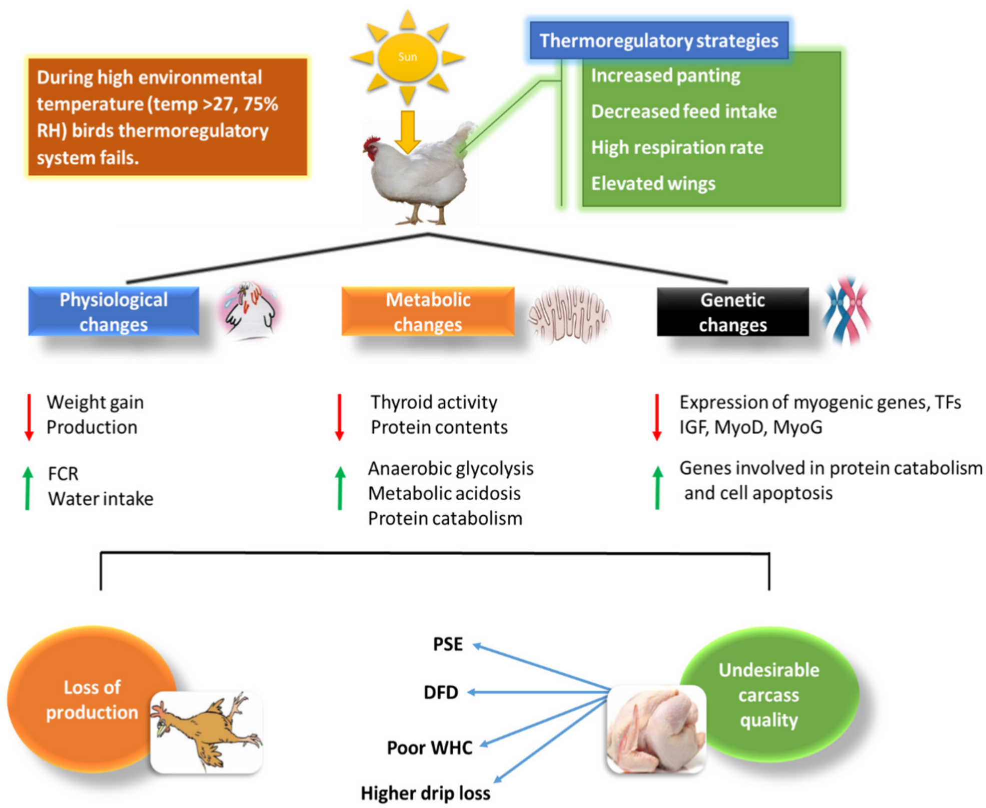

FIGURE 1 | Relationship of HS with physiological and biochemical changes in chicken and how it affects broiler chickens' meat quality.

Furthermore, glucocorticoids initiate the hydrolysis of circulating triglycerides, intensifying the activity of lipoprotein lipase that leads to an increase in lipolysis. Moreover, anabolic factors like insulin growth factor (IGF-1) are negatively regulated by glucocorticoids to worsen the skeletal muscle damage. HPA is considered a better indicator of HS than corticosterone as it could be secreted in many other conditions like fear of invading animals etc. (30, 31). Corticosterone is secreted from both the HPA axis and the pituitary gland, corticosterone's secretion rate is relatively as slow compared to adrenaline but displays more compound and prominent effect during HS (32, 33). Long-term secretion of corticosterone during chronic HS is linked to many deleterious consequences in broiler chicken, including compromised immunity, muscle breakdown, cardiac issues, and depression (**Figure 2**). HS also induces infertility by disturbing reproductive hormones, severely affecting poultry gut health (leaky gut), as well as the altering of the immune functioning by triggering inflammatory cytokines (34).

THERMOREGULATORY APPARATUS IN CHICKEN

All homoeothermic organisms have an optimal temperature range considered as the thermo-neutral zone unlike poikilotherms whose body temperature varies greatly depending on environmental temperature. In case the environmental temperature increases, the birds require more energy to maintain their body temperature (35). During HS conditions, metabolic heat increases, and animal succumbs to hyperthermia. Birds do not have sweat glands unlike mammals, but they have developed some behavioral adaptations to cope with heat, including elevated respiration rate, panting and raised wings (35, 36). In commercial poultry, high production always remained a priority that made the broiler more vulnerable to environmental stressors. The insulation provided by feathers in commercial poultry is one of the major hindrances in birds' thermoregulation (35, 37). To sum up, high ambient temperature beyond the thermo-neutral zone during the production phases badly affect meat production, meat quality and cause severe immune problems in the broiler flocks.

IMPACT OF HS ON POULTRY MEAT PRODUCTION

Reduction in Feed Intake and Poor Weight Gain

Reduction in feed intake in HS animals is an adaptive mechanism to minimize metabolic heat production. A significant decrease in feed intake, body weight gain, and feed efficiency has been reported in many studies conducted on birds and other

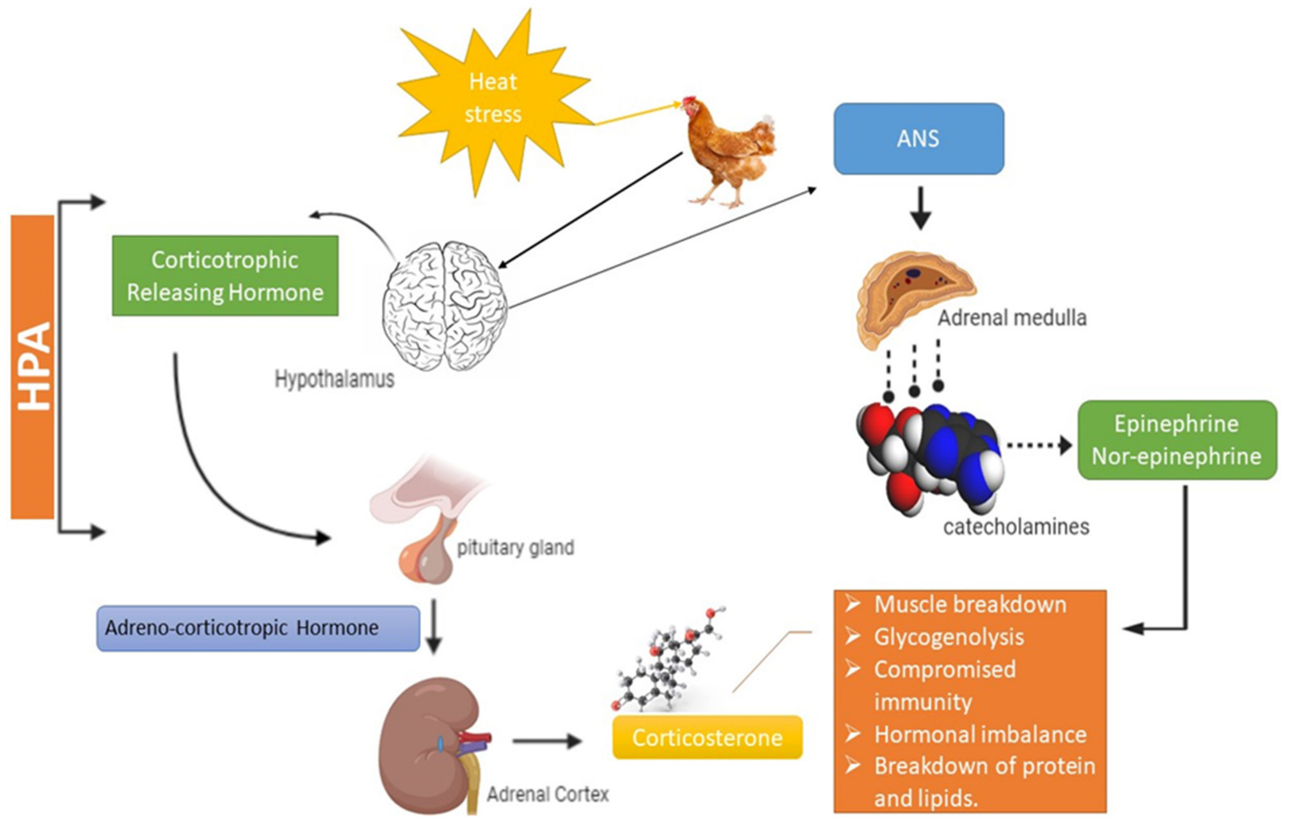

FIGURE 2 | Mechanism of heat stress in broiler. HPA, Hypothalamic pituitary adrenal axis; ANS, Autonomic nervous system.

animals. During stress conditions, the priority of every living organism is to survive rather than growth. A recent study on broilers revealed that both cyclical and continuous heat stress significantly compromises growth performance by reducing protein digestibility up to 9.7%. Broilers under heat stress (32°C) have shown increased metabolizable energy intake (20.3%) and heat production (35.5%), and decreased energy retention (20.9%) and energy efficiency (32.4%) as compared to control group (38). Another study in laying hens reported a significant decrease in weight gain as average body weight (BW) of heat stressed hens was recorded 1.233 kg as compared to 1.528 kg BW of control group after 5 weeks of chronic heat stress (35°C). The significant decrease in weight is possibly due to reduced feed intake as birds under heat conditions ate less feed in relation to the control ones (39). This reduction in feed intake and nutrient digestibility severely compromises production efficiency and product quality. Chicken meat quality deteriorates since poor nutrients availability causes a sharp decline in muscle glycogen reserves, leading to dark, firm, and dry (DFD) meat (16).

Increase in Fat and Reduction in Protein Contents of Poultry Meat

High ambient temperature disrupts normal lipid metabolism (lipolysis) by downregulating the enzymes involved in lipid breakdown resulting in more fat deposition and reduced protein content in muscles (40, 41). Many publications reported increased fat content in chicken under HS that seems to be an adaptive mechanism in birds as they store more energy in the form of fat to avoid further heat production during metabolism. A study conducted by Zhang et al. (14) reported that broiler birds raised under CHS (34–36°C) showed reduction in breast muscle mass (31.53%) and thigh muscle (11.17%) as compared to the normal control group. Considerable reduction in breast muscle mass was characterized by a significant change in chemical composition with higher fat quantity and lower protein concentration in muscles. Another study also concluded that cyclical HS (33°C for 9 h, 25°C for 15 h 1–42 days) in broiler reduced breast muscle weight by 16% (42). Lu et al. (43) reported higher intramuscular fat, increased activity of pyruvate kinase and lactate dehydrogenase in pectoral muscles of broilers under HS (32°C for 14 days). Moreover, CHS reduces the rate of aerobic metabolism by disturbing the mitochondrial functioning, decreasing aerobic metabolic activity and promoting glycolysis consequently leading to more fat deposition in muscles, which ultimately deteriorates meat quality (44). A study reported more fatness and low protein content in HS-broiler-chicken as compared to those maintained at the thermo-neutral condition (45). Production and quality losses in broiler chicken are not merely due to the reduced intake. Many other factors, including physiological, biochemical, and hormonal changes, are equally involved in all these losses to the poultry industry.

EXCESSIVE HEAT BURDEN TRIGGERS METABOLIC STRESS THAT DETERIORATES MEAT QUALITY

Excessive Production of ROS Impairs Meat Quality

Genetic modifications for rapid growth in broiler chicken has made the chicken more vulnerable to environmental stressors (17, 46). Oxidative stress is among the major stressors, which can potentially halt chicken growth, having severe consequences on the broiler's meat quality. Increased ROS liberation is potentially damaging as it aggravates the aging of muscles, protein degradation and inactivates the nuclear proteins, including DNA and RNA. HS induces ROS production by impairing mitochondrial function leading to reduced aerobic metabolism of fat and glucose and enhanced glycolysis, which ultimately results in poor meat quality characterized by low pH and high drip loss (47). Living tissues have many antioxidants to cope with oxidants, if the balance among antioxidants and oxidants disturbs and oxidants exceed a certain limit within the body, this condition indicates oxidative stress. Mostly oxidants are produced during cellular metabolism in the mitochondria of living cells. Cellular metabolism is not the only source of oxidants, some external sources, including feed comprised of oxidized lipids and fats, are responsible for producing reactive oxygen species (48). According to Mujahid et al. (49), leakage of electrons from the mitochondrial respiratory chain during oxidative phosphorylation is the main source of ROS. HS increases ROS production by compromising the electron transport chain's functioning, which is necessary for energy production in the muscles (50).

ROS changes calcium sensitivity by oxidizing the thiol groups in the ryanodine receptor and damages an enzyme sarcoendoplasmic reticulum Ca^{+2}-ATPase (SERCA). This enzyme maintains calcium balance within the sarcoplasmic reticulum by removing extra calcium. Due to ROS, this system for calcium control collapses leading to overwhelming muscle contractions, culminating in muscle dystrophy (51–53). Numerous studies reported that oxidative stress leads to cell death and causes oxidation of protein and lipids, which ultimately deteriorates production efficiency and quality. Production of ROS in mitochondria leads to cellular oxidative stress, and it has severe consequences on physiological and behavioral characteristics in birds, which ultimately reduces the performance efficiency of the commercial meat birds. In short, oxidative stress lowers ATP production, creates calcium imbalance, and oxidizes several proteins within mitochondria along with mitochondrial membrane disruption (48, 53, 54).

Acidosis Lowers Water Holding Capacity (WHC) and Damages Meat Texture

Rapid pH reduction in chicken muscle is also associated with HS, and it has severe implications on meat quality or texture. Multiple studies indicated HS to potentially reduce muscle pH leading to harmful changes in muscles (55). HS triggers anaerobic glycolysis within the muscles during and after slaughtering of the animal, thus, more H^+ and lactic acid accumulate in the muscles due to hydrolysis of ATP during the anaerobic glycolysis. This result in a rapid drop in the pH of muscles leading to low water holding capacity which then develop into an abnormal condition called pale, soft, and exudative meat (56, 57).

Thyroid Hormone Imbalance Under HS Impairs Skeletal Muscle Development

Thyroid hormone plays crucial role in the thermogenesis of avian via the thermoregulation by controlling metabolic heat production that is crucial to maintain normal body temperature. Tri-iodothyronine (T3) and tetra-iodothyronine (T4) enhance basal metabolism by modifying the mitochondrial function and assists skeletal muscles to acclimatize with a changing environment. Recent study regarding thyroid hormones in heat-stressed chicken found that high ambient temperature reduces both activity and size of thyroid. Lower level of thyroid hormone has observed in different studies conducted on heat stressed (38 °C for 24 h) quail (58) and domestic fowl (59). Thyroid hormones from external sources have also been observed to have lower survival time during HS (60). In broiler chicken, the thyroid gland's size, along with activity, decreases by high ambient temperatures and vice-versa (15). High ambient temperature normally responsible for drop in T3 and T4 plasma concentration. This mechanism is an adaptive tool to escape extra heat load, by decreasing metabolic heat production, plummeting maintenance energy requirements and increasing fat deposition by discouraging lipolysis (45, 61).

How Does Meat Quality Deteriorate?

Drip Loss

After slaughtering, when muscle converts to meat, it loses some of its contents, including water, myofibers, iron, and proteins. Loss of muscle contents during which meat tends to lose its original texture and taste are often referred as drip loss (62). When frozen meat is being thawed, it loses its texture and taste due to loss of water contents and leakage of other nutritional contents through the water. Drip loss is related to overall meat quality as it reduces meat palatability, juiciness, and acceptability of meat. It is one of the major meat quality defects, of which experts are trying to resolve, most particularly in pork and chicken (63). HS before slaughtering of bird increases metabolic rate and rigor mortis that results in protein denaturation. As protein is involved in the water-binding capacity of meat, so protein damage due to high carcass temperature hinders protein ability to bind water that culminates into pronounced reduction in poor water-holding ability characterized by higher drip loss and cooking loss (64). According to a recent study, constant high temperature harms water-holding capacity because it increases drip loss in poultry meat. The researchers found that broiler birds' meat under high temperatures had increased value of cook loss, shear force, and decreased pH. Birds under cyclic heat had higher cook loss value in breast muscles as compared to those raised under the thermo-neutral environment (14). Practical observations and studies have demonstrated both AHS and CHS during the housing period of broiler to be responsible for poor water-holding capacity.

Development of Pale, Soft, and Exudative Meat

In chicken, the development of PSE is mainly due to the rapid decrease in pH after birds' slaughtering. Birds with high metabolic activities and efficient growth rates often have poor thermoregulatory ability; consequently, these birds are more prone to HS during the growing period (65). HS, during the broiler's growth period especially, causes multiple problems, including muscle atrophy, acid-base imbalance, and poor meat quality. In chicken, mostly muscles are comprised of fast twitching fibers. Fast twitching fibers are mainly dependent on anaerobic glycolysis (66). HS before slaughtering accelerates the anaerobic glycolysis in muscles and lowers pH during the conversion of muscles into the meat while the body temperature is high (67). High carcass temperature with low pH causes protein degradation and develops PSE condition (68). The processing capability of PSE meat is poor making processed meat more dry and brittle due to lack of proper WHC and protein extractability (69, 70). During hot weather, the broiler industry reports extensive losses in meat production due to reduced water holding capacity, poor meat texture, and pale color (57).

Production of Protein Carbonyls

AHS downregulates the protein synthesis at the transcriptional level, and it alters both ribosomal gene transcription and protein synthesis, consequently reducing protein deposition. The different durations of HS have different implications on the protein metabolism of hyperthermic animals (15). Short duration HS increases protein catabolism (marked by an increased plasma uric acid level), reduces protein synthesis and N retention, which decreases plasma concentrations of aspartic acid (Asp), serine (Ser), tyrosine (Tyr), and cysteine (Cys) (71). However, CHS knockdown protein synthesis in various muscles, decreases protein breakdown, with lower levels of plasma amino acids (especially sulfur and branched-chain amino acids) and higher serum levels of Asp, glutamic acid (Glu), and phenylalanine (Phe) (45, 72).

THE GENETIC BASIS OF MUSCLE DEVELOPMENT AND HEAT STRESS

Skeletal muscles contribute up to 40–60% of total animal body weight and play a crucial role in the movement, respiration, and homeostasis of the animal body (73). Moreover, they play significant role in the food industry and have significant economic importance. Especially in meat-producing animals, scientists and researchers are busy finding multiple ways to enhance skeletal muscle mass (74). Each muscle cell in skeletal muscle is termed as myofibril having multiple nuclei. This myofibril arises from the fusion of mesoderm progenitor cells called myoblast. In almost every major species, the number of myofibrils set at the time of birth and cannot be increased after birth, but muscle size can be increased (75). In chickens, muscle growth after birth is only due to hypertrophy, characterized by proliferation and fusion of activated satellite cells with muscle fibers and increased protein synthesis ability. Myogenesis is an intricate process having multiple steps determined by numerous myogenic factors including transcription factors, adhesion, molecules, growth hormones, and myogenic regulatory factors (76). **Figure 3** illustrates the stepwise process of muscle cell formation and highlights genetic factors, which regulate the myofiber formation at every step.

Myogenic Regulatory Factors (MRFs), namely; Myf5, MyoD, myogenin, and MRF4, are members of the basic helix-loop-helix family of transcription factors that control the determination and differentiation of skeletal muscle cells during embryogenesis and postnatal myogenesis (77). MRFs form a family of transcription factors whose function and activity represent a paradigm where a series of molecular switches determine an entire cell lineage's fate. The MRFs are a group of muscle-specific proteins that act at multiple points in the muscle lineage to cooperatively establish the skeletal muscle phenotype by regulating the muscle cell proliferation, irreversible cell cycle arrest of precursor cells, followed by a regulated activation of sarcomeric and muscle-specific genes to facilitate differentiation and sarcomere assembly (78). A study on the mouse model has shown that MyoD, Myf5, and MRF4 are responsible for a myogenic determination as in the absence of these factors, there will be no skeletal muscle formation. At the same time, myogenin works as a differentiation factor. As myogenesis initiates, Myf5 is the first regulatory gene to be activated near the neural tube while Mrf4 is also activated during the early stages, but later on, it expresses only during the differentiation of the skeletal muscles (79, 80).

Constituents of the endocrine system, such as growth hormone (GH), IGF-1, and androgens, are the principal regulators of muscle metabolism. These endocrine components significantly impact muscle growth and act as anabolic factors, the major regulators of muscle's bulk (81). IGF-1 is considered to play key roles in fetal development and growth up to adolescence and in maintaining homeostasis in adult tissues by regulating cell proliferation, differentiation, and survival (82). IGF-1 exhibits a direct and crucial influence on muscle growth and differentiation during skeletal muscle development. Numerous studies have reported that IGF-1 is a positive regulator of myogenesis, which tightly controls the whole process of myogenic development. It is involved in various phases of myogenesis and muscle regeneration: triggering satellite cell proliferation, increasing protein synthesis, and promoting differentiation (82, 83).

MRFs are key regulators in skeletal muscle development, and numerous studies reported that HS has negative implications on myogenic regulatory factors. Low expression levels of MyoD, myogenin had been observed in chicken embryos at high temperatures (84). HS, during the embryonic development phase, postpones the formation of myofibers, consequently affecting the muscle proliferation and differentiation at later stages. The number of muscle fibers is fixed at the time of birth and can never increase; the only size of those fibers increases, and major muscle growth in chicken is carried out by hypertrophy lead by protein deposition (85, 86). A study on muscle development reported that HS impairs muscle hypertrophy by reducing the IGF-1 gene expression level and circulating IGF-1 concentration. HS also

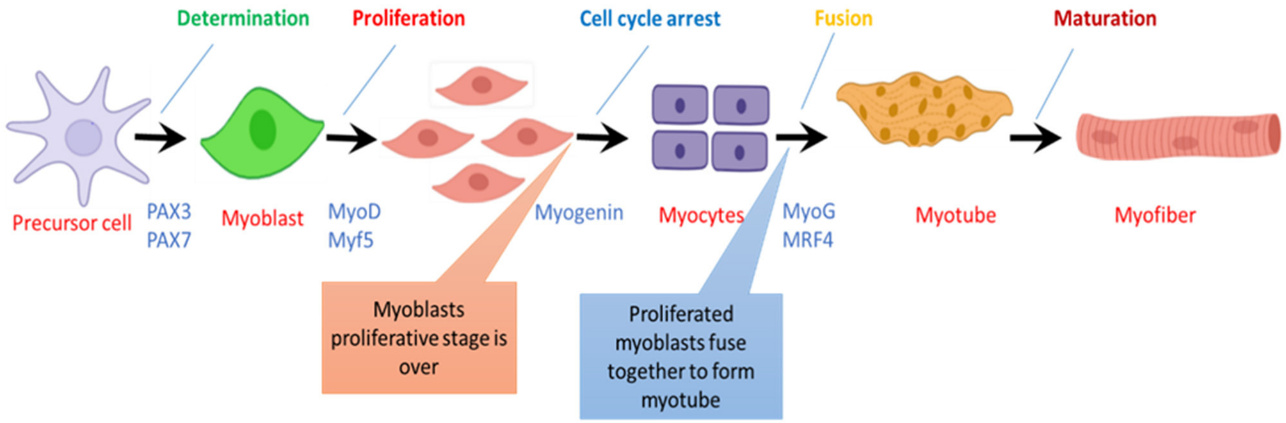

FIGURE 3 | Step wise progress of myofiber formation during skeletal muscle development.in chicken.

decreases the expression of MyoD, MyoG, and consequently hinders muscle hypertrophy by inhibiting S6K1. S6K1 plays a major role in cell growth regulation and muscle hypertrophy (84, 87). A study demonstrated that knockdown of S6K1 in rats caused a significant reduction in muscle size. This molecule is responsible for cell growth by increasing muscle cell size without affecting the cell number (84, 88).

To conclude, HS reduces growth performance, breast muscle mass, and yield in broilers. HS also reduced the mRNA expressions of IGF-1 and its downstream genes in breast muscle, thereby induced inactivity of mTOR and its downstream target S6K1 that regulates MRFs to decrease muscle hypertrophy. Meanwhile, the reduction of muscle protein synthesis is caused by reductions in both muscle amino acid uptake and the expressions of specific transporter isoforms due to the inactivity of mTOR and S6K1 (84).

HEAT SHOCK PROTEINS DURING HEAT STRESS

Heat shock proteins are widely considered as stress proteins found within the cells of all living organisms. During the high ambient temperature, living cells trigger a response named "heat shock response," which activates the specific set of proteins to protect cells from stressors like heat (89). The primary function of heat shock proteins is housekeeping, they maintain order in the cell by synthesizing other proteins while during stressful environment or any pathological condition, their expression level increases and they incline to attract immune cells at the respective site or organ (90, 91). HSPs comprised six members classified and named on the basis of molecular weight, including HSP40, HSP70, HSP90, HSP100, small HSPs, and chaperonins. HSPs originate from an extracellular environment and function in specific parts of the body as stress signals and trigger immune cells during any stress and unfavorable conditions. HSPs also play an important role in protein formation and degradation by regulating folding/unfolding and translocation of proteins. All living organisms produce heat shock proteins under HS environment as these proteins are only produced under the stimuli of any stressor such as high temperature (91–94).

Role of HSPs as Stress Indicator and Cell Protector

HSP70 is very crucial for cell recovery after the damage done by HS (95). Increased expression levels of HSPs during HS is an adaptive phenomenon that improves tolerance level against HS in living cells as the studies on the transcriptional behavior of heat shock proteins have revealed that HSPs are the heat polypeptides produced due to high temperature. HSP70 and 90 are more extensively studied families among heat shock proteins, and these two families exhibit a plethora of functions from involving in cell tolerance to control cell cycle (96–99). Exposure to high ambient temperature enhances the production of heat shock proteins, which are synthesized in respective cells experiencing stress, and helps synthesize other proteins. It also regulates many processes, including protein refolding, translocation, and prevents the oxidative breakdown and apoptosis of damaged proteins during stress conditions. All these functions carried out by Heat shock proteins are very handy for cell recovery after stress (96, 100–102). Studies revealed that HSPs play regulatory roles in various types of immunity. Production of HSPs during HS is mainly to attract immune cells. Numerous studies on differentially expressed genes during HS have shown that HSPs are related to birds' immune functioning during HS (103).

HSPs Protect the Muscle Cells From Damage

In an HS environment, HSPs repair the damaged proteins. In normal climatic conditions, HSP 70 is present in low concentrations as molecular chaperones, while the level of HSPs increases rapidly in muscles during cellular stress (hyperthermia, oxidative stress, changes in pH). An increase of HSPs leads to significant changes in gene expression leading to remodeling of skeletal muscles (104). Numerous studies in broiler chickens reported that the HSP family is playing a key role to repair the damaged cells, and it has observed during acute stress, HSP70 expressed in the muscles, liver, heart, kidney, and blood vessels (101, 105). During AHS, an upregulated gene expression of HSP70 and 90 have been observed in muscle cells of broiler chicken. Moreover, AHS triggers both protein and mRNA expression of HSP70 and 90 in the kidney of chicken. A study conducted on Taiwanese roosters under acute HS has revealed the upregulation of HSP70 and 90 in Taiwanese roosters' testes. In contrast, another study reported depression in the expression level of HSP 90 and HSP25, which are believed to be involved in protein folding (101, 106, 107).

HSPs Regulates Meat Quality by Inhibiting Muscle Apoptosis

After the slaughtering of animals, muscles undergo cell apoptosis due to the unavailability of oxygen and nutrients within muscle cells. All those factors involved in the apoptotic activity of muscle cells are considered to control the animal's ultimate meat quality. Multiple studies reported the role of small heat shock proteins as an anti-apoptotic factor in muscle cells during post-mortem changes in muscles of slaughtered animals and influences the meat quality attributes including color, tenderness, juiciness, and the meat flavor (108, 109). After the muscle undergoes cell death due to apoptosis, the number of small heat shock protein increases at that side and lowers the rate of apoptosis and unfolding of proteins in muscles (110). They delay protein degradation in muscle cells and try to maintain homeostasis at the cellular level. In this way, small heat shock proteins impede the aging process and play a crucial part in developing meat quality (110, 111).

DEALING WITH HS TO IMPROVE MEAT PRODUCTION AND MEAT QUALITY

Dietary Supplementation

Multiple nutritional strategies have been suggested to alleviate HS destructive effects in the poultry industry. Previous studies revealed that protein metabolism is severely affected by chronic HS and leads to reduced protein deposition in muscles. This dwindling protein level cannot be compensated through dietary protein because it further aggravates HS by producing more metabolic heat (112, 113). On the other hand, reducing protein concentration in diet culminates in to poor weight gain and lower feed efficiency. Chickens on a low protein diet often consume more feed to fulfill their protein requirements and the consumption of more feed results in poor feed efficiency. It has suggested that feed with more fat supplementation and low protein contents could minimize HS mischievous impact (114, 115). A similar study (116) proposed that feed supplemented with 5% fat and 4% palm oil can improve broiler production performance under the HS environment by lowering feed retention and optimizing the nutrient utilization. Secondly, Feed restrictions during the early period of life in chicken have been proved handy in reducing HS's damaging effects. A study demonstrated that feed restriction during early days of broiler chicken (4–6 days after birth) promotes heat tolerance later in life (35–40 days of age) (7, 116). Early feed reduction (EFR) and fat supplemented feed have a beneficial impact on heat-stressed broiler birds.

Thirdly, ample supplementation of vitamins is obligatory for better broiler production, especially amid harsh environment (10, 116, 117). Vitamin supplementation through drinking water is common practice in some poultry farms that have proved helpful to boost immunity and enhance heat-stressed broilers' performance. Diets containing vitamin A help broilers to fight against oxidative injuries induced by high environmental temperature (118). Kucuk et al. (118) also reported that vitamin A fortification has positive effects on production status as it enhances body weight gain, feed efficiency and reduces oxidative damage. Poultry birds can synthesize Vitamin C by itself and does not seek an external supply of vitamin C during normal conditions. However, under stress conditions, the additional supplementation of vitamin C might be fruitful for broilers' better performance as it promotes fatty acid oxidation instead of protein breakdown and reduces respiratory quotient (119). Studies reported increased hunger of birds for vitamin C during HS as vitamin C promotes fatty acid oxidation instead of protein breakdown and reduces respiratory quotient (119, 120). Moreover, it enhances meat quality by producing meat with high protein and low-fat contents and maintains redox status during high temperature because of its ability to be one of the best antioxidants. A study based on vitamin E diet supplementation reported that vitamin E supplementation promotes the phagocytic activity of macrophages and increases serum antibodies (IgM and IgG) levels in broiler under HS (121).

Use of Herbs

There has been much attention placed on how herbal feed additives can be used in alleviating the adverse effects of HS, which in a way will help to enhance the production and performance of other animals, including poultry, pigs, and rabbits (122). The advantages of herbal additives include pharmacological and nutritional values and amelioration of many animal diseases. For example, there was noticeable recovery reported in animals which suffered harmful HS sequence after dietary supplementation of some herbs such as Ginger, Fennel, Black seed, hot red pepper, *Artemisia annua*, Rosemary, Moringa, *Radix bupleurum*, Chicory, and Dill (123).

Ginger (*Zingiber officinale*) as widely known to be used in the treatment of lots of disorders (119), contains compounds such as gingerdione, gingerdiol, and shagaols, which possess quite a lot of antioxidant and antimicrobial activities (119, 124). The addition of ginger (2%) to heat-stressed broilers significantly improved the biochemical blood parameters and the growth performance in comparison to the control whereby the changes which emanated were attributed to antibacterial potential of the supplement, which in effect improved the digestibility, palatability, metabolism, and health status of the chicken (119, 124). HS is noted to affect the poultry by reducing the villus height in quail (125) and broiler chicks (126). However, broilers supplemented with 2 and 4 g/kg garlic diets revealed the highest intestinal villi and most significant crypt depth in comparison to the control as reported by Shewita and Taha (127) although negative impacts on body weight, FCR, and FI at higher levels

(6 g/kg) were reported. A report by Khonyoung et al. (128) showed that dietary supplemented with fermented-dried ginger products at 1% can help reduce abdominal fat, which in effect can help improve the health of heat-stressed broilers. For Fennel (*Foeniculum vulgare*), lots of research showcases the role that its essential oil plays as an antioxidant, antimicrobial, and a potent hepatoprotective agent (129, 130). A study conducted by Ragab et al. (131) revealed an improvement of feed intake, meat breast (%), and leukocytes of heat-stressed Ross broilers after 1 or 2% of this herb. Correspondingly, fennel fruits supplementation at 10 or 20 g/kg diet in heat-stressed laying hens significantly improved the quality of eggs, reduced the malondialdehyde (MDA) contents, carboxyl levels in eggs, and again reduced the triglyceride and cholesterol contents (132). Again, Mohammed and Abbas (133) also observed that feeding of chicks with 1, 2, and 3 g fennel/kg diet significantly increased the RBCs, Hb, and PCV in comparison to the control.

Again, *Nigella sativa*, commonly known as the black seed, has been used in many HS research of poultry, and effect has shown encouraging results due to the higher nutritional values it carries. Active materials such as thymoquinone, nigellone, and thymohydroquinone, which aids in exerting antitoxic and antimicrobial properties through increased defense mechanisms against infectious diseases, are reported to be contained in black seed (134). Heat stressed pigeon which was fed with 2% black seed aided in weight gain and body weight improvement, hepatic lesion protection, which led to mild vascular congestion and vacuolization of the hepatocyte without creating damages to sinusoids in comparison to the control [EL (135)]. Heat stressed broilers subjected to a 1% black seed diet increased the feed intake, dressing percentage, body gain while reducing the panting behavior, water to feed ratio, corticosterone, and T3 levels (136). Judging from these, ginger, fennel and black seed herb among others can be used to reduce the bad effects HS is noted to have on the poultry production.

Probiotic Effects on HS in Poultry

The supplementation of feed additives such as probiotics, prebiotics, and symbiotics has been used lately to curb the negative impacts HS poses in birds (9). Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (137)." Lots of research have been conducted proving that probiotics administration in diets is a sure way of improving the growth, immune response, digestive enzyme activity, disease resistance, gut microbiota in aquatic animals (138–141) chicken (142), pigs (143), etc. Given this, probiotics have gained lots of attentions from scientists in the poultry industry as the addition of this additive is a sure way of enhancing the intestinal morphology, physiological conditions, immunity; thus, the overall well-being and performance of heat-stressed poultry as previously reported (144, 145). A study conducted by Zulkifli et al. (146) reported that a probiotic-enhanced water acidifier (*S. faecium* and *L. acidophilus* + citric acid + sorbic acid + sodium citrate + sodium chloride + zinc sulfate + ferrous sulfate + potassium chloride + cellulase +magnesium sulfate) aided in the restoration of Na and K levels in broilers after 1 day HS. Broilers subjected to HS saw an increase in T_3 (147) and T_4 (148) in the serum after administering probiotics.

There has been a report that "Protexin® Boost," a probiotic treatment, improved serum uric acid levels of heat-stressed birds. Uric acid plays a critical role as an antioxidative agent (149); thus, an increase in its level after the probiotic treatment depicts that the probiotic exerts some mechanism in alleviating the oxidative damage after the HS in birds. Hasan et al. (150) observed an increase in hemoglobins in heat-stressed birds after dietary supplementation of probiotics (Protexin® Boost). Furthermore, probiotics have also been revealed to improve the immune system of HS birds (151). It has been established that, the administration of probiotics enhances not only the responses of antibody (146, 151, 152) but also leukocytes count (153) in heat-stressed birds. Intraepithelial lymphocyte (IEL), an important host immune system component, is noted to respond rapidly when host organisms are infected (154). An experiment executed by Deng et al. (151) revealed a lower IEL number in the cecum and ileum of laying hens at week 61. Hasan et al. (155) revealed that the lymphoid organ's involution due to HS in poultry could be prevented by *B. subtilis* supplementation. Correspondingly, Lei et al. (156) observed a reduction in the corticosterone levels, which causes lymphoid organ involution after HS. Studies show that probiotics' dietary supplementation enhances the intestinal composition after HS conditions (144). Many studies on the health and well-being of heat-stressed poultry after supplementation have been established, as some have been discussed. **Table 1** also enlists other research performed previously, which reveals the positive effects of probiotics in improving microbiota, morphology, and immune response of heat-stressed poultry.

Introducing Heat Tolerant Traits From Indigenous Breeds Into Commercial Breeds

Introduction of new technologies such as genomics provides valuable data and new approaches to address these challenges. The commercial broiler industry's focus largely remained only on fast weight gain and feed efficiency from previous two decades. Commercial breeds capable of gaining more weight in thermo-neutral conditions when raised under a high-temperature environment fail to maintain their growth performance (15). Genetic selection for heat tolerance in broilers needs to be taken into account, especially in tropical and subtropical regions of the world. A specific phenotype "frizzled feather," characterized by curly feathers waving outside, was reported by Darwin (163). It was proposed that this type of chicken gives the best protection against the severe environment and the specific gene revealing such characteristics expresses in many chicken breeds (164). A study reported that 69-bp deletion in KRT6A was responsible for frizzle character in chicken. On the other hand, our research group conducted study on local Chinese frizzle breed found a 15-bp deletion in the KRT75L4 gene (165). This natural mutation in the chicken genome is reflected as an adaptive mechanism as these birds can tolerate heat better and are mostly found in warm regions. Data on the country-wise distribution of different animal

TABLE 1 | Role of different probiotics to counter the damaging impact of heat stress in poultry.

Probiotics name	Poultry strain	Findings which aided in countering heat stress	Country of Investigation	Sample size	References
Multi strains probiotics (*L. plantarum, L. bulgaricus, L. acidophilus, L. rhamnosus, B. bifidum, S. thermophiles)*	White layer (Hy-line variety)	(1) Strengthens antibody titer against SRBC	Iran	60	(157)
Probiotic *B. licheniformis*	Hy-line Brown	(1) Enhanced mucosal immunity (IgA-secreting cells) in heat-stressed chicken (2) Overturned the increased levels of serum TNF-α and IL-1 due to HS (3) Improved IEL counts in the ileum and cecum of heat-stressed chicken (4) Counter the increased number of mast cells in the ileum and cecum of birds due to HS	China	96	(151)
Probiotic mixture (*L. pentosus* ITA23 and *L. acidophilus* ITA44)	Broiler chicken (Cobb-500)	(1) Improved antioxidant ability of liver in chicken raised at high ambient temperature (2) Improved the population of following bacteria in heat-stressed chicken a. *Bifidobacteria* b. *Lactobacillus* c. *Enterococcus*	Malaysia	192	(145)
B. subtilis and *B. licheniformis*	Duck (cherry valley pekin Ducks)	(1) Augmented expression levels and enzyme action of LXRα, which wheels the functional specialty of splenic macrophages in ducks	China	750	(158)
Probiotic *S. cerevisiae*	Broiler chicken (Cobb-400)	(1) Enhanced the villus height in the duodenum of broilers raised under HS (2) Reduced the number of *Salmonella* and *E. coli* in excreta and gut of HSed broilers	Turkey	175	(152)
Probiotic mixture (*L. acidophilus*, *L. casei*, *E. faecium,* and *B. bifidium*)	Broiler chicken (Ross-308)	(1) Improved antibody responses to Newcastle disease (ND), Bronchitis, and Gumboro disease in broilers under cyclic HS	Iran	96	(159)
Lactobacillus sp. and yeast culture	Arbor Acres broiler	(1) Reduced the population of *E. coli* and *Salmonella pullorum* (2) Lessened the pH of the intestine (duodenum, jejunum, ileum, and cecum) in a heat-stressed broiler		300	(147)
Lactobacillus-based probiotics (*L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*, *B. bifidum*, *S. thermophilus*, *E. faecium*, *A. oryzae,* and *C. pintolopesii*)	Broiler chicken (Hubbard)	(1) Regained villus height and crypt depth in duodenum and ileum of a heat-stressed broiler (2) Maintained the activity of goblet cells	Pakistan	250	(160)
Probiotic *B. subtilis*	Hubbard broiler	(1) Improved the population of useful Intestinal bacteria (*Lactobacillus* and *Bifidobacterium*) (2) Renovated the reduced villus-crypt structure	Jordan	480	(144)
Probiotic mixture (*B. licheniformis*, *B. subtilis,* and *L. plantarum*)	Ross-308	(1) Improved the viable counts of small intestinal *Lactobacillus* and *Bifidobacterium*, and reduced coliforms in a heat-stressed broiler (2) Enhanced villus height in the jejunum and improved intestinal barrier function	China	360	(161)
Lactobacillus-based probiotics (*L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*,	Hubbard	(1) Ameliorated the inflammatory response (decreased excessive numbers of IEL) in all intestinal segments of heat-stressed broilers (2) Increased the count of goblet cells in the intestine (duodenum and jejunum) of heat-stressed broilers	Pakistan	250	(160)

(Continued)

TABLE 1 | Continued

Probiotics name	Poultry strain	Findings which aided in countering heat stress	Country of Investigation	Sample size	References
B. bifidum, *S. thermophilus*, *E. faecium*, *A. oryzae,* and *C. pintolopesii*)					
Probiotic mixture (*L. plantarum*, *L. delbrueckii* ssp. *Bulgaricus*, *L. acidophilus*, *L. rhamnosus*, *B. bifidum,* and *S. salivarius* ssp.)	Ross-708	(1) Developed intestinal microarchitecture (villus width and surface area) of heat-stressed broilers	United States	450	(162)

IEL, Intraepithelial lymphocyte.

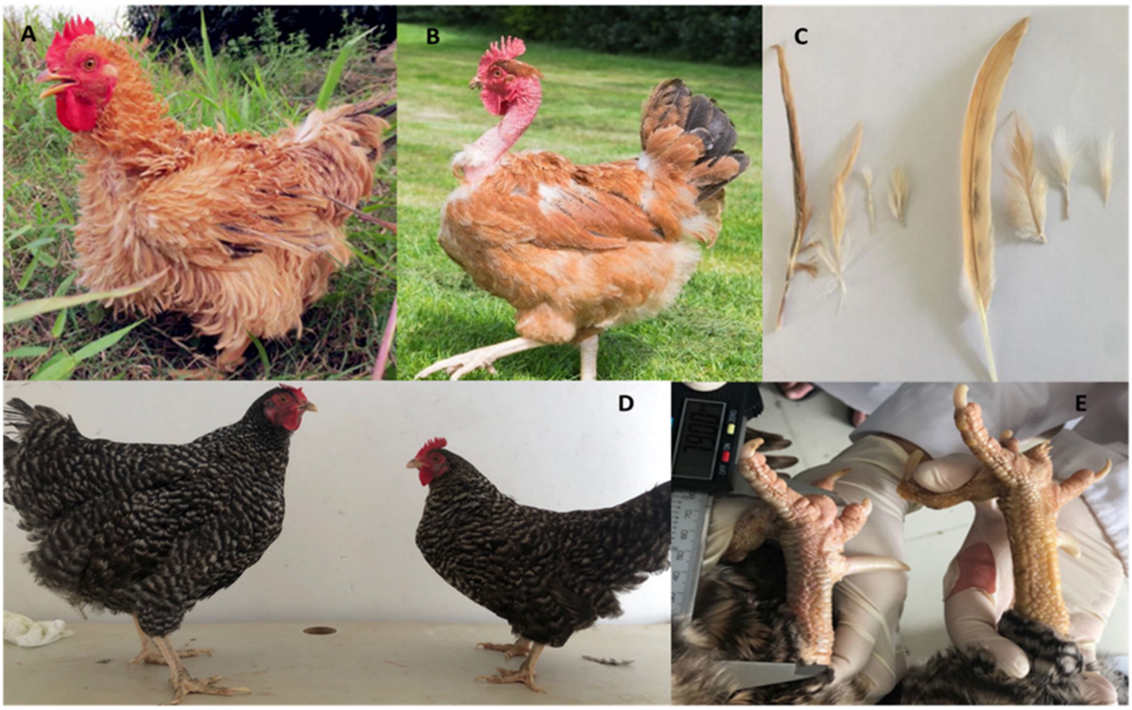

FIGURE 4 | (A) Frizzled feather chicken **(B)** Naked neck chicken **(C)** Comparison among normal and frizzle feathers, frizzle feathers on the left side, normal feathers on right side **(D)** Dwarf size plymouth rock chicken with normal Plymouth rock chicken **(E)** Shank length of dwarf chicken as compared to normal chicken (These pictures have been taken in Guangdong Ocean University, Zhanjiang, China by our research group).

breeds on the FAO website revealed both naked neck and frizzled feather chicken found worldwide. The naked neck gene has also been observed to withstand extreme climatic changes like high temperature (116).

Naked neck (Na), Frizzle (F, candidate gene: KRT6A and KRT75L4), and Dwarf (Dw, candidate gene: GHR) genes in poultry are considered candidate genes to tolerate thermal stress. Naked neck gene reduces the feather mass up to 40% and lowers the chances of heat insulation due to more feathers on the skin (166, 167). Studies reported that Na chicken perform better under heat stress compared to birds with normal feathers. Better immunity and production performance have also been observed in the Na chicken line (168). Lack of feathers on the neck provides more space for heat dissipation and discourages heat insulation, helping birds tolerate the harsh temperature. Na gene has a considerable positive role in production performance and immunity development in birds. It also minimizes the fat deposition in the breast region, promoting heat dissipation, leading to heat tolerance (166, 169, 170). The dwarf (GHR) gene is also considered a heat-tolerant gene as it reduces body size from 30 to 40%. Na, F, and Dw genes could prove beneficial for the commercial poultry industry in tropical and subtropical parts of the world (171). **Figure 4** shows different heat-tolerant breeds, including naked neck, frizzled feather, and dwarf chicken.

CONCLUSION

With time, HS issue is becoming more challenging for poultry industry. Genotype selection in broiler birds for higher growth rates to meet ever-increasing food requirement has made broiler chicken vulnerable to HS. Unluckily, the detrimental consequences of heat stress for poultry health and production are likely to continue and to be acquired by next generation during gestation if selection for only production traits is prioritized against heat tolerance and climate adaption according to current trends of global warming. High producers, commercial broiler breeds cannot withstand HS resulting in substantial economic losses to the industry, which triggers food security issues. Genetic selection for heat tolerance in poultry is the only durable solution to curb HS's negative implications. Realizing this threat to food security, scientists and industry's concerted efforts will be required to overcome this problem. These efforts should include (a) Genotype profiling of heat-tolerant breeds along with comprehensive studies on the interaction between genotype and phenotype in both heat tolerant and susceptible broiler breeds. (b) To explore the complete molecular mechanism of muscle development and muscle growth during HS environment. (c) Crossing frizzled feathers chicken breed to dwarf breed may give more apparent illustration about molecular and genetic mechanisms underlying heat resistance. Apart from breeding strategies, adopting modern managerial and environmental strategies could minimize the deleterious effects of heat on meat production and quality.

AUTHOR CONTRIBUTIONS

AHN did the majority of the writing by communicating with KA and coordinated the document editing. LZ provided advice on the research input to the review article and performed significant edits to the document as well the funding acquisition. QYL, JHZ, and WLZ helped to gather and analyze information regarding the topic of review.

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REFERENCES

1. Mottet A, Tempio G. Global poultry production: current state and future outlook and challenges. *Worlds Poult Sci J.* (2017) 73:245–56. doi: 10.1017/S0043933917000071
2. Bahri SIS, Ariffin AS, Mohtar S. Critical review on food security in Malaysia for broiler industry. *Int J Acad Res Bus Soc Sci.* (2019) 9:869–76. doi: 10.6007/IJARBSS/v9-i7/6186
3. FAO. *Meat Market Review*. Rome (2018).
4. Nardone A, Ronchi B, Lacetera N, Ranieri MS, Bernabucci U. Effects of climate changes on animal production and sustainability of livestock systems. *Livest Sci.* (2010) 130:57–69. doi: 10.1016/j.livsci.2010.02.011
5. Sejian V, Bhatta R, Gaughan JB, Dunshea FR, Lacetera N. Review: adaptation of animals to heat stress. *Animal.* (2018) 12:431–44. doi: 10.1017/S1751731118001945
6. Afsal A, Sejian V, Bagath M, Devaraj C, Bhatta R. Heat stress and livestock adaptation: neuro-endocrine regulation. *Int J Vet Anim Med.* (2018) 2:1–7. doi: 10.31021/ijvam.20181108
7. Renaudeau D, Collin A, Yahav S, de Basilio V, Gourdine JL, Collier RJ. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal.* (2012) 6:707–28. doi: 10.1017/S1751731111002448
8. St-Pierre NR, Cobanov B, Schnitkey G. Economic losses from heat stress by US livestock industries. *J Dairy Sci.* (2003) 86:52–77. doi: 10.3168/jds.S0022-0302(03)74040-5
9. Lara L, Rostagno M. Impact of heat stress on poultry production. *Animals.* (2013) 3:356–69. doi: 10.3390/ani3020356
10. Pawar SS, Basavaraj S, Dhansing LV, Pandurang KN, Sahebrao KA, Vitthal NA, et al. Assessing and mitigating the impact of heat stress in poultry. *Adv Anim Vet Sci.* (2016) 4:332–41. doi: 10.14737/journal.aavs/2016/4.6.332.341
11. Adu-Asiamah P, Zhang Y, Amoah K, Leng QY, Zheng JH, Yang H, et al. Evaluation of physiological and molecular responses to acute heat stress in two chicken breeds. *Animal.* (2021) 15:100106. doi: 10.1016/j.animal.2020.100106
12. Abd El-Hack ME, Abdelnour SA, Taha AE, Khafaga AF, Arif M, Ayasan T, et al. Herbs as thermoregulatory agents in poultry: an overview. *Sci Total Environ.* (2020) 703:134399. doi: 10.1016/j.scitotenv.2019.134399
13. Gregory NG. How climatic changes could affect meat quality. *Food Res Int.* (2010) 43:1866–73. doi: 10.1016/j.foodres.2009.05.018
14. Zhang ZY, Jia GQ, Zuo JJ, Zhang Y, Lei J, Ren L, et al. Effects of constant and cyclic heat stress on muscle metabolism and meat quality of broiler breast fillet and thigh meat. *Poult Sci.* (2012) 91:2931–7. doi: 10.3382/ps.2012-02255
15. Gonzalez-Rivas PA, Chauhan SS, Ha M, Fegan N, Dunshea FR, Warner RD. Effects of heat stress on animal physiology, metabolism, and meat quality: a review. *Meat Sci.* (2020) 162:108025. doi: 10.1016/j.meatsci.2019.108025
16. Song DJ, King AJ. Effects of heat stress on broiler meat quality. *Worlds Poult Sci J.* (2015) 71:701–9. doi: 10.1017/S0043933915002421
17. Altan Ö, Pabuçcuoglu A, Altan A, Konyalioglu S, Bayraktar H. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *Br Poult Sci.* (2003) 44:545–50. doi: 10.1080/00071660310001618334
18. Kumar B. Stress and its impact on farm animals. *Front Biosci.* (2012) 4:e496. doi: 10.2741/e496
19. Cirule D, Krama T, Vrublevska J, Rantala MJ, Krams I. A rapid effect of handling on counts of white blood cells in a wintering passerine bird: a more practical measure of stress? *J. Ornithol.* (2012) 153:161–6. doi: 10.1007/s10336-011-0719-9
20. Nawab A, Ibtisham F, Li G, Kieser B, Wu J, Liu W, et al. Heat stress in poultry production: mitigation strategies to overcome the future challenges facing the global poultry industry. *J Therm Biol.* (2018) 78:131–9. doi: 10.1016/j.jtherbio.2018.08.010
21. Virden WS, Kidd MT. Physiological stress in broilers: ramifications on nutrient digestibility and responses. *J Appl Poult Res.* (2009) 18:338–47. doi: 10.3382/japr.2007-00093
22. Mack LA, Felver-Gant JN, Dennis RL, Cheng HW. Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poult Sci.* (2013) 92:285–94. doi: 10.3382/ps.2012-02589

23. Chen Y, Arsenault R, Napper S, Griebel P. Models and methods to investigate acute stress responses in cattle. *Animals.* (2015) 5:1268–95. doi: 10.3390/ani5040411
24. Minton JE. Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. *J Anim Sci.* (1994) 72:1891–8. doi: 10.2527/1994.7271891x
25. Roach PJ. Control of glycogen synthase by hierarchal protein phosphorylation. *FASEB J.* (1990) 4:2961–8. doi: 10.1096/fasebj.4.12.2168324
26. Kuo T, Harris CA, Wang J-C. Metabolic functions of glucocorticoid receptor in skeletal muscle. *Mol Cell Endocrinol.* (2013) 380:79–88. doi: 10.1016/j.mce.2013.03.003
27. Shini S, Kaiser P, Shini A, Bryden WL. Biological response of chickens (*Gallus gallus* domesticus) induced by corticosterone and a bacterial endotoxin. *Comp Biochem Physiol Part B Biochem Mol Biol.* (2008) 149:324–33. doi: 10.1016/j.cbpb.2007.10.003
28. Bell RAV, Al-Khalaf M, Megeney LA. The beneficial role of proteolysis in skeletal muscle growth and stress adaptation. *Skelet Muscle.* (2016) 6:16. doi: 10.1186/s13395-016-0086-6
29. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. *FEBS J.* (2013) 280:4294–314. doi: 10.1111/febs.12253
30. Cockrem JF. Stress, corticosterone responses and avian personalities. *J Ornithol.* (2007) 148:169–78. doi: 10.1007/s10336-007-0175-8
31. Ognik K, Sembratowicz I. Stress as a factor modifying the metabolism in poultry. A review. *Ann UMCS Zootech.* (2012) 30:34–43. doi: 10.2478/v10083-012-0010-4
32. Mormède P, Andanson S, Aupérin B, Beerda B, Guémené D, Malmkvist J, et al. Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. *Physiol Behav.* (2007) 92:317–39. doi: 10.1016/j.physbeh.2006.12.003
33. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dial Clin Neurosci.* (2006) 8:383–95. doi: 10.31887/DCNS.2006.8.4/ssmith
34. Romero LM, Platts SH, Schoech SJ, Wada H, Crespi E, Martin LB, et al. (2012). Understanding stress in the healthy animal – potential paths for progress. *Stress.* (2015) 18:491–7. doi: 10.3109/10253890.2015.1073255
35. Spiers DE. Physiological basics of temperature regulation in domestic animals. In: R. J. Collier, J. L. Collier, editors. *Environmental Physiology of Livestock.* Oxford: Wiley-Blackwell (2012). p. 17–34. doi: 10.1002/9781119949091.ch2
36. Lin H, Zhang HF, Du R, Gu XH, Zhang ZY, Buyse J, et al. Thermoregulation responses of broiler chickens to humidity at different ambient temperatures. II Four weeks of age. *Poult Sci.* (2005) 84:1173–8. doi: 10.1093/ps/84.8.1173
37. Eppley ZA. Development of thermoregulation in birds: physiology, interspecific variation and adaptation to climate. In: Johnston IA, Bennett AF, editors. *Animals and Temperature.* Cambridge University Press (1996). p. 313–46. doi: 10.1017/CBO9780511721854.014
38. de Souza LFA, Espinha LP, de Almeida EA,,Lunedo R, Furlan RL, Macari M. How heat stress (continuous or cyclical) interferes with nutrient digestibility, energy and nitrogen balances and performance in broilers. *Livest Sci.* (2016) 192:39–43. doi: 10.1016/j.livsci.2016.08.014
39. Mashaly MM, Hendricks GL, Kalama MA, Gehad AE, Abbas AO, Patterson PH. Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poult Sci.* (2004) 83:889–94. doi: 10.1093/ps/83.6.889
40. Cahaner A, Pinchasov Y, Nir I, And Nitsan Z. Effects of dietary protein under high ambient temperature on body weight, breast meat yield, and abdominal fat deposition of broiler stocks differing in growth rate and fatness. *Poult Sci.* (1995) 74:968–75. doi: 10.3382/ps.0740968
41. Lu Z, He XF, Ma BB, Zhang L, Li JL, Jiang Y, et al. Increased fat synthesis and limited apolipoprotein B cause lipid accumulation in the liver of broiler chickens exposed to chronic heat stress. *Poult Sci.* (2019) 98:3695–704. doi: 10.3382/ps/pez056
42. Shakeri M, Cottrell J, Wilkinson S, Ringuet M, Furness J, Dunshea F. Betaine and antioxidants improve growth performance, breast muscle development and ameliorate thermoregulatory responses to cyclic heat exposure in broiler chickens. *Animals.* (2018) 8:162. doi: 10.3390/ani8100162
43. Lu Q, Wen J, Zhang H. Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. *Poult Sci.* (2007) 86:1059–64. doi: 10.1093/ps/86.6.1059
44. Zaboli G, Huang X, Feng X, Ahn DU. How can heat stress affect chicken meat quality? – A review. *Poult Sci.* (2019) 98:1551–6. doi: 10.3382/ps/pey399
45. Geraert PA, Padilha JCF, Guillaumin S. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: biological and endocrinological variables. *Br J Nutr.* (1996) 75:205–16. doi: 10.1079/BJN19960125
46. Sihvo H-K, Immonen K, Puolanne E. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. *Vet Pathol.* (2014) 51:619–23. doi: 10.1177/0300985813497488
47. Estévez M. Oxidative damage to poultry: from farm to fork. *Poult Sci.* (2015) 94:1368–78. doi: 10.3382/ps/pev094
48. Cadenas E, Davies KJA. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med.* (2000) 29:222–30. doi: 10.1016/S0891-5849(00)00317-8
49. Mujahid A, Akiba Y, Warden CH, Toyomizu M. Sequential changes in superoxide production, anion carriers and substrate oxidation in skeletal muscle mitochondria of heat-stressed chickens. *FEBS Lett.* (2007) 581:3461–7. doi: 10.1016/j.febslet,.2007.06.051
50. Huff Lonergan E, Zhang W, Lonergan SM. Biochemistry of postmortem muscle — lessons on mechanisms of meat tenderization. *Meat Sci.* (2010) 86:184–95. doi: 10.1016/j.meatsci.2010.05.004
51. Favero TG, Zable AC, Abramson JJ. Hydrogen peroxide stimulates the Ca2+ Release channel from skeletal muscle sarcoplasmic reticulum. *J Biol Chem.* (1995) 270:25557–63. doi: 10.1074/jbc.270.43.25557
52. Küchenmeister U, Kuhn G, Ender K. Preslaughter handling of pigs and the effect on heart rate, meat quality, including tenderness, and sarcoplasmic reticulum Ca2+ transport. *Meat Sci.* (2005) 71:690–5. doi: 10.1016/j.meatsci.2005.05.020
53. Zissimopoułos S, Lai FA. Redox regulation of the ryanodine receptor/calcium release channel. *Biochem Soc Trans.* (2006) 34:919–21. doi: 10.1042/BST0340919
54. Belhadj Slimen I, Najar T, Ghram A, Dabbebi H, Ben Mrad M, Abdrabbah M. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. *Int J Hyperth.* (2014) 30:513–23. doi: 10.3109/02656736.2014.971446
55. Wang Y, Du W, Lei K, Wang B, Wang Y, Zhou Y, et al. Effects of dietary bacillus licheniformis on gut physical barrier, immunity, and reproductive hormones of laying hens. *Probiotics Antimicrob Proteins.* (2017) 9:292–9. doi: 10.1007/s12602-017-9252-3
56. Strasburg GM, Chiang W. Pale, soft, exudative turkey—the role of ryanodine receptor variation in meat quality. *Poult Sci.* (2009) 88:1497–505. doi: 10.3382/ps.2009-00181
57. Van Laack RLJ, Liu C-H, Smith MO, Loveday HD. Characteristics of pale, soft, exudative broiler breast meat. *Poult Sci.* (2000) 79:1057–61. doi: 10.1093/ps/79.7.1057
58. Del Vesco AP, Gasparino E, Zancanela V, Grieser DO, Stanquevis CE, Pozza PC, et al. Effects of selenium supplementation on the oxidative state of acute heat stress-exposed quails. *J Anim Physiol Anim Nutr.* (2017) 101:170–9. doi: 10.1111/jpn.12437
59. Huston TM, Edwards HM, Williams JJ. The effects of high environmental temperature on thyroid secretion rate of domestic fowl. *Poult Sci.* (1962) 41:640–5. doi: 10.3382/ps.0410640
60. Bowen SJ, Washburn KW. Thyroid and adrenal response to heat stress in chickens and quail differing in heat tolerance. *Poult Sci.* (1985) 64:149–54. doi: 10.3382/ps.0640149
61. Collin A, Cassy S, Buyse J, Decuypere E, Damon M. Potential involvement of mammalian and avian uncoupling proteins in the thermogenic effect of thyroid hormones. *Domest Anim Endocrinol.* (2005) 29:78–87. doi: 10.1016/j.domaniend.2005.02.007
62. Ponsuksili S, Jonas E, Murani E, Phatsara C, Srikanchai T, Walz C, et al. Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. *BMC Genomics.* (2008) 9:367. doi: 10.1186/1471-2164-9-367
63. Forrest JC, Morgan MT, Borggaard C, Rasmussen AJ, Jespersen BL, Andersen JR. Development of technology for the early post mortem prediction of

water holding capacity and drip loss in fresh pork. *Meat Sci.* (2000) 55:115–22. doi: 10.1016/S0309-1740(99)00133-3
64. Woelfel RL, Owens CM, Hirschler EM, Martinez-Dawson R, Sams AR. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. *Poult Sci.* (2002) 81:579–84. doi: 10.1093/ps/81.4.579
65. Petracci M, Mudalal S, Soglia F, Cavani C. Meat quality in fast-growing broiler chickens. *Worlds Poult Sci J.* (2015) 71:363–74. doi: 10.1017/S0043933915000367
66. Zhang X, Owens CM, Schilling MW. Meat: the edible flesh from mammals only or does it include poultry, fish, and seafood? *Anim. Front.* (2017) 7:12–8. doi: 10.2527/af.2017.0437
67. Yost J, Kenney P, Slider S, Russell R, Killefer J. Influence of selection for breast muscle mass on myosin isoform composition and metabolism of deep pectoralis muscles of male and female turkeys. *Poult Sci.* (2002) 81:911–7. doi: 10.1093/ps/81.6.911
68. Wilhelm AE, Maganhini MB, Hernández-Blazquez FJ, Ida EI, Shimokomaki M. Protease activity and the ultrastructure of broiler chicken PSE (pale, soft, exudative) meat. *Food Chem.* (2010) 119:1201–4. doi: 10.1016/j.foodchem.2009.08.034
69. Barbut S, Sosnicki AA, Lonergan SM, Knapp T, Ciobanu DC, Gatcliffe LJ, et al. Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. *Meat Sci.* (2008) 79:46–63. doi: 10.1016/j.meatsci.2007.07.031
70. Owens CM, Alvarado CZ, Sams AR. Research developments in pale, soft, and exudative turkey meat in North America. *Poult Sci.* (2009) 88:1513–7. doi: 10.3382/ps.2009-00008
71. Emadi M, Kaveh K, Jahanshiri F, Hair-Bejo M, Ideris A, Alimon AR. Dietary tryptophan effects on growth performance and blood parameters in broiler chicks. *J Anim Vet Adv.* (2010) 9:700–4. doi: 10.3923/javaa.2010.700.704
72. Temim S, Chagneau A-M, Peresson R, Tesseraud S. Chronic heat exposure alters protein turnover of three different skeletal muscles in finishing broiler chickens fed 20 or 25% protein diets. *J Nutr.* (2000) 130:813–9. doi: 10.1093/jn/130.4.813
73. Yin H, Zhang S, Gilbert ER, Siegel PB, Zhu Q, Wong EA. Expression profiles of muscle genes in postnatal skeletal muscle in lines of chickens divergently selected for high and low body weight. *Poult Sci.* (2014) 93:147–54. doi: 10.3382/ps.2013-03612
74. Ge X, Zhang Y, Park S, Cong X, Gerrard DE, Jiang H. Stac3 inhibits myoblast differentiation into myotubes. *PLoS ONE.* (2014) 9:e95926. doi: 10.1371/journal.pone.0095926
75. Yin H, Price F, Rudnicki MA. Satellite cells and the muscle stem cell niche. *Physiol Rev.* (2013) 93:23–67. doi: 10.1152/physrev.00043.2011
76. Bryson-Richardson RJ, Currie PD. The genetics of vertebrate myogenesis. *Nat Rev Genet.* (2008) 9:632–46. doi: 10.1038/nrg2369
77. Hernández-Hernández JM, García-González EG, Brun CE, Rudnicki MA. The myogenic regulatory factors, determinants of muscle development, cell identity and regeneration. *Semin Cell Dev Biol.* (2017) 72:10–8. doi: 10.1016/j.semcdb.2017.11.010
78. Buckingham M, Rigby PWJ. Gene regulatory networks and transcriptional mechanisms that control myogenesis. *Dev Cell.* (2014) 28:225–38. doi: 10.1016/j.devcel.2013.12.020
79. Fong AP, Tapscott SJ. Skeletal muscle programming and re-programming. *Curr Opin Genet Dev.* (2013) 23:568–73. doi: 10.1016/j.gde.2013.05.002
80. Moncaut N, Rigby PWJ, Carvajal JJ. Dial M(RF) for myogenesis. *FEBS J.* (2013) 280:3980–90. doi: 10.1111/febs.12379
81. Fink J, Schoenfeld BJ, Nakazato K. The role of hormones in muscle hypertrophy. *Phys Sportsmed.* (2018) 46:129–34. doi: 10.1080/00913847.2018.1406778
82. Winston BW, Ni A, Arora RC. Insulin-like growth factors. In: Laurent GJ, Shapiro SD, editors. *Encyclopedia of Respiratory Medicine*. Elsevier (2006). p. 339–46. doi: 10.1016/B0-12-370879-6/00453-1
83. Clemmons DR. Role of IGF-I in skeletal muscle mass maintenance. *Trends Endocrinol Metab.* (2009) 20:349–56. doi: 10.1016/j.tem.2009.04.002
84. Ma B, He X, Lu Z, Zhang L, Li J, Jiang Y, et al. Chronic heat stress affects muscle hypertrophy, muscle protein synthesis and uptake of amino acid in broilers via insulin like growth factor-mammalian target of rapamycin signal pathway. *Poult Sci.* (2018) 97:4150–8. doi: 10.3382/ps/pey291
85. Knapp JR. Loss of myogenin in postnatal life leads to normal skeletal muscle but reduced body size. *Development.* (2006) 133:601–10. doi: 10.1242/dev.02249
86. Rehfeldt C, Te Pas MFW, Wimmers K, Brameld JM, Nissen PM, Berri C, et al. Advances in research on the prenatal development of skeletal muscle in animals in relation to the quality of muscle-based food. I Regulation of myogenesis and environmental impact. *Animal.* (2011) 5:703–17. doi: 10.1017/S1751731110002089
87. Ohanna M, Sobering AK, Lapointe T, Lorenzo L, Praud C, Petroulakis E, et al. Atrophy of S6K1-/- skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. *Nat Cell Biol.* (2005) 7:286–94. doi: 10.1038/ncb1231
88. Yin H, Li D, Wang Y, Zhao X, Liu Y, Yang Z, et al. Myogenic regulatory factor (MRF) expression is affected by exercise in postnatal chicken skeletal muscles. *Gene.* (2015) 561:292–9. doi: 10.1016/j.gene.2015.02.044
89. Frier BC, Locke M. Heat stress inhibits skeletal muscle hypertrophy. *Cell Stress Chaperones.* (2007) 12:132. doi: 10.1379/CSC-233R.1
90. Lindquist S. The heat-shock response. *Annu Rev Biochem.* (1986) 55:1151–91. doi: 10.1146/annurev.bi.55.070186.005443
91. Shankar K, Mehendale HM. Heat-shock proteins. In: Wexler P, editor. *Encyclopedia of Toxicology*. Bethesda, MD: Elsevier (2014). p. 830–1. doi: 10.1016/B978-0-12-386454-3.00320-1
92. Bernabòl P, Rebecchi L, Jousson O, Martínez-Guitarte JL, Lencioni V. Thermotolerance and hsp70 heat shock response in the cold-stenothermal chironomid Pseudodiamesa branickii (NE Italy). *Cell Stress Chaperones.* (2011) 16:403–10. doi: 10.1007/s12192-010-0251-5
93. De Maio A, Vazquez D. Extracellular heat shock proteins. *Shock.* (2013) 40:239–46. doi: 10.1097/SHK.0b013e3182a185ab
94. Hartl FU. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science.* (2002) 295:1852–8. doi: 10.1126/science.1068408
95. Ming J, Xie J, Xu P, Liu W, Ge X, Liu B, et al. Molecular cloning and expression of two HSP70 genes in the Wuchang bream (Megalobrama amblycephala Yih). *Fish Shellfish Immunol.* (2010) 28:407–18. doi: 10.1016/j.fsi.2009.11.018
96. Bakthisaran R, Tangirala R, Rao CM. Small heat shock proteins: role in cellular functions and pathology. *Biochim Biophys Acta Proteins Proteomics.* (2015) 1854:291–319. doi: 10.1016/j.bbapap.2014.12.019
97. Pockley AG. Heat shock proteins as regulators of the immune response. *Lancet.* (2003) 362:469–76. doi: 10.1016/S0140-6736(03)14075-5
98. Vamvakopoulos NO. Tissue-specific expression of heat shock proteins 70 and 90: potential implication for differential sensitivity of tissues to glucocorticoids. *Mol Cell Endocrinol.* (1993) 98:49–54. doi: 10.1016/0303-7207(93)90235-C
99. Wegele H, Müller L, Buchner J. Hsp70 and Hsp90—a relay team for protein folding. In: Mager T, editor. *Reviews of Physiology, Biochemistry and Pharmacology*. Berlin; Heidelberg: Springer Berlin Heidelberg (2004). p. 1–44. doi: 10.1007/s10254-003-0021-1
100. Jiang S, Mohammed AA, Jacobs JA, Cramer TA, Cheng HW. Effect of synbiotics on thyroid hormones, intestinal histomorphology, and heat shock protein 70 expression in broiler chickens reared under cyclic heat stress. *Poult Sci.* (2020) 99:142–50. doi: 10.3382/ps/pez571
101. Pei-Ming S, Yu-Tian L, Qing-Hua W, Zhi-Liang W, En-Dong B. HSP70 and HSP70 mRNA localization in heat-stressed tissues of broilers. *Chinese J Agric Biotechnol.* (2007) 4:181–6. doi: 10.1017/S1479236207001726
102. Yu J, Bao E. Effect of acute heat stress on heat shock protein 70 and its corresponding mRNA expression in the heart, liver, and kidney of broilers. *Asian Australas J Anim Sci.* (2008) 21:1116–26. doi: 10.5713/ajas.2008.70703
103. Shevtsov M, Multhoff G. Heat shock protein–peptide and hsp-based immunotherapies for the treatment of cancer. *Front Immunol.* (2016) 7:171. doi: 10.3389/fimmu.2016.00171
104. Abdelnour SA, Abd El-Hack ME, Khafaga AF, Arif M, Taha AE, Noreldin AE. Stress biomarkers and proteomics alteration to thermal stress in ruminants: a review. *J Therm Biol.* (2019) 79:120–34. doi: 10.1016/j.jtherbio.2018.12.013
105. Zhang WW, Kong LN, Zhang XQ, Luo QB. Alteration of HSF3 and HSP70 mRNA expression in the tissues of two chicken breeds during acute heat stress. *Genet Mol Res.* (2014) 13:9787–94. doi: 10.4238/2014.November.27.6
106. Lei L, Yu J, Bao E. Expression of heat shock protein 90 (Hsp90) and transcription of its corresponding mRNA in broilers exposed to high temperature. *Br Poult Sci.* (2009) 50:504–11. doi: 10.1080/00071660903110851

107. Wang SH, Cheng CY, Tang PC, Chen CF, Chen HH, Lee YP, et al. Differential gene expressions in testes of L2 strain Taiwan country chicken in response to acute heat stress. *Theriogenology.* (2013) 79:374–82.e7. doi: 10.1016/j.theriogenology.2012.10.010
108. Balan P, Kim YHB, Blijenburg R. Small heat shock protein degradation could be an indicator of the extent of myofibrillar protein degradation. *Meat Sci.* (2014) 97:220–2. doi: 10.1016/j.meatsci.2014.01.019
109. Lomiwes D, Farouk MM, Wiklund E, Young OA. Small heat shock proteins and their role in meat tenderness: a review. *Meat Sci.* (2014) 96:26–40. doi: 10.1016/j.meatsci.2013.06.008
110. Herrera-Mendez CH, Becila S, Boudjellal A, Ouali A. Meat ageing: reconsideration of the current concept. *Trends Food Sci Technol.* (2006) 17:394–405. doi: 10.1016/j.tifs.2006.01.011
111. Ouali A, Herrera-Mendez CH, Coulis G, Becila S, Boudjellal A, Aubry L, et al. Revisiting the conversion of muscle into meat and the underlying mechanisms. *Meat Sci.* (2006) 74:44–58. doi: 10.1016/j.meatsci.2006.05.010
112. Attia YA, Al-Harthi MA, Sh. Elnaggar A. Productive, physiological and immunological responses of two broiler strains fed different dietary regimens and exposed to heat stress. *Ital J Anim Sci.* (2018) 17:686–97. doi: 10.1080/1828051X.2017.1416961
113. Daghir N. Nutritional strategies to reduce heat stress in broilers and broiler breeders. *Lohmann Inf.* (2009) 44:6–15. Available online at: https://thepoultrypunch.com/2019/05/nutritional-strategies-to-alleviate-the-effect-of-heat-stress-in-broilers/
114. Ghazalah AA, Abd-Elsa MO, Ali AM. Influence of dietary energy and poultry fat on the response of broiler chicks to heat therm. *Int J Poult Sci.* (2008) 7:355–9. doi: 10.3923/ijps.2008.355.359
115. Naga Raja Kumari K, Narendra Nath D. Ameliorative measures to counter heat stress in poultry. *Worlds Poult Sci J.* (2018) 74:117–30. doi: 10.1017/S0043933917001003
116. Fisinin VI, Kavtarashvili AS. Heat stress in poultry. II methods and techniques for prevention and alleviation (review). *Sel'skokhozyaistvennaya Biol.* (2015) 50:431–43. doi: 10.15389/agrobiology.2015.4.431eng
117. Sahin K, Smith MO, Onderci M, Sahin N, Gursu MF, Kucuk O. Supplementation of zinc from organic or inorganic source improves performance and antioxidant status of heat-distressed quail. *Poult Sci.* (2005) 84:882–7. doi: 10.1093/ps/84.6.882
118. Kucuk O, Sahin N, Sahin K. Supplemental zinc and vitamin a can alleviate negative effects of heat stress in broiler chickens. *Biol Trace Elem Res.* (2003) 94:225–36. doi: 10.1385/BTER:94:3:225
119. Khan RU, Naz S, Nikousefat Z, Selvaggi M, Laudadio V, Tufarelli V. Effect of ascorbic acid in heat-stressed poultry. *Worlds Poult Sci J.* (2012) 68:477–90. doi: 10.1017/S004393391200058X
120. Mahmoud UT, Mootaz AM, Abdel-Rahman MHAD. Effects of propolis, ascorbic acid and vitamin E on thyroid and corticosterone. *J Adv Vet Res.* (2014) 4:18–27. Available online at: https://www.cabdirect.org/cabdirect/abstract/20143121467
121. Habibian M, Ghazi S, Moeini MM. Effects of dietary selenium and vitamin e on growth performance, meat yield, and selenium content and lipid oxidation of breast meat of broilers reared under heat stress. *Biol Trace Elem Res.* (2016) 169:142–52. doi: 10.1007/s12011-015-0404-6
122. Liu P, Wang X, Hu C, Hu T. Inhibition of proliferation and induction of apoptosis by trimethoxyl stilbene (TMS) in a lung cancer cell line. *Asian Pac J Cancer Prev.* (2011) 12:2263–9. Available online at: https://europepmc.org/article/MED/22296367
123. Abd El-Hack ME, Alagawany M, Noreldin AE. Managerial and nutritional trends to mitigate heat stress risks in poultry farms. *Handb Environ Chem.* (2019) 77:325–38. doi: 10.1007/698_2018_290
124. Rehman Z, Chand N, Khan RU, Naz S, Alhidary IA. Serum biochemical profile of two broiler strains supplemented with vitamin E, raw ginger (*Zingiber officinale*) and L-carnitine under high ambient temperatures. *S Afr J Anim Sci.* (2019) 48:935. doi: 10.4314/sajas.v48i5.13
125. Sandikci M, Eren U, Onol AG, Kum S. The effect of heat stress and the use of Saccharomyces cerevisiae or (and) bacitracin zinc against heat stress on the intestinal mucosa in quails. *Rev Med Vet.* (2004) 155:552–6. doi: 10.5281/zenodo.1072271
126. Mitchell MA, Carlisle AJ. The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl (*Gallus domesticus*). *Comp Biochem Physiol Part A Physiol.* (1992) 101:137–42. doi: 10.1016/0300-9629(92)90641-3
127. Shewita RS, Taha AE. Effect of dietary supplementation of different levels of black seed (*Nigella sativa* L) on growth performance, immunological, hematological and carcass parameters of broiler chicks. *World Acad Sci Eng Technol.* (2011) 5:31325. Available online at: https://www.revmedvet.com/artdes-us.php?id=1273
128. Khonyoung D, Yamauchi K, Buwjoom T, Maneewan B, Thongwittaya N. Effects of dietary dried fermented ginger on growth performance, carcass quality, and intestinal histology of heat-stressed broilers. *Can J Anim Sci.* (2012) 92:307–17. doi: 10.4141/cjas2011-129
129. Özbek H, Ugraş S, Dülger H, Bayram I, Tuncer I, Öztürk G, et al. Hepatoprotective effect of *Foeniculum vulgare* essential oil. *Fitoterapia.* (2003) 74:317–19. doi: 10.1016/S0367-326X(03)00028-5
130. Ruberto G, Baratta MT, Deans SG, Dorman HJD. Antioxidant and antimicrobial activity of foeniculum vulgare and *Crithmum maritimum* essential oils. *Planta Med.* (2000) 66:687–93. doi: 10.1055/s-2000-9773
131. Ragab MS, Namra MMM, Aly MMM, Fathi MA. Impact of inclusion fennel seeds and thyme dried leaves in broiler diets on some productive and physiological performance during summer season. *Egypt Poult Sci.* (2016) 33:197–219. Available online at: https://www.researchgate.net/publication/338623480_IMPACT_OF_INCLUSION_FENNEL_SEEDS_AND_THYME_DRIED_LEAVES_IN_BROILER_DIETS_ON_SOME_PRODUCTIVE_AND_PHYSIOLOGICAL_PERFORMANCE_DURING_SUMMER_SEASON
132. Gharaghani H, Shariatmadari F, Torshizi M. Effect of fennel (*Foeniculum vulgare* Mill.) used as a feed additive on the egg quality of laying hens under heat stress. *Rev Bras Ciência Avícola.* (2015) 17:199–207. doi: 10.1590/1516-635x1702199-208
133. Mohammed AA, Abbas RJ. The effect of using fennel seeds (*Foeniculum vulgare* L.) on productive performance of broiler chickens. *Int J Poult Sci.* (2009) 8:642–4. doi: 10.3923/ijps.2009.642.644
134. Forouzanfar F, Fazly Bazzaz BS, Hosseinzadeh H. Black cumin *(Nigella sativa)* and its constituent (thymoquinone): a review on antimicrobial effects. *Iran J Basic Med Sci.* (2014) 17:929–38.
135. Shoukary RDEL, Sayed RK, Hassan RI. Behavioral, hepato-morphological, and biochemical studies on the possible protective effect of black seed and water bath against change-mediated heat stress on pigeon. *J Basic Appl Zool.* (2018) 79:23. doi: 10.1186/s41936-018-0035-5
136. El-Shoukary RDM, Darwish MHA, Abdel-Rahman MAM. Behavioral, Performance, carcass traits and hormonal changes of heat stressed broilers feeding black and coriander seeds. *Adv Vet Res.* (2014) 4:97–101. Available online at: https://advetresearch.com/index.php/AVR/article/view/79
137. FAO/WHO. *WHO Working Group Guidelines for the Evaluation of Probiotics 734 in Food*. London (2002).
138. Abarike ED, Jian J, Tang J, Cai J, Yu H, Lihua C, et al. Influence of traditional Chinese medicine and Bacillus species (TCMBS) on growth, immune response and disease resistance in *Nile tilapia*, *Oreochromis niloticus*. *Aquac Res.* (2018) 49:2366–75. doi: 10.1111/are.13691
139. Amoah K, Dong X, Tan B, Zhang S, Chi S, Yang Q, et al. Administration of probiotic *Bacillus licheniformis* induces growth, immune and antioxidant enzyme activities, gut microbiota assembly and resistance to *Vibrio parahaemolyticus* in *Litopenaeus vannamei*. *Aquac Nutr.* (2020) 26:1604–22. doi: 10.1111/anu.13106
140. Amoah K, Huang Q, Dong X, Tan B, Zhang S, Chi S, et al. *Paenibacillus polymyxa* improves the growth, immune and antioxidant activity, intestinal health, and disease resistance in Litopenaeus vannamei challenged with Vibrio parahaemolyticus. *Aquaculture.* (2020) 518:734563. doi: 10.1016/j.aquaculture.2019.734563
141. Amoah K, Dong X, Tan B, Zhang S, Kuebutornye FKA, Chi S, et al. *In vitro* assessment of the safety and potential probiotic characteristics of three *Bacillus* strains isolated from the intestine of hybrid grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂). *Front Vet Sci.* (2021) 8:675962. doi: 10.3389/fvets.2021.675962
142. Xu S, Lin Y, Zeng D, Zhou M, Zeng Y, Wang H, et al. Bacillus licheniformis normalize the ileum microbiota of chickens infected with necrotic enteritis. *Sci Rep.* (2018) 8:1744. doi: 10.1038/s41598-018-20059-z
143. Li X-Q, Zhu Y-H, Zhang H-F, Yue Y, Cai Z-X, Lu Q-P, et al. Risks associated with high-dose *Lactobacillus rhamnosus* in an *Escherichia coli* model of piglet

diarrhoea: intestinal microbiota and immune imbalances. *PLoS ONE.* (2012) 7:e40666. doi: 10.1371/journal.pone.0040666
144. Al-Fataftah A-R, Abdelqader A. Effects of dietary Bacillus subtilis on heat-stressed broilers performance, intestinal morphology and microflora composition. *Anim Feed Sci Technol.* (2014) 198:279–85. doi: 10.1016/j.anifeedsci.2014.10.012
145. Faseleh Jahromi M, Wesam Altaher Y, Shokryazdan P, Ebrahimi R, Ebrahimi M, Idrus Z, et al. Dietary supplementation of a mixture of Lactobacillus strains enhances performance of broiler chickens raised under heat stress conditions. *Int J Biometeorol.* (2016) 60:1099–110. doi: 10.1007/s00484-015-1103-x
146. Zulkifli I, Juriah K, Htin NN, Norazlina I. Response of heat-distressed broiler chickens to virginiamycin and probiotic-enhanced water acidifier (Acid-Pak 4-Way^{TM}) supplementation, and early age feed restriction. *Arch fur Geflugelkd.* (2006) 70:119–26. Available online at: https://www.european-poultry-science.com/Response-of-heat-distressed-broiler-chickens-to-virginiamycin-and-probiotic-enhanced-water-acidifier-Acid-Pak-4-WaysupTMsup-supplementation-and-early-age-feed-restriction,QUlEPTQyMTY5MjcmTUlEPTE2MTAxNA.htm
147. Tollba AAH, Sabry MM, Medani GG. Effect of microbial probiotics on the performance of broiler chicks under normal or heat stress conditions. 2 - Bacteria concentration or yeast culture Egypt. *Poult Sci J.* (2004) 24:351–67. Available online at: https://www.mendeley.com/catalogue/d49176f5-db18-3496-b59d-6962b1d43672/?utm_source=desktop&utm_medium=1.19.8&utm_campaign=open_catalog&userDocumentId=%7B9a293449-c6bd-4921-9274-20c701a5a90b%7D
148. Sohail MU, Ijaz A, Yousaf MS, Ashraf K, Zaneb H, Aleem M, et al. Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. *Poult Sci.* (2010) 89:1934–8. doi: 10.3382/ps.2010-00751
149. de Oliveira EP, Burini RC. High plasma uric acid concentration: causes and consequences. *Diabetol Metab Syndr.* (2012) 4:12. doi: 10.1186/1758-5996-4-12
150. Hasan S, Hossain MM, Alam J, Bhuiyan MER. Benificial effects of probiotic on growth performance and hemato-biochemical parameters in broilers during heat stress. *Int J Innov Appl Stud ISSN.* (2015) 10:244–9. Available online at: https://www.semanticscholar.org/paper/Influences-of-prebiotic-on-growth-performance-and-Hasan-Hossain/3e1a9b73e675c80752f3ec81b50b7bada9214ac2?p2df
151. Deng W, Dong XF, Tong JM, Zhang Q. The probiotic *Bacillus licheniformis* ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. *Poult Sci.* (2012) 91:575–82. doi: 10.3382/ps.2010-01293
152. Haldar S, Ghosh TK, Toshiwati, Bedford MR. Effects of yeast (*Saccharomyces cerevisiae*) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with *Salmonella enteritidis*. *Anim Feed Sci Technol.* (2011) 168:61–71. doi: 10.1016/j.anifeedsci.2011.03.007
153. Rahimi S, Khaksefidi A. A comparison between the effects of a probiotic (Bioplus 2B) and an antibiotic (virginiamycin) on the performance of broiler chickens under heat stress condition. *Iran J Vet Res.* (2006) 7:23–8. Available online at: https://www.cabdirect.org/cabdirect/abstract/20063194510
154. Sheridan BS, Lefrançois L. Intraepithelial lymphocytes: to serve and protect. *Curr Gastroenterol Rep.* (2010) 12:513–21. doi: 10.1007/s11894-010-0148-6
155. Hasan S, Hossain M, Miah A, Bhuiyan M. Influences of prebiotic on growth performance and hemato-biochemical parameters in broiler during heat stress. *Bangladesh J Vet Med.* (2014) 12:121–5. doi: 10.3329/bjvm.v12i2.21273
156. Lei K, Li YL, Yu DY, Rajput IR, Li WF. Influence of dietary inclusion of *Bacillus licheniformis* on laying performance, egg quality, antioxidant enzyme activities, and intestinal barrier function of laying hens. *Poult Sci.* (2013) 92:2389–95. doi: 10.3382/ps.2012-02686
157. Asli MM, Shariatmadari F, Hosseini SA, Lotfollahian H. Effect of Probiotics, Yeast, Vitamin E and Vitamin C supplements on performance and immune response of laying hen during high environmental temperature. *Int J Poult Sci.* (2007) 6:895–900. doi: 10.3923/ijps.2007.895.900
158. Huang Z, Mu C, Chen Y, Zhu Z, Chen C, Lan L, et al. Effects of dietary probiotic supplementation on LXRα and CYP7α1 gene expression, liver enzyme activities and fat metabolism in ducks. *Br Poult Sci.* (2015) 56:218–24. doi: 10.1080/00071668.2014.1000821
159. Landy N, Kavyani A. Effects of using a multi-strain probiotic on performance, immune responses and cecal microflora composition in broiler chickens reared under cyclic heat stress condition. *IJAS.* (2013) 3:703–8. Available online at: https://www.semanticscholar.org/paper/Effects-of-Using-a-Multi-Strain-Probiotic-on-Immune-Landy-Kavyani/e674fb972adac50cf74304868f13734841d1cbb2
160. Ashraf S, Zaneb H, Yousaf MS, Ijaz A, Sohail MU, Muti S, et al. Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. *J Anim Physiol Anim Nutr.* (2013) 97:68–73. doi: 10.1111/jpn.12041
161. Song J, Xiao K, Ke YL, Jiao LF, Hu CH, Diao QY, et al. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. *Poult Sci.* (2014) 93:581–8. doi: 10.3382/ps.2013-03455
162. Sohail MU, Hume ME, Byrd JA, Nisbet DJ, Ijaz A, Sohail A, et al. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poult Sci.* (2012) 91:2235–40. doi: 10.3382/ps.2012-02182
163. Darwin CR. *The Variation of Animals and Plants Under Domestication,* Vol. 1, 1st ed. London, John Murray (1868). Available online at: http://darwin-online.org.uk/content/frameset?itemID=F877.1andviewtype=textandpageseq=1
164. Duah KK, Essuman EK, Boadu VG, Olympio OS, Akwetey W. Comparative study of indigenous chickens on the basis of their health and performance. *Poult Sci.* (2020) 99:2286–92. doi: 10.1016/j.psj.2019.11.049
165. Dong J, He C, Wang Z, Li Y, Li S, Tao L, et al. A novel deletion in KRT75L4 mediates the frizzle trait in a Chinese indigenous chicken. *Genet Sel Evol.* (2018) 50:68. doi: 10.1186/s12711-018-0441-7
166. Patra BN, Bais RKS, Prasad RB, Singh BP. Performance of naked neck versus normally feathered coloured broilers for growth, carcass traits and blood biochemical parameters in tropical climate. *Asian-Australasian J Anim Sci.* (2002) 15:1776–83. doi: 10.5713/ajas.2002.1776
167. Yunis R, Cahaner A. The effects of the naked neck (Na) and frizzle (F) genes on growth and meat yield of broilers and their interactions with ambient temperatures and potential growth rate. *Poult Sci.* (1999) 78:1347–52. doi: 10.1093/ps/78.10.1347
168. Lin H, Jiao HC, Buyse J, Decuypere E. Strategies for preventing heat stress in poultry. *Worlds Poult Sci J.* (2006) 62:71–86. doi: 10.1079/WPS200585
169. Cahaner A, Deeb N, And Gutman M. Effects of the plumage-reducing naked neck (na) gene on the performance of fast-growing broilers at normal and high ambient temperatures. *Poult Sci.* (1993) 72:767–75. doi: 10.3382/ps.0720767
170. Raju MVLN, Shyam Sunder G, Chawak MM, Rama Rao SV, Sadagopan VR. Response of naked neck (Nana) and normal (nana) broiler chickens to dietary energy levels in a subtropical climate. *Br Poult Sci.* (2004) 45:186–93. doi: 10.1080/00071660410001715786
171. Ouyang J, Xie L, Nie Q, Zeng H, Peng Z, Zhang D, et al. The effects of different sex-linked dwarf variations on Chinese native chickens. *J Integr Agric.* (2012) 11:1500–8. doi: 10.1016/S2095-3119(12)60150-6

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Amino Acid Supplementation to Reduce Environmental Impacts of Broiler and Pig Production: A Review

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Poultry and swine farming are large contributors to environmental impacts, such as climate change, eutrophication, acidification, and air and water pollution. Feed production and manure management are identified as the main sources of these impacts. Reducing dietary crude protein levels is a nutritional strategy recognized to both decrease the use of high-impact feed ingredients and alter manure composition, reducing emissions of harmful components. For a successful implementation of this technique, feed-grade amino acid supplementation is crucial to maintaining animal performance. Reducing crude protein lowers nitrogen excretion, especially excess nitrogen excreted in urea or uric acid form, improving nitrogen efficiency. At the feed-gate, low–crude protein diets can reduce the carbon footprint of feed production through changes in raw material inclusion. The magnitude of this reduction mainly depends on the climate change impact of soybean meal and its land-use change on the feed-grade amino acids used. Reducing dietary crude protein also lowers the environmental impact of manure management in housing, storage, and at spreading: nitrogen emissions from manure (ammonia, nitrates, nitrous oxide) are reduced through reduction of nitrogen excretion. Moreover, synergetic effects exist with nitrogen form, water excretion, and manure pH, further reducing emissions. Volatilization of nitrogen is more reduced in poultry than in pigs, but emissions are more studied and better understood for pig slurry than poultry litter. Ammonia emissions are also more documented than other N-compounds. Low–crude protein diets supplemented with amino acids is a strategy reducing environmental impact at different stages of animal production, making life cycle assessment the best-suited tool to quantify reduction of environmental impacts. Recent studies report an efficient reduction of environmental impacts with low–crude protein diets. However, more standardization of limits and methods used is necessary to compare results. This review summarizes the current knowledge on mitigation of environmental impacts with low–crude protein diets supplemented with amino acids in poultry and swine, its quantification, and the biological mechanisms involved. A comparison between pigs and poultry is also included. It provides concrete information based on quantified research for decision making for the livestock industry and policy makers.

Keywords: crude protein, amino acids, broiler, pig, nitrogen, environmental impacts, life cycle assessment

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INTRODUCTION

The environmental impact from animal production has become a major concern in the past decades (1, 2). Simultaneously, an increasing world population and shift toward more meat-based diets in developing countries will increase demand for animal products by an estimated 50% by 2050 (3). Pork and chicken are the most consumed meats today and will continue to grow (4), making the transition to less impactful practices crucial for these productions. Furthermore, societal demand for environmentally friendly production is rising and should be taken into account by industry actors in the sector of broiler and pig production.

Environmental impacts of animal rearing are mostly caused by feed production and emissions from manure (5, 6). At the feed production step, the main impacts are climate change linked to energy consumption, nitrous oxide emissions from the fields, and the land-use change (LUC) impact of crops cultivated on recently converted forests or grasslands. This mostly concerns soybean meal (SBM) produced in South America and used widely in Europe and Asia as a source of protein for animal feed. Emissions from the field, due to fertilization, are also an important contributor to acidification and eutrophication. Emissions from manure cause climate change (methane, nitrous oxide), acidification [ammonia (NH_3)], eutrophication (phosphorus, nitrates, NH_3), air pollution (NH_3, particles), and water pollution (nitrates). Nitrogen (N) emissions are involved in all those impacts and are the leading cause of pollution from broiler and pig manure. Those emissions can happen on the farm, in the barn or during manure storage, or at the field after spreading. Other sources of environmental impact are less important in the case of monogastrics. They include energy consumption on the farm and production of enteric methane for pigs. The main processes involved in broiler and pig production and their associated resource use and impacts are summarized in **Figure 1**.

Nutrition is one of the most effective levers to reduce environmental impact as it can affect emissions from feed production and modify manure composition, limiting emissions in housing and during storage and spreading. Reduction of crude protein (CP) content of feed is a method that has been widely studied and implemented in pig production in order to reduce environmental impacts (7, 8). For broilers, it has been explored more recently with studies focusing on animal performance rather than on implications for environmental impacts, and its practical implementation is still in the early stages (9, 10). In the European Union, it is recognized as a best available technique to reduce NH_3 emissions from pig and broiler farms (11). It is also a recommended method to reduce eutrophication impact due to nitrate leaching. Indeed, it allows reducing N excretion with a maintained animal performance thanks to feed-grade amino acid (AA) supplementation to cover animal requirements. This reduces N emissions from manure. Low-CP diets supplemented with AA gradually replace protein sources, generally SBM, with cereals and feed-grade AA and possibly alternative protein sources and co-products. In contexts in which SBM associated with LUC is used, this allows tackling the environmental impact of feed production.

This work aims to review the current knowledge on the mitigating effects of low-CP diets supplemented with AA on the environmental impacts of broiler and pig production. Effects of this strategy are considered at different stages of production: animal performance and excretion, feed production, and manure management. Evaluations of the strategy through life cycle assessment (LCA) are also presented, which allows evaluating the effects on the whole system. The review covers both mechanisms involved and available quantification, highlighting areas in which more research is needed and comparing effects between the two species.

IMPACT OF LOW-CP DIETS AND FEED-GRADE AA INCLUSION ON ANIMAL PERFORMANCE AND METABOLISM

Animal Performance

Dietary CP reduction in swine and broiler diets is performed by replacing protein-rich feedstuffs, generally SBM, by cereals and feed-grade AA. This reduces CP and, thus, N content of the diet while maintaining an adequate supply of indispensable AA.

Low-CP diets formulated with an adequate dietary AA supply are shown to maintain growth performance of growing and finishing pigs consistently in the past decades (12–19). These results are confirmed in recent trials even with a dietary CP reduction of more than 30 g/kg (20–22). Feed-grade AA supplementation is shown to be essential to ensure constant animal performance and successful implementation of a low-CP strategy in swine (22, 23).

In broilers, multiple recently published trials show that reducing dietary CP formulated with an adequate dietary AA supply does not affect growth performance (9, 10, 24–26). When balancing all indispensable AA, it seems possible to reduce dietary CP by 30 g/kg in broiler chickens without affecting growth, intake, feed efficiency, or carcass traits. Lowering dietary CP is also shown to improve animal welfare based on foot pad lesion indicators (10), thanks to a lower litter moisture.

In both species, lowering dietary CP requires a holistic nutritional approach as not only protein and AA, but also fiber, electrolyte balance, and energy sources are affected (27). A careful control of those parameters is, thus, recommended to optimize performance of pigs and poultry fed low-CP diets.

Nitrogen Balance and Animal Metabolism

Various meta-analyses have been published in recent years to synthesize the extensive literature available on the effect of CP reduction on N excretion for both pigs and broilers. In swine, one estimated from nine trials shows a reduction of N excretion by 7.5% per each 10 g/kg CP reduction (28); another reported from 59 publications an average reduction of N excretion of 28.5% with low-CP strategies (29); and the last, based on 27 trials, shows a reduction of N excretion by 8.2% per each 10 g/kg CP reduction (8) (**Figure 2**). In broilers, a reduction of N excretion by 9% per each 10 g/kg CP reduction was estimated from 107 trials (27) (**Figure 3**). The reduction of total N excretion with low CP diets is, thus, of similar extent in broilers and pigs.

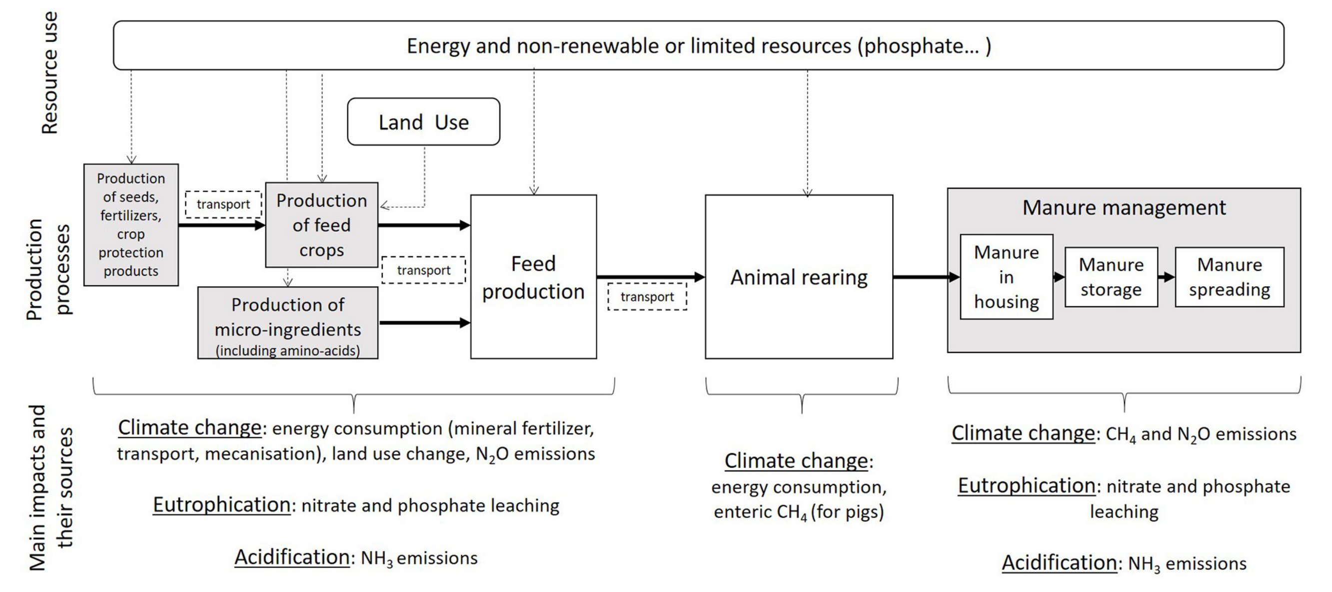

FIGURE 1 | Resource use and environmental impacts associated with the main steps of broiler and pig production.

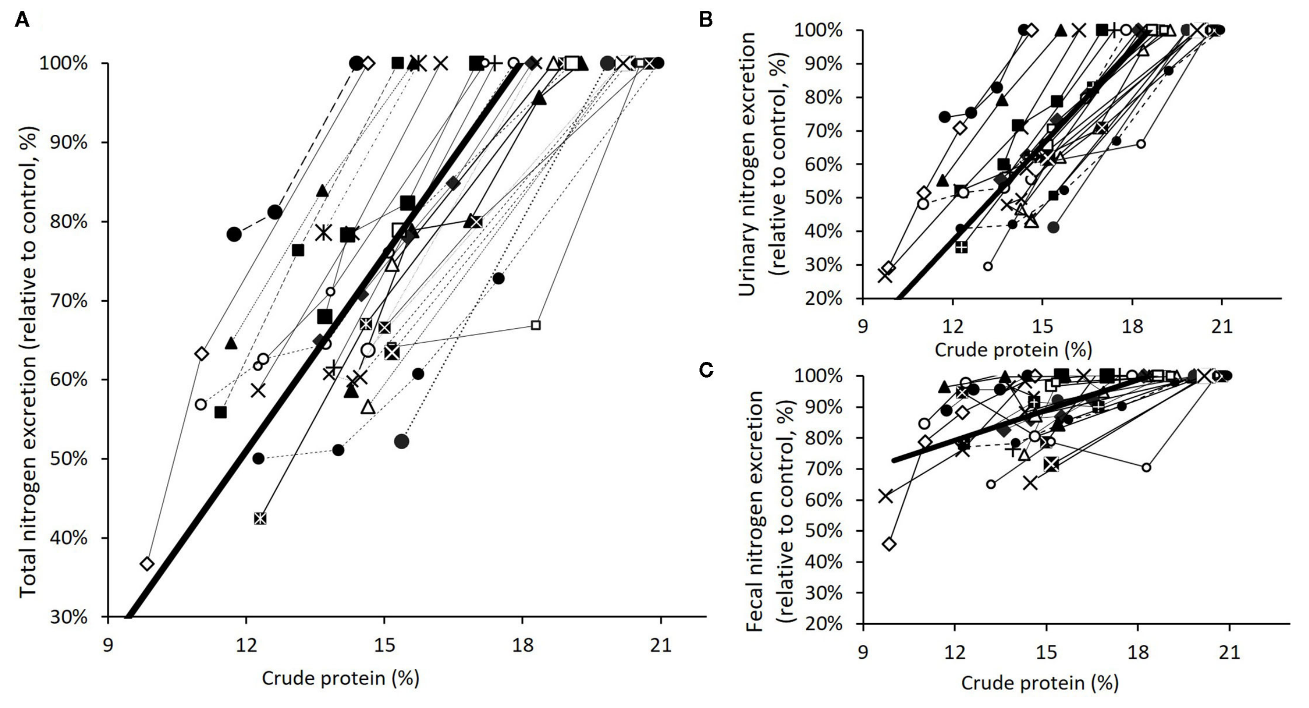

FIGURE 2 | Meta-analysis of the effect of dietary CP reduction on nitrogen excretion in pigs: total excretion **(A)**, urinary excretion **(B)** and fecal excretion **(C)** [adapted from Cappelaere et al. (8)].

Reduction of dietary CP lowers N content of diets and, thus, N intake. As growth performance is not affected, N retention is kept constant. Consequently, N efficiency is improved by 1.6 percentage points per each 10 g/kg CP reduction in swine (8) and 2.3 percentage points in broilers (27). Dietary CP reduction with AA supplementation improves valorization of N from feedstuffs used by livestock.

Dietary CP reduction lowers the supply of excess AA, thanks to a better balanced, indispensable AA profile reached with the use of feed-grade AA and a reduction of the dispensable AA

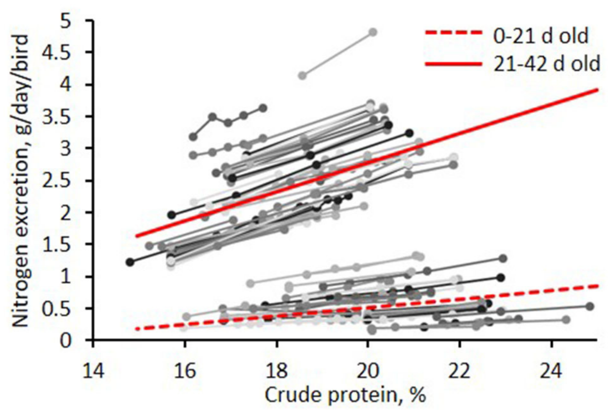

FIGURE 3 | Meta-analysis of the effect of dietary CP reduction on nitrogen excretion in broilers [adapted from Alfonso-Avila et al. (27)].

content of the diet. This reduces the AA catabolism as evidenced by the reduction of plasma uric acid concentration in broilers (30–33) and serum or plasma urea N concentration in pigs (16, 18, 34). Indeed, N excretion pathways differ between broilers and pigs. Mammals are ureotelic animals, and birds are uricotelic, meaning that N is excreted mainly as urea in the former and as uric acid in the latter. Urea excretion requires more water than uric acid excretion as the first has to be solubilized in urine at a non-toxic concentration while the second is not soluble, less toxic, and excreted directly in solid form in the cloaca, mixed with feces. Conversely, nitrogen excretion in birds is more complex and requires more energy than in mammals.

In pigs, the separation between undigested N excreted in feces and catabolized N excreted in urine allows for easily measuring the contribution of reduction of excess dietary AA to lower N excretion. It also allows differentiating between organic N, which is a stable form of N, and urea N, quickly degraded into ammonia. A meta-analysis (29) reports an average reduction of urinary N excretion by 39.6% with low-CP strategies although fecal N excretion was only reduced by 10.4%. Similarly, another meta-analysis (8) reports a reduction of urinary N excretion by 10% and of fecal N excretion by 3.1% per each 10 g/kg CP reduction (**Figure 2**). Thus, the share of N excreted as urea is reduced by 2.5 percentage points per each 10 g/kg CP reduction. This meta-analysis also shows that the share of N excreted as urea is very well-predicted by N efficiency, tightly correlated to excess N. A limited effect of classic CP-reduction strategies on fecal excretion is explained by a limited impact on N digestibility. This is not the case when fiber-rich ingredients, such as rapeseed meal, dried distiller's grain with solubles or sugar beet pulp, are used, resulting in a more important shift from urinary to fecal N excretion and an increased fecal excretion (35–37).

This differentiation is not possible in broilers as undigested and catabolized N are excreted together. However, the share of N excreted as uric acid should also decrease in broilers fed low-CP diets as the biological mechanisms involved are similar in broilers and pigs.

Dietary CP reduction also leads to lower water intake and water excretion in both pigs and broilers (27, 38–41). Lower AA catabolism reduces the quantity of water needed for N excretion in both species (42, 43). Dietary CP reduction is also associated with a lower potassium content and electrolyte balance as SBM is a high contributor to dietary potassium, and feed-grade lysine is rich in chlorure. This has the added advantage of lower water intake and excretion with low-CP diets (27, 44, 45).

ENVIRONMENTAL IMPACT OF FEED PRODUCTION FOR LOW-CP DIETS SUPPLEMENTED WITH AA

Feed production is the main contributor to the climate change impact of pig and broiler production, accounting for around 60–85% (46–48). These data are consistent across the literature as the evaluation of climate change impacts has been harmonized, following IPCC guidelines (49). Contribution of feed production to the final product's acidification and eutrophication impacts is also significant but varies depending on the allocation of emissions from manure to animal or vegetal production. Furthermore, this contribution reflects the variety of practices and also the characterization methods used for those impacts (50) that are less robust and homogenized between official methods than for climate change (51).

Several feed LCAs focusing on low-CP diets have been performed in recent years for pigs and broilers and are summarized in **Table 1**. Most of those studies are performed in a European context and test the effect of taking into consideration or not LUC. All studies that included a LUC impact reported a decrease in climate change potential when reducing CP levels, but this decrease was always <5% per each 10 g/kg CP reduction. CP reduction and feed-grade AA inclusion also decrease energy use for feed production (52, 55). The effect on acidification potential is contrasted between publications. It mostly depends on NH_3 volatilization during crop fertilization and, thus, on agricultural practices considered. Eutrophication potential is consistently decreased with dietary CP reduction.

Dietary CP reduction generally replaces SBM with cereals and feed-grade AA. SBM is mostly produced in North and South America. South American SBM is generally associated with deforestation and has a high LUC impact, especially in Center-West Brazil, leading to a high climate change impact (56, 57). This is not the case for SBM produced in North America. Thus, dietary CP reduction is mainly implemented to reduce the climate change impact of feed when Brazilian SBM is used.

In contexts in which the SBM used has an LUC impact (South America, Europe, China), CP reduction allows reducing the climate change impact associated with SBM inclusion. In low-CP diets, impact values attributed to feed-grade AA are also important even if their inclusion rate is low because they are much higher compared with crops due to the processes used and the high energy demand (55, 58). These values can vary with the origin of the product, type of energy used, and C and N sources for fermentation. Due to these influencing factors, the climate change impact of feed production has been

TABLE 1 | Methodology and results of recent broiler and pig feed LCAs.

publication	animals considered	farm location	crop origin	LUC	scenario	CP levels	climate change	acidification	eutrophication	energy demand
Méda et al. (52)	finishing broilers	France	Europe	yes						
					S19	19	100%	100%	100%	100%
					S17	17	91%	103%	96%	100%
					S15	15	80%	105%	92%	99%
Cherubini et al. (53)	finishing pigs	Brazil	Brazil	no						
						18	100%			
						16	103%			
						15	107%			
						13	110%			
Meul at al. (54)	fattening pigs	Europe	Europe, Brazilian SBM	no						
					reference	15.7	100%			
					low crude protein	13	102%			
				yes						
					reference	15.7	100%			
					low crude protein	13	93%			
				yes + indirect LUC						
					reference	15.7	100%			
					low crude protein	13	91%			
Mosnier et al. (55)	fattening pigs	France	Europe, Brazilian SBM	no						
					standard (noAA)		100%	100%	100%	100%
					biphase (noAA)		99%	98%	98%	94%
					biphase low CP	16.5/15	101%	90%	87%	94%
					biphase least cost with AA		101%	90%	87%	94%
	broilers	France	Europe, Brazilian SBM	no						
					only Met		100%	100%	100%	100%
					Met, Lys		100%	98%	98%	100%
					Met, Lys, Thr		105%	93%	94%	100%
	fattening pigs	France	Europe, Brazilian SBM	yes						
					standard (noAA)		100%	100%	100%	100%
					biphase (noAA)		97%	96%	98%	94%
					biphase low CP	16.5/15	94%	87%	89%	88%
					biphase least cost with AA		93%	85%	89%	86%
	broilers	France	Europe, Brazilian SBM	yes						
					only Met		100%	100%	100%	100%
					Met, Lys		98%	97%	98%	97%
					Met, Lys, Thr		100%	92%	94%	96%

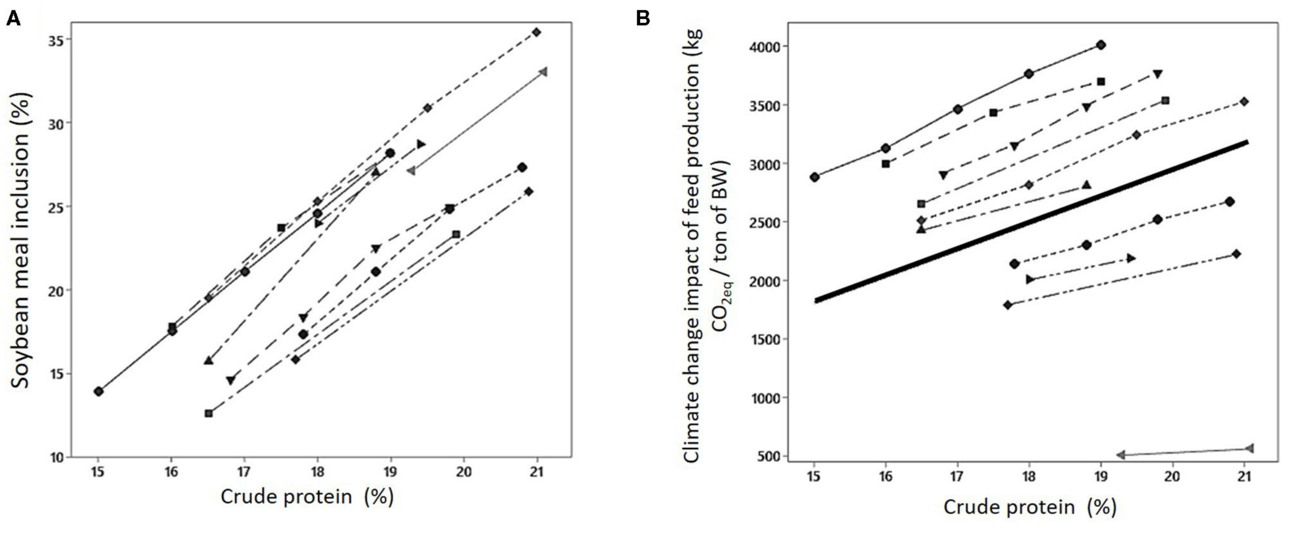

FIGURE 4 | Effect of CP reduction and supplementation with AA on SBM use **(A)** and climate change impact of feed production **(B)** [adapted from Le Cour Grandmaison et al. (61, 62)].

shown to slightly increase with CP reduction in some specific contexts (53, 59). Monteiro et al. (60) shows that, with a farm-gate LCA, CP reduction and AA inclusion decreased climate change impact of French and Brazilian pig production when SBM associated with recent deforestation was used but increased it when SBM was not associated with LUC. Furthermore, the effect of the strategy was more pronounced when SBM was the sole source of protein compared with diets with a mix of protein sources. Similarly, Kebreab et al. (48) evaluate the sensitivity to geographical context and inclusion or not of LUC of the benefits from AA inclusion in pig and broiler production. When LUC was excluded, variation of the feed production climate change impact was low and depended on the geographical region and the species, whereas when LUC was included, climate change impact consistently decreased. In a European context using Brazilian SBM associated with LUC, Le Cour Grandmaison et al. (61) calculated, from recent performance trials, that a 10 g/kg dietary CP reduction reduced climate change impact of a ton of feed by 101 kg CO_2eq. It corresponds to an 8% decrease of climate change impact and, for broilers, to a reduction of 226 kg CO_2eq per ton of live weight (62). This is associated with a reduction of SBM inclusion of 39 kg/t in fattening pigs and 35 kg/t in broilers for each point of dietary CP reduction (**Figure 4**).

Beyond the context of production, quantitative benefits of low-CP strategies for the climate change impact of diets relies heavily on the value taken for LUC impact of SBM. Those values vary greatly between LCA databases, even for equivalent products. For example, the average Brazilian SBM climate change impact triples between EcoAlim (ADEME, 2016) and GFLI (Global Feed LCA institute, 2019) or Agri-footprint 5.0 (Blonk Consultants, 2019) databases. Da Silva et al. (57) also frequently uses in LCAs reports of even lower climate change impacts with a low contribution of LUC (under 30%). Climate change impact values of Brazilian soybeans are presented in **Table 2**. Efforts have been made to propose a standardized method to calculate LUC (63), and those guidelines should be broadly used to improve the consistency and comparability of studies.

Some approaches have also taken into consideration indirect LUC, i.e., LUC caused by the displacement of other crops, pushed by the increased demand for crop cultivated on existing cropland (54), reducing the benefits of using feedstuffs from non-deforested areas. Beyond LUC, CP reduction is shown to reduce land use by up to 10% (64, 65), reducing the pressure for arable land in all production regions.

Reducing CP level using AA also allows for more flexibility on raw materials used. Thus, inclusion of alternative protein sources is made easier, permitting an increase in use of local feedstuffs and reducing the impact of feed transportation and also to valorize more by-products (54).

LOW-CP STRATEGIES REDUCE ENVIRONMENTAL IMPACT OF MANURE MANAGEMENT

Composition of Manure

Commercial broilers across the world are mainly reared on litter, and manure is managed in solid form (66). Conversely, most pigs are reared on slatted floors, and urine and feces are mixed to produce liquid manure, i.e., slurry (11). Feeding animals low-CP diets reduces their N excretion and, in turn, manure N content. A reduction of total N content of pig manure by 3.5% per each 10 g/kg CP reduction was quantified (67). A meta-analysis calculated a higher reduction of N concentration of pig slurry by 5% per each 10 g/kg CP reduction (8), which could be explained by the inclusion of more recent experiments and the selection of trials on iso-digestible lysine diets. Litter N content has also been shown to decrease with low-CP diets in broilers (9, 10, 33, 68).

TABLE 2 | Average Brazilian soybean climate change value in several databases and publications.

	AgriFootPrint 5.0 and GFLI	EcoInvent 3.6	EcoAlim V7	Da Silva et al. (57)
Climate change impact (kg CO_2eq/kg)	5.6	2.6	1.35	0.51–0.96

The chemical form of N in manure is affected by CP reduction. Uric acid and urea, to a greater extent, are quickly degraded by bacteria into NH_3, and N contained in undigested proteins is very stable. Consequently, total ammoniacal N (TAN) content of manure is greatly reduced with low-CP diets correctly supplemented with feed-grade AA. Meta-analyses have estimated a reduction of TAN content of pig slurry by 7–8% per each 10 g/kg CP reduction (8, 69). In broilers, litter TAN has been less studied, and more research is needed on this parameter. An article reported no statistical effect of CP reduction on litter TAN content (10) although a trial in breeders showed a 9% reduction of TAN concentration with a 15 g/kg CP reduction (70).

The lower water excretion of animals with low-CP diets reduces manure moisture and increases dry matter (DM) content and, consequently, reduces manure production expressed as volume or weight. In pigs, slurry volume is reduced by 2.8% per each 10 g/kg CP reduction (8). Some studies report a higher DM content of slurry with low-CP diets (39, 71), but other dietary parameters, such as fiber content, have a higher impact and interact with the protein level (72, 73). Hence, the impact on DM content depends on the formulation choices made when implementing CP reduction and their consequences on ingredient inclusion. In broilers, a reduction of litter moisture by 12 g/kg and a reduction of litter weight by 3.3% per each 10 g/kg CP reduction was quantified by meta-analysis (27). Reduction of CP results in drier and more friable litter with a higher DM content (10).

The magnitude of the benefits, during manure management, of changes in manure composition due to nutritional strategies depends on manure management practices implemented (slurry separation, composting, biological treatment, etc.).

Modifications of pig slurry composition with manipulation of dietary CP have been modeled (74), allowing a precise prediction of the effects of nutritional strategies. Such an approach has not been yet developed in broilers.

Dietary CP reduction also reduces slurry pH in swine (67) by 0.15 points per each 10 g/kg CP reduction (8). This can be explained by a lowered urinary pH (38, 75) due to a lower electrolytic balance. Fecal pH can also be lowered when alternative protein sources rich in fiber are added as hindgut fermentation producing volatile fatty acids is increased (36, 76). In broilers, no effect of CP level on litter pH has been found (10, 68).

Volatilization of N Components and Biological Processes Involved

The nitrogen content of pig and broiler manure is degraded by microorganisms, resulting in different N compounds emitted in the environment with various negative impacts. When manure is produced, the main forms of N are undigested and microbial protein (organic N), urea or uric acid, depending on the species (simple forms of organic N), and ammoniacal N (mineral N). Uric acid is degraded into urea by aerobic bacteria. Urea is quickly degraded into NH_3 by urease produced by microorganisms present in manure. Mineralization of undigested protein is slower and requires specific organisms. It happens mostly in soils when manure storage is short. With long-term storage of slurry or litter or when it undergoes treatments, such as composting, a significant share of the organic matter is degraded before spreading. Ammoniacal N is present in manure in an acid-base equilibrium between ammonium (NH_4^+) and NH_3. It is degraded into N oxides: nitrites (NO_2^-) and then nitrates (NO_3^-) during the nitrification process. This step of the N cycle takes place in aerobic conditions and, thus, mainly concerns litter compared with slurry, which is an anaerobic environment. Nitrates are degraded into N_2 during denitrification. Nitric oxide (NO) and nitrous oxide (N_2O) are also produced during this nitrification–denitrification process due to incomplete biological reactions. Intensity of these processes depends on numerous regulating factors of microbial activity: substrate availability, aerobic or anaerobic conditions, humidity, temperature, pH. Gaseous N compounds (NH_3, NO, N_2O, N_2) are volatilized when exposed to the atmosphere. As slurry is an anaerobic environment, on-farm emissions are in NH_3 form for the majority. In poultry litter, the entire N mineralization process can take place, and emissions are more diversified. The volatilization rate depends on chemical and physical parameters, such as temperature, pH, concentration, and air flow (77). These mechanisms are summed up in **Figure 5**.

The first effect of CP reduction is to decrease manure N content and, thus, substrate to produce harmful N compounds, particularly TAN, as presented above. Synergies with other impacts on manure composition are also involved as CP reduction influences humidity and pH of manure as well as C/N ratio. In pigs, the main effect is the one of pH as a more acidic slurry favors the left-hand side of the NH_4^+/NH_3 chemical equilibrium and limits volatilization of NH_3. This effect is limited and was not caught by the meta-analysis by (8), as CP reduction was not shown to have an effect on the emission factor of TAN into NH_3. Water content of slurry does not have a significant effect on ammonia emissions as slurry remains an anaerobic environment in which water is not limiting to biological processes when water content decreases. However, a higher DM content of slurry is shown to reduce emission factors (77). Conversely, water content of broiler litter largely impacts N volatilization (78) as it is a limiting factor for the biological breakdown of uric acid into NH_3. Belloir et al. (9) measured a reduction of the share of N volatilized from broiler litter with CP reduction of between 3.9 and 6.4 percentage points per each 10

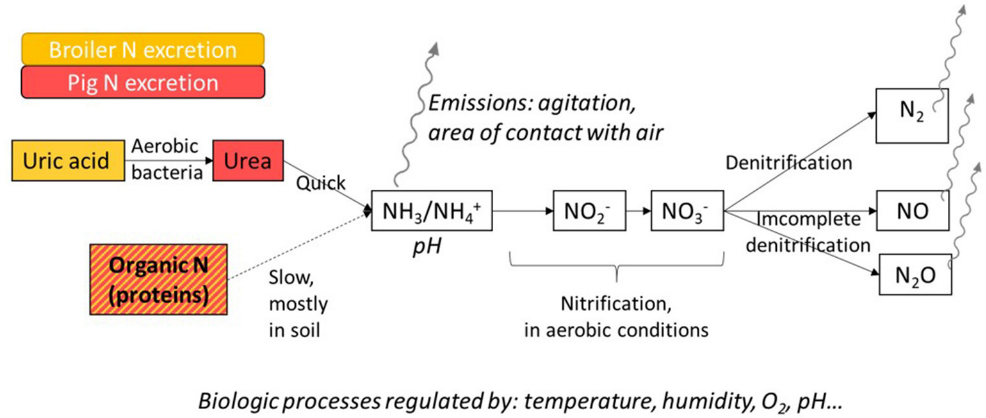

FIGURE 5 | Biochemical processes of the degradation of nitrogen excreted by pigs and broilers.

g/kg CP reduction. However, the form in which N was volatilized was not studied, and thus, no conclusion on NH_3, NO, or N_2O volatilization can be drawn without strong hypotheses. The effect of litter humidity and pH on emissions introduces a synergetic effect between CP reduction and SBM inclusion reduction as the later reduces dietary potassium and, thus, water intake and excretion as well as electrolytic balance and, thus, excreta pH as detailed in previous parts.

These synergetic effects mostly concern on-farm emissions. After spreading, manure is incorporated into the soil and becomes part of a more complex system with many interfering factors (soil type, climate, meteorological conditions, agronomic practices, etc.).

For pig production, the most studied on-farm emissions are NH_3 emissions. Meta-analyses show a reduction of on-farm NH_3 emissions by 10% (8) or 11% (69) per each 10 g/kg CP reduction. Reduction of NH_3 emissions in the barn and at storage ranging from 24 to 65% with low CP strategies are reported (29, 67).

In poultry, quantification of NH_3 emissions in the context of low-CP strategies is not as advanced. Reduction of NH_3 emission by 16% was observed in commercial settings with a 15 g/kg CP reduction (29). A synergy was observed with litter moisture as N excretion was only reduced by 4.8% due to impaired performance. With a similar CP reduction, another trial measured a 9% reduction of NH_3 emissions and an 11% reduction of total N volatilization with breeder broilers (70). A meta-analysis estimated a reduction of NH_3 emissions by 20% with low-CP strategies (66).

For other N compounds, empirical data on the effect of CP reduction is scarcer. A reduction of N_2O emissions from pig manure compost by 39% is shown with a 25 g/kg CP reduction (79) with no effect on the emission factor. Conversely, two studies identify no effect on N_2O emissions with up to a 30 g/kg CP reduction (80, 81).

The effect of low-CP diets on emissions during and after manure spreading are also rarely studied. Portejoie et al. (39) did so, measuring a 53% reduction of NH_3 emissions following pig slurry application for a CP reduction from 200 to 120 g/kg but no significant difference between 160 and 200 g/kg CP treatments. Furthermore, models proposed by international guidelines use fixed emission factors—depending on the animal species, type of manure, country, and climate—based on TAN (82) or total N (49). With these hypotheses, reduction of N_2O, NO, and nitrate emissions are of the same magnitude as reduction of N excretion. However, predicting the fate of N in organic fertilizer is much more complicated as it interacts with the soil and crops. Reducing dietary CP decreases TAN content of manure that is readily available for fertilized crops, and increases the share of organic N, slowly degraded and made available for plants. Without an effective prediction of nutrients available long-term, associated with good fertilization management, changes in manure composition achieved with low-CP strategies can lead to a reduction of emissions at spreading but an increase of long-term emissions, such as nitrate leaching (83).

More research is needed to accurately model the effects of low-CP diets on N emission factors and the type of N compound volatilized. This is particularly true for poultry production and for emissions other than NH_3.

Associated with the reduction of these emissions, the environmental impact of manure management can be greatly decreased. An LCA study estimated a reduction of the eutrophication potential linked to manure management by 20–35% in pigs and 19–49% in broilers with low-CP diets supplemented with feed-grade AA, depending on the continent considered (48). Acidification potential was reduced by 30–35% in pigs and 51–53% in broilers.

FARMGATE LIFE CYCLE ASSESSMENT ALLOWS AGGREGATING THE VALUE CREATED ALONG THE PRODUCTION CHAIN

Dietary CP reduction is a multifactorial strategy that reduces environmental impacts from feed production and also from manure management. To correctly take into consideration those benefits, the whole pig or broiler production chain has to be assessed, from production of feedstuffs to manure spreading. LCA is the best-suited method to do so, thanks to its normalized methodological framework (84, 85) designed to measure the environmental impact of a product or a system throughout its life cycle with a holistic approach.

Dietary CP reduction strategies have been thoroughly evaluated with LCA approaches in pigs since the early 2000s (86, 87), but they have only been recently studied in broilers with few publications. **Supplementary Table 1** summarizes methodology used and results of recent pig LCA studies and available broiler LCA studies focusing on the effect of CP reduction and feed-grade AA supplementation. Similar results were obtained for pigs and broilers. All but one (52) study took into consideration the impact of manure spreading, using the system extension method, meaning that the manure is considered to be used to fertilize crops fed to the animals studied. This allows fully grasping the effect of the mitigation measure. With this method, use of animal manure for fertilization is generally considered to avoid production of mineral fertilizer. This gives a bonus for manure production on energy demand, which is decreased with reduction of N excretion, penalizing low-CP diets for this indicator.

Most studied contexts are European and Brazilian animal productions, which are major broiler and pig producers, but more research effort should be carried out for North American and Asian contexts. The method used was fairly similar between publications. The functional unit used is generally a ton of live weight, but some studies also used the animal (59).

All LCAs considered in this review report a positive effect of CP reduction on the environmental parameters studied (**Figure 6**): climate change, acidification, eutrophication, and land use. The effect on climate change was low when no LUC was considered (48, 64), confirming the conclusions of feed LCAs. Acidification and eutrophication impacts are consistently reduced (7) regardless of context. This is explained by the fact that those impacts are mainly caused by emissions from manure (48, 65). Energy demand increased in some studies, particularly due to energy used for feed-grade AA production (48, 52, 64). Compatibilization of manure used as fertilizer is also involved.

An LCA (65) studied in a French context showed the effect of numerous interacting factors on reduction of environmental impact with low-CP diets supplemented with feed-grade AA: type of manure management, source of protein, interaction with phase-feeding, and LUC value of SBM. CP reduction had a positive effect in all of the contexts. The main differences between liquid and solid manure management were the contribution of manure emissions to climate change impact with higher emissions of N_2O from manure in solid manure systems. As a consequence, reduction of climate change impact is more important in solid manure systems. Other factors had a low influence on the effect of nutritional modifications. Climate change impact was highly affected by the origin of SBM and N_2O emission factors.

DISCUSSION

State-of-the-Art and Future Research

Dietary CP reduction is possible without decreasing performance with adequate feed-grade AA supplementation. Very low-CP diets need to be further explored to keep lowering the environmental impact of animal production. Not impairing performance is of the first importance to keep the economic value of animal production and also to improve environmental performance as feed efficiency is one of the first levers to do so (88). Variability in animal performance has been identified to affect results of dietary solutions (60).

The impact of CP reduction on N balance has been largely studied for both species. Accurate quantification of the amount of N excreted with low-CP diets supplemented with feed-grade AA is available and should be used to predict N emissions from manure. However, the form of in which N is found in manure is also important. Reactive N responsible for emissions is mainly in ammoniacal form. In pigs, the share of N in ammoniacal form can be predicted from urinary excretion, and models exist to estimate it with CP reduction. Due to metabolic differences, this is not the case for broilers for which the share of N excreted as uric acid is not well-documented. This discrepancy remains at the manure level as effects of CP reduction on pig slurry composition are well-quantified, and effects on poultry litter remain to be explored. Furthermore, slurry is a simpler environment to study compared with dry manure as only anaerobic processes happen in the former. Several models for N volatilization have been developed for pigs, including an effect of dietary protein or manure N. This does not exist in broilers, and the effect of reducing CP on N emissions has only been quantified in a few trials. Most publications study NH_3 emissions, but more work is needed for other types of emissions, especially nitrous oxide. Moreover, studies on reducing CP diets focus on emissions at the farm, and few data is available on the effects at the field after spreading. Agronomic valorization of the organic fertilizer produced should also be taken into account to evaluate nutritional strategies. Reducing dietary CP will be more interesting in a territory producing excess organic N fertilizer compared with a territory where it is in demand.

LCA studies on low-CP diets were first performed at the feed level (86). This measures the effects of the strategy on the impact of feed production. The context of production influences greatly the results of all those studies as LUC impact has the highest weight (60). To have comparable data, a standardized method should be used, and LCA values of crops and feed ingredients (especially feed-grade AA and SBM) should be harmonized between databases to avoid cherry-picking of values. The PEFCR method for evaluation of feed provides guidelines and should be used more broadly. In European and South American contexts, a low-CP diet supplemented with amino acids is shown to reduce the environmental impacts of feed production, but the magnitude of this impact is low. In contexts in which SBM associated with recent LUC is not used, low-CP strategies do not intend to reduce environmental impacts of feed production and have a low potential of doing so. In those cases, the interest of low-CP

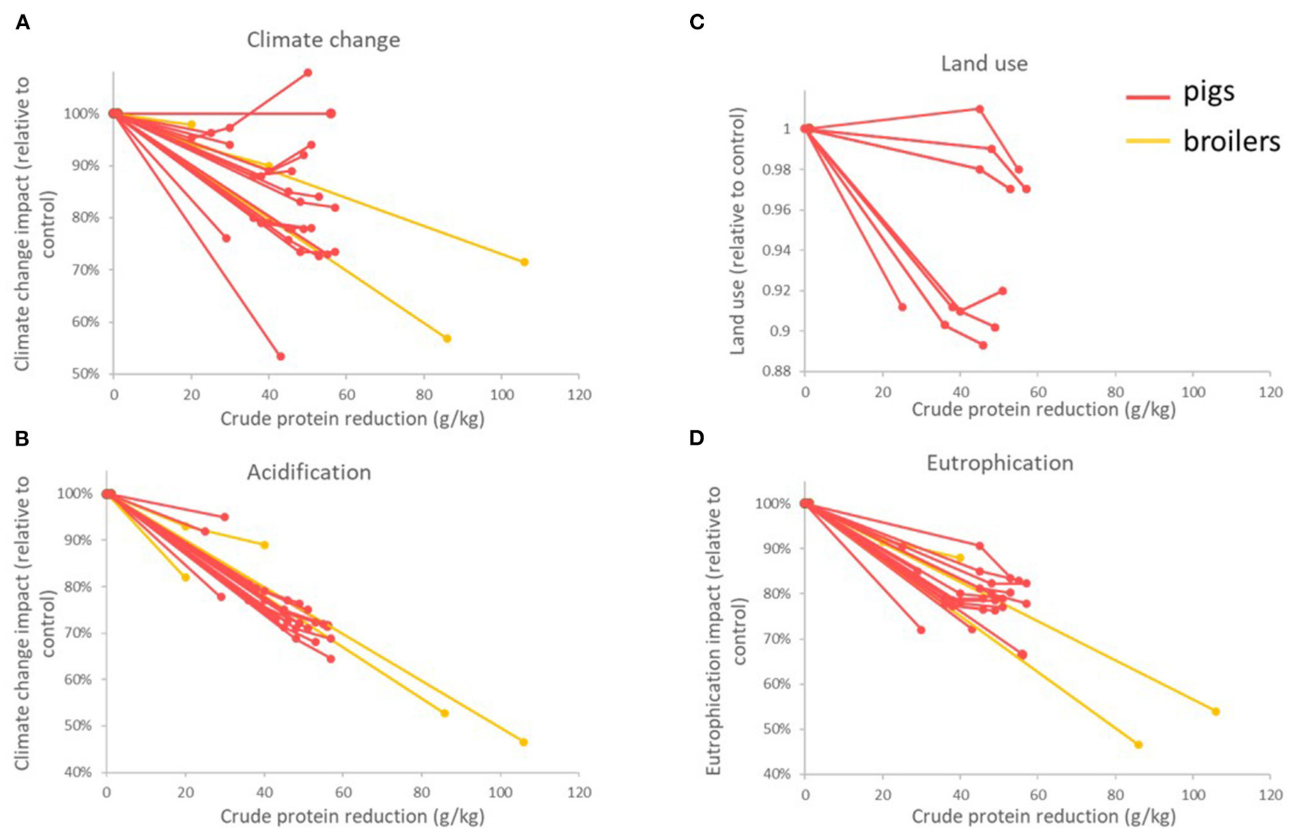

FIGURE 6 | Reduction of environmental impacts with CP reduction in recent pig and broiler LCAs: climate change **(A)**, acidification **(B)**, land use **(C)** and eutrophication **(D)**.

strategies is mostly in the reduction on environmental impacts arising from N excretion.

Thus, it is crucial to aggregate the different effects of the strategy to evaluate its benefits. To fully encompass the effects of low-CP diets on environmental impacts, a farm-scale LCA is the most appropriate tool. It should include the impact of manure spreading to represent accurately the benefits of low-CP diets. However, this is complicated as it requires modifying feed production data to avoid accounting twice for emissions from fertilization. A first step is to, at least, take into account emissions up to manure storage in product LCAs. The LCA method has been increasingly used for pig production since the early 2000s (89). In broiler production, environmental impact has been more recently raised as a concern, and thus, few LCAs are available, even fewer on a specific topic such as low-CP diets supplemented with feed-grade AA (90). The scope and method applied are similar between studies, allowing for comparison. Results are all in accordance to indicate a positive effect of CP reduction on climate change, acidification, and eutrophication impacts.

Perspectives for the Broiler and Pig Sectors

Dietary CP reduction is an effective method to reduce the environmental impact of broiler and pig production. Protein inclusion has already been substantially decreased in pig diets, especially in European production. This process is only starting for broiler production across the world. Current knowledge on animal nutrition and experimental data leaves room to keep reducing dietary CP levels in both species and, doing so, reducing environmental impact. Innovation, such as precision feeding, will allow further decreasing CP levels as individual requirements will be met. Precision feeding can significantly contribute to improving environmental performance of livestock (91, 92). Synergy with the reduction of dietary CP further drives down the environmental impacts of poultry and pigs. Precision feeding also has the added advantage to be able target multiple problematics, such as phosphorus or energy supply, in a holistic approach.

To keep improving the environmental performance of animal production, quantified impacts should be included in the decision-making process at the farm or on the company scale (93) and for policy making (94). In this context, specificity and accuracy of data used to model animal performance and emissions is key to have reliable quantification of the effects of a strategy (95), and further research is encouraged to fill the gaps identified. Interaction with types of manure management and other mitigating measures has to be studied (precision feeding, technological measures, manure treatment, anaerobic digestion for energy production, etc.) as these techniques have to be combined to achieve optimized environmental performance. To be able to compare mitigating techniques, the same scope should be used for LCAs estimating their benefits (89). The whole animal production system from feed production to manure management should be considered to be as inclusive as possible.

Including feed LCA values in feed formulation is shown to significantly decrease environmental impacts of feed compared with the least-cost formulation currently in use (46, 94). It supports further reduction of CP levels and inclusion of feed-grade AA. To increase accuracy of the predicted impact of dietary solutions on environmental impacts, a farm-scale LCA model should be used, but it is harder to implement as we exit the linear programming domain. Inclusion of other indicators should be also considered to encompass societal and environmental demands: economics, animal health and welfare, and antibiotic use (96).

CONCLUSION

Low-CP diets supplemented with feed-grade AA reduce the environmental impact of broiler and pig production, acting on both impact of feed production and emissions from manure. Concerning impact of feed ingredients, the amplitude of the mitigation effect of the strategy depends on the raw material and geographical context. Implementation of harmonized methods is necessary to have reproducible and comparable data. For effects on manure emissions, mechanisms involved and quantification of the effects of CP reduction have been thoroughly studied for pigs. It is not the case for broilers for which only the effect on N excretion is well-quantified. For both species, a higher focus on molecules other than NH_3 is also needed, especially N_2O, for which climate change impact is well-known but for which emissions factors have a lot of uncertainty.

AUTHOR CONTRIBUTIONS

LC, WL, and NM worked on the conception of the review. LC performed the literature review and drafted the article. WL, JL, and NM provided critical review of the paper. LC, JL, NM, and WL approved the final draft of the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, Rosales M, et al. *Livestock's Long Shadow: Environmental Issues and Options*. Rome: Food & Agriculture Org. (2006).
2. De Vries M, de Boer IJ. Comparing environmental impacts for livestock products: a review of life cycle assessments. *Livest Sci.* (2010) 128:1–11. doi: 10.1016/j.livsci.2009.11.007
3. Alexandratos N, Bruinsma J. *World Agriculture Towards 2030/2050: The 2012 Revision*. Rome: Food and Agricultural Organization of the United Nations (2012).
4. Mottet A, Tempio G. Global poultry production: current state and future outlook and challenges. *Worlds Poult Sci J.* (2017) 73:245–56. doi: 10.1017/S0043933917000071
5. Dourmad J-Y, Ryschawy J, Trousson T, Bonneau M, Gonzàlez J, Houwers H, et al. Evaluating environmental impacts of contrasting pig farming systems with life cycle assessment. *Animal.* (2014) 8:2027–37. doi: 10.1017/S1751731114002134
6. Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, et al. *Tackling Climate Change Through Livestock: A Global Assessment of Emissions and Mitigation Opportunities*. Rome: Food and Agriculture Organization of the United Nations (FAO) (2013).
7. Monteiro AR, Dourmad J-Y, Pozza PC. Life cycle assessment as a tool to evaluate the impact of reducing crude protein in pig diets. *Ciência Rural.*, Universidade Federal de Santa Maria (2017) 47. doi: 10.1590/0103-8478cr20161029
8. Cappelaere L, Van Milgen J, Syriopoulos K, Simongiovanni A, Lambert W. *Quantifying Benefits of Reducing Dietary Crude Protein on Nitrogen Emissions of Fattening Pigs: A Meta-Analysis*. Paris; Journées de la Recherche Porcine (2021).
9. Belloir P, Méda B, Lambert W, Corrent E, Juin H, Lessire M, et al. Reducing the CP content in broiler feeds: impact on animal performance, meat quality and nitrogen utilization. *Animal.* (2017) 11:1881–9. doi: 10.1017/S1751731117000660
10. Van Harn J, Dijkslag M, Van Krimpen M. Effect of low protein diets supplemented with free amino acids on growth performance, slaughter yield, litter quality, and footpad lesions of male broilers. *Poult Sci.* (2019) 98:4868–77. doi: 10.3382/ps/pez229
11. IRPP B. *Best Available Techniques (BAT) Reference Document for the Intensive Rearing of Poultry or Pigs*. Luxembourg: Publications Office of the European Union (2017).
12. Valaja J, Alaviuhkola T, Suomi K. Reducing crude protein content with supplementation of synthetic lysine and threonine in barley-rapeseed meal-pea diets for growing pigs. *Agric Food Sci.* (1993) 2:117–23. doi: 10.23986/afsci.72640
13. Le Bellego L, van Milgen J, Dubois S, Noblet J. Energy utilization of low-protein diets in growing pigs. *J Anim Sci.* (2001) 79:1259–71. doi: 10.2527/2001.7951259x
14. Figueroa JL, Miller PS, Fischer RL, Diedrichsen RM, Gomez RS, Lewis AJ. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. *J Anim Sci.* (2002) 80:2911–9. doi: 10.2527/2002.80112911x
15. Quiniou N, Primot Y, Peyronnet C, Quinsac A. Des aliments porcs moins riches en protéines et formulés à base de tourteau de colza et d'acides aminés de synthèse, dont la L-Valine, permettent de réduire le recours au tourteau de soja. *J Rech Porcine.* (2011) 43:135–6.
16. Roux ML, Donsbough AL, Waguespack AM, Powell S, Bidner TD, Payne RL, et al. Maximizing the use of supplemental amino acids in corn-soybean meal diets for 20- to 45-kilogram pigs. *J Anim Sci.* (2011) 89:2415–24. doi: 10.2527/jas.2010-3756
17. Ball MEE, Magowan E, McCracken KJ, Beattie VE, Bradford R, Gordon FJ, et al. The effect of level of crude protein and available lysine on finishing pig performance, nitrogen balance and nutrient digestibility. *Asian-Australas J Anim Sci.* (2013) 26:564–72. doi: 10.5713/ajas.2012.12177
18. Nørgaard JV, Hansen MJ, Soumeh EA, Adamsen APS, Poulsen HD. Effect of protein level on performance, nitrogen utilisation and carcass composition in finisher pigs. *Acta Agric Scand Sec A Anim Sci.* (2014) 64:123–9. doi: 10.1080/09064702.2014.943280

19. Morales A, Araiza BA, Castillo G, Buenabad L, Arce N, Cervantes M, et al. Low-protein amino acid–supplemented diets for growing pigs: Effect on expression of amino acid transporters, serum concentration, performance, and carcass composition1. *J Anim Sci.* (2015) 93:2154–64. doi: 10.2527/jas.2014-8834
20. Wang YM, Yu HT, Zhou JY, Zeng XF, Wang G, Cai S, et al. Effects of feeding growing-finishing pigs with low crude protein diets on growth performance, carcass characteristics, meat quality and nutrient digestibility in different areas of China. *Anim Feed Sci Technol.* (2019) 256:114256. doi: 10.1016/j.anifeedsci.2019.114256
21. Yamazaki M, Inoue H, Matsumoto M, Kaji Y. Effect of feeding a low crude protein diet on growth performance of finishing pigs at a high ambient temperature. *Japan Agricu Res Q JARQ.* (2019) 53:47–50. doi: 10.6090/jarq.53.47
22. Zhao Y, Tian G, Chen D, Zheng P, Yu J, He J, et al. Effect of different dietary protein levels and amino acids supplementation patterns on growth performance, carcass characteristics and nitrogen excretion in growing-finishing pigs. *J Anim Sci Biotechnol.* (2019) 10:75. doi: 10.1186/s40104-019-0381-2
23. Ma W, Mao P, Guo L, Qiao S. Crystalline amino acids supplementation improves the performance and carcass traits in late-finishing gilts fed low-protein diets. *Anim Sci J.* (2020) 91:e13317. doi: 10.1111/asj.13317
24. Chrystal PV, Moss AF, Khoddami A, Naranjo VD, Selle PH, Liu SY. Impacts of reduced-crude protein diets on key parameters in male broiler chickens offered maize-based diets. *Poult Sci.* (2020) 99:505–16. doi: 10.3382/ps/pez573
25. Chrystal PV, Moss AF, Khoddami A, Naranjo VD, Selle PH, Liu SY. Effects of reduced crude protein levels, dietary electrolyte balance, and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein, and amino acid metabolism. *Poult Sci.* (2020) 99:1421–31. doi: 10.1016/j.psj.2019.10.060
26. Ospina-Rojas I, Murakami A, Duarte C, Eyng C, Oliveira C, Janeiro V. Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. *Br Poult Sci.* (2014) 55:766–73. doi: 10.1080/00071668.2014.970125
27. Alfonso-Avila A, Cirot O, Lambert W, Létourneau-Montminy M. Effect of low protein diets on nitrogen utilization efficiency, daily water consumption, and litter moisture in broilers through meta-analysis approach. *EAAP Scientific Series.* Wageningen: Wageningen Academic Publishers (2019). p. 344–50. doi: 10.3920/978-90-8686-891-9_18
28. Wang Y, Zhou J, Wang G, Cai S, Zeng X, Qiao S. Advances in low-protein diets for swine. *J Anim Sci Biotechnol.* (2018) 9:60. doi: 10.1186/s40104-018-0276-7
29. Wang H, Long W, Chadwick D, Velthof GL, Oenema O, Ma W, et al. Can dietary manipulations improve the productivity of pigs with lower environmental and economic cost? A global meta-analysis. *Agricu Ecosystems Environ.* (2020) 289:106748. doi: 10.1016/j.agee.2019.106748
30. Kriseldi R, Tillman PB, Jiang Z, Dozier WA, III. Effects of feeding reduced crude protein diets on growth performance, nitrogen excretion, and plasma uric acid concentration of broiler chicks during the starter period. *Poult Sci.* (2018) 97:1614–26. doi: 10.3382/ps/pex395
31. Corzo A, Fritts C, Kidd M, Kerr B. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. *Anim Feed Sci Technol.* (2005) 118:319–27. doi: 10.1016/j.anifeedsci.2004.11.007
32. Namroud NF, Shivazad M, Zaghari M. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. *Poult Sci.* (2008) 87:2250–8. doi: 10.3382/ps.2007-00499
33. Kamran Z, Sarwar M, Nisa M, Nadeem MA, Mahmood S. Effect of low levels of dietary crude protein with constant metabolizable energy on nitrogen excretion, litter composition and blood parameters of broilers. *Int J Agric Biol.* (2010) 12:401–5.
34. Martínez-Aispuro M, Figueroa-Velasco JL, Zamora-Zamora V, Cordero-Mora JL, Narciso-Gaytán C, Sánchez-Torres MT, et al. Effect of CLA supplementation to low-protein diets on the growth performance, carcass characteristics, plasma urea nitrogen concentration, and fatty acid profile in the meat of pigs. *Braz Arch Biol Technol.* (2014) 57:742–54. doi: 10.1590/S1516-8913201401407
35. Jarret G, Martinez J, Dourmad JY. Effect of biofuel co-products in pig diets on the excretory patterns of N and C and on the subsequent ammonia and methane emissions from pig effluent. *Animal.* (2011) 5:622–31. doi: 10.1017/S1751731110002041
36. Lynch MB, O'Shea CJ, Sweeney T, Callan JJ, O'Doherty JV. Effect of crude protein concentration and sugar-beet pulp on nutrient digestibility, nitrogen excretion, intestinal fermentation and manure ammonia and odour emissions from finisher pigs. *Animal.* (2008) 2:425–34. doi: 10.1017/S1751731107001267
37. O'Shea CJ, Lynch B, Lynch MB, Callan JJ, O'Doherty JV. Ammonia emissions and dry matter of separated pig manure fractions as affected by crude protein concentration and sugar beet pulp inclusion of finishing pig diets. *Agricu Ecosystems Environ.* (2009) 131:154–60. doi: 10.1016/j.agee.2009.01.019
38. Carpenter DA, O'Mara FP, O'Doherty JV. The effect of dietary crude protein concentration on growth performance, carcass composition and nitrogen excretion in entire grower-finisher pigs. *Ir J Agricu Food Res.* (2004) 43:227–36.
39. Portejoie S, Dourmad JY, Martinez J, Lebreton Y. Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs. *Livest Prod Sci.* (2004) 91:45–55. doi: 10.1016/j.livprodsci.2004.06.013
40. Alleman F, Leclercq B. Effect of dietary protein and environmental temperature on growth performance and water consumption of male broiler chickens. *Br Poult Sci.* (1997) 38:607–10. doi: 10.1080/00071669708418044
41. Hernández F, López M, Martinez S, Megias M, Catala P, Madrid J. Effect of low-protein diets and single sex on production performance, plasma metabolites, digestibility, and nitrogen excretion in 1-to 48-day-old broilers. *Poult Sci.* (2012) 91:683–92. doi: 10.3382/ps.2011-01735
42. Pfeiffer A, Henkel H, Verstegen M, Philipczyk I. The influence of protein intake on water balance, flow rate and apparent digestibilty of nutrients at the distal ileum in growing pigs. *Livest Prod Sci.* (1995) 44:179–87. doi: 10.1016/0301-6226(95)00070-4
43. Collett SR. Nutrition and wet litter problems in poultry. *Anim Feed Sci Technol.* (2012) 173:65–75. doi: 10.1016/j.anifeedsci.2011.12.013
44. Mushtaq M, Pasha T, Mushtaq T, Parvin R. Electrolytes, dietary electrolyte balance and salts in broilers: an updated review on growth performance, water intake and litter quality. *Worlds Poult Sci J.* (2013) 69:789–802. doi: 10.1017/S0043933913000810
45. Shaw MI, Beaulieu AD, Patience JF. Effect of diet composition on water consumption in growing pigs1. *J Anim Sci.* (2006) 84:3123–32. doi: 10.2527/jas.2005-690
46. Garcia-Launay F, Dusart L, Espagnol S, Laisse-Redoux S, Gaudré D, Méda B, et al. Multiobjective formulation is an effective method to reduce environmental impacts of livestock feeds. *Br J Nutr.* (2018) 120:1298–309. doi: 10.1017/S0007114518002672
47. Dourmad J-Y, Ryschawy J, Trousson T, Bonneau M, Gonzalez J, Houwers W, et al. Évaluation par analyse de cycle de vie de la durabilité environnementale de systèmes contrastés de production porcine en Europe. *45èmes journées de la recherche porcine.* Paris, FRA: 45 Journées de la Recherche Porcine (2013) p. 109–14.
48. Kebreab E, Liedke A, Caro D, Deimling S, Binder M, Finkbeiner M. Environmental impact of using specialty feed ingredients in swine and poultry production: a life cycle assessment. *J Anim Sci.* (2016) 94:2664–81. doi: 10.2527/jas.2015-9036
49. IPCC. *Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories.* Geneva: The Intergovernmental Panel on Climate Change (2019).
50. Cherubini E, Franco D, Zanghelini GM, Soares SR. Uncertainty in LCA case study due to allocation approaches and life cycle impact assessment methods. *Int J Life Cycle Assess.* (2018) 23:2055–70. doi: 10.1007/s11367-017-1432-6
51. European Comission JRC. *Analysing of existing Environmental Impact Assessment Methodologies for Use in Life Cycle Assessment.* Luxembourg: Joint Research Centre Institute for Environment and Sustainability, ILCD handbook (2010).
52. Méda B, Belloir P, Lessire M, Juin H, Corrent E, Lambert W, et al. Environmental implications of decreasing dietary crude protein content in finishing broilers. *12e Journées de la Recherche Avicole et Palmipèdes à Foie Gras (JRA-JRPFG 2017), 5 & 6 avril 2017.* Tours, France. (2017). p. 356–60.
53. Cherubini E, Zanghelini GM, Tavares JMR, Belettini F, Soares SR. The finishing stage in swine production: influences of feed composition on carbon footprint. *Environ Dev Sustain.* (2015) 17:1313–28. doi: 10.1007/s10668-014-9607-9

54. Meul M, Ginneberge C, Van Middelaar CE, de Boer IJ, Fremaut D, Haesaert G. Carbon footprint of five pig diets using three land use change accounting methods. *Livest Sci.* (2012) 149:215–23. doi: 10.1016/j.livsci.2012.07.012
55. Mosnier E, Van der Werf H, Boissy J, Dourmad J-Y. Evaluation of the environmental implications of the incorporation of feed-use amino acids in the manufacturing of pig and broiler feeds using Life Cycle Assessment. *Animal.* (2011) 5:1972–83. doi: 10.1017/S1751731111001078
56. Castanheira ÉG, Freire F. Greenhouse gas assessment of soybean production: implications of land use change and different cultivation systems. *J Clean Prod.* (2013) 54:49–60. doi: 10.1016/j.jclepro.2013.05.026
57. Da Silva VP, van der Werf HM, Spies A, Soares SR. Variability in environmental impacts of Brazilian soybean according to crop production and transport scenarios. *J Environ Manage.* (2010) 91:1831–9. doi: 10.1016/j.jenvman.2010.04.001
58. Marinussen M, Kool A. *Environmental Impacts of Synthetic Amino Acid Production*. Netherlands: Blonk Milieu Advies BV. (2010).
59. Ogino A, Osada T, Takada R, Takagi T, Tsujimoto S, Tonoue T, et al. Life cycle assessment of Japanese pig farming using low-protein diet supplemented with amino acids. *Soil Sci Plant Nutr.* (2013) 59:107–18. doi: 10.1080/00380768.2012.730476
60. Monteiro ANTR, Garcia-Launay F, Brossard L, Wilfart A, Dourmad J-Y. Effect of feeding strategy on environmental impacts of pig fattening in different contexts of production: evaluation through life cycle assessment1. *J Anim Sci.* (2016) 94:4832–47. doi: 10.2527/jas.2016-0529
61. Le Cour Grandmaison J, Martin N, Lambert W, Corrent E. Benefits of low dietary crude protein strategies on feed global warming potential in broiler and pig production systems: a case study. *12th International Conference on Life Cycle Assessment of Food*. Berlin, Germany (2020).
62. Le Cour Grandmaison J, Bertrand M, Nicolas M, Lambert W, Corrent E, editors. Benefits of low crude protein diets in broilers: beyond CO2, the importance of N and NH3 assessment. *EAAP Annual Meeting*. Virtual Conference. (2020).
63. BSI. *PAS 2050:2011 Specification for the Assessment of the Life Cycle Greenhouse Gas Emissions of Goods and Services*. (2012). London: British Standards Institution.
64. Monteiro A, Bertol TM, de Oliveira PAV, Dourmad JY, Coldebella A, Kessler AM. The impact of feeding growing-finishing pigs with reduced dietary protein levels on performance, carcass traits, meat quality and environmental impacts. *Livest Sci.* (2017) 198:162–9. doi: 10.1016/j.livsci.2017.02.014
65. Garcia-Launay F, van der Werf HMG, Nguyen TTH, Le Tutour L, Dourmad JY. Evaluation of the environmental implications of the incorporation of feed-use amino acids in pig production using Life Cycle Assessment. *Livest Sci.* (2014) 161:158–75. doi: 10.1016/j.livsci.2013.11.027
66. Wang Y, Xue W, Zhu Z, Yang J, Li X, Tian Z, et al. Mitigating ammonia emissions from typical broiler and layer manure management – a system analysis. *Waste Manage.* (2019) 93:23–33. doi: 10.1016/j.wasman.2019.05.019
67. Hou Y, Velthof GL, Oenema O. Mitigation of ammonia, nitrous oxide and methane emissions from manure management chains: a meta-analysis and integrated assessment. *Glob Change Biol.* (2015) 21:1293–312. doi: 10.1111/gcb.12767
68. Hernández F, Rivas MDM, Femenia JO, López M, Madrid J. Effect of dietary protein level on retention of nutrients, growth performance, litter composition and NH3 emission using a multi-phase feeding programme in broilers. *Span J Agricu Res.* (2013) 11:736–46. doi: 10.5424/sjar/2013113-3597
69. Sajeev EPM, Amon B, Ammon C, Zollitsch W, Winiwarter W. Evaluating the potential of dietary crude protein manipulation in reducing ammonia emissions from cattle and pig manure: a meta-analysis. *Nutr Cycling in Agroecosys.* (2018) 110:161–75. doi: 10.1007/s10705-017-9893-3
70. van Emous RA, Winkel A, Aarnink AJA. Effects of dietary crude protein levels on ammonia emission, litter and manure composition, N losses, and water intake in broiler breeders. *Poult Sci.* (2019) 98:6618–25. doi: 10.3382/ps/pez508
71. Hansen MJ, Nørgaard JV, Adamsen APS, Poulsen HD. Effect of reduced crude protein on ammonia, methane, and chemical odorants emitted from pig houses. *Livest Sci.* (2014) 169:118–24. doi: 10.1016/j.livsci.2014.08.017
72. Le PD, Aarnink AJA, Jongbloed AW, van der Peet-Schwering CMC, Ogink NWM, Verstegen MWA. Interactive effects of dietary crude protein and fermentable carbohydrate levels on odour from pig manure. *Livest Sci.* (2007) 114:48–61. doi: 10.1016/j.livsci.2007.04.009
73. Seradj AR, Balcells J, Morazan H, Alvarez-Rodriguez J, Babot D, De la Fuente G. The impact of reducing dietary crude protein and increasing total dietary fiber on hindgut fermentation, the methanogen community and gas emission in growing pigs. *Anim Feed Sci Technol.* (2018) 245:54–66. doi: 10.1016/j.anifeedsci.2018.09.005
74. Rigolot C, Espagnol S, Pomar C, Dourmad J-Y. Modelling of manure production by pigs and NH 3, N 2 O and CH 4 emissions. Part I: animal excretion and enteric CH 4, effect of feeding and performance. *Animal.* (2010) 4:1401–12. doi: 10.1017/S1751731110000492
75. Pierce KM, Callan JJ, McCarthy P, O'Doherty JV. The interaction between lactose level and crude protein concentration on piglet post-weaning performance, nitrogen metabolism, selected faecal microbial populations and faecal volatile fatty acid concentrations. *Anim Feed Sci Technol.* (2007) 132:267–82. doi: 10.1016/j.anifeedsci.2006.02.010
76. Jarret G, Cerisuelo A, Peu P, Martinez J, Dourmad J-Y. Impact of pig diets with different fibre contents on the composition of excreta and their gaseous emissions and anaerobic digestion. *Agricu Ecosystems Environ.* (2012) 160:51–8. doi: 10.1016/j.agee.2011.05.029
77. Rigolot C, Espagnol S, Robin P, Hassouna M, Béline F, Paillat J-M, et al. Modelling of manure production by pigs and NH 3, N 2 O and CH 4 emissions. Part II: effect of animal housing, manure storage and treatment practices. *Animal.* (2010) 4:1413–24. doi: 10.1017/S1751731110000509
78. Meda B, Hassouna M, Aubert C, Robin P, Dourmad J-Y. Influence of rearing conditions and manure management practices on ammonia and greenhouse gas emissions from poultry houses. *Worlds Poult Sci J.* (2011) 67:441–56. doi: 10.1017/S0043933911000493
79. Osada T, Takada R, Shinzato I. Potential reduction of greenhouse gas emission from swine manure by using a low-protein diet supplemented with synthetic amino acids. *Anim Feed Sci Technol.* (2011) 166–167:562–74. doi: 10.1016/j.anifeedsci.2011.04.079
80. Le PD, Aarnink AJA, Jongbloed AW. Odour and ammonia emission from pig manure as affected by dietary crude protein level. *Livest Sci.* (2009) 121:267–74. doi: 10.1016/j.livsci.2008.06.021
81. Wang Y, Dong H, Zhu Z, Gerber PJ, Xin H, Smith P, et al. Mitigating greenhouse gas and ammonia emissions from swine manure management: a system analysis. *Environ Sci Technol.* (2017) 51:4503–11. doi: 10.1021/acs.est.6b06430
82. EMEP/EEA. *Air Pollutant Emission Inventory Guidebook (3. B Manure Management)*. (2019). Luxembourg: Publications Office of the European Union.
83. Rees B, Sylvester-Bradley R, Olesen J, Vallejo A. *Field Application of Organic and Inorganic Fertilizers and Manure*. Luxembourg: Publications office of the European Union (2019)
84. I. *Environmental Management: Life Cycle Assessment; Principles and Framework*. Geneva: International Organization for Standardization (2006).
85. I. *Environmental Management: Life Cycle Assessment; Requirements and Guidelines*. Geneva: International Organization for Standardization (2006).
86. van der Werf HMG, Petit J, Sanders J. The environmental impacts of the production of concentrated feed: the case of pig feed in Bretagne. *Agricu Syst.* (2005) 83:153–77. doi: 10.1016/j.agsy.2004.03.005
87. Eriksson IS, Elmquist H, Stern S, Nybrant T. Environmental systems analysis of pig production-the impact of feed choice (12 pp). *Int J Life Cycle Assess.* (2005) 10:143–54. doi: 10.1065/lca2004.06.160
88. Nguyen TLT, Hermansen JE, Mogensen L. Fossil energy and GHG saving potentials of pig farming in the EU. *Energy Policy.* (2010) 38:2561–71. doi: 10.1016/j.enpol.2009.12.051
89. McAuliffe GA, Chapman DV, Sage CL. A thematic review of life cycle assessment (LCA) applied to pig production. *Environ Impact Assess Rev.* (2016) 56:12–22. doi: 10.1016/j.eiar.2015.08.008
90. Méda B, Belloir P, Narcy A, Wilfart A. Improving environmental sustainability of poultry production using innovative feeding strategies. *Proc 22nd Eur Sympo Poult Nutr.* Gdansk, Poland. (2019). p. 82–92.
91. Garcia-Launay F, Delage C, Quiniou N, Brossard L, Marcon M, Espagnol S, editors. Analyse environnementale de stratégies d'alimentation de précision

des porcs charcutiers. *52 Journées de la Recherche Porcine*. Paris: IFIP-Institut du Porc (2020).

92. Andretta I, Pomar C, Rivest J, Pomar J, Radünz J. Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. *Animal.* (2016) 10:1137–47. doi: 10.1017/S1751731115003067
93. Meul M, Van Middelaar CE, de Boer IJ, Van Passel S, Fremaut D, Haesaert G. Potential of life cycle assessment to support environmental decision making at commercial dairy farms. *Agricu Syst.* (2014) 131:105–15. doi: 10.1016/j.agsy.2014.08.006
94. de Boer IJ, Cederberg C, Eady S, Gollnow S, Kristensen T, Macleod M, et al. Greenhouse gas mitigation in animal production: towards an integrated life cycle sustainability assessment. *Curr Opin Environ Sustain.* (2011) 3:423–31. doi: 10.1016/j.cosust.2011.08.007
95. Monteiro ANTR, Dourmad J-Y, Moreira CA, Rossi RM, Ferreira LFM, Costa AER, et al. The source of performance and excretion data affects the environmental impact of pig rearing estimated by life cycle assessment. *Canad J Anim Sci*. (2020) 100:184–92. doi: 10.1139/cjas-2019-0022
96. Tallentire CW, Edwards SA, Van Limbergen T, Kyriazakis I. The challenge of incorporating animal welfare in a social life cycle assessment model of European chicken production. *Int J Life Cycle Assess.* (2019) 24:1093–104. doi: 10.1007/s11367-018-1565-2

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A Novel Estimation of Unobserved Pig Growth Traits for the Purposes of Precision Feeding Methods

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Recent technological advances make it possible to deliver feeding strategies that can be tailored to the needs of individual pigs in order to optimise the allocation of nutrient resources and contribute toward reducing excess nutrient excretion. However, these efforts are currently hampered by the challenges associated with: (1) estimation of unobserved traits from the available data on bodyweight and feed consumption; and (2) characterisation of the distributions and correlations of these unobserved traits to generate accurate estimates of individual level variation among pigs. Here, alternative quantitative approaches to these challenges, based on the principles of inverse modelling and separately inferring individual level distributions within a Bayesian context were developed and incorporated in a proposed precision feeding modelling framework. The objectives were to: (i) determine the average and distribution of individual traits characterising growth potential and body composition in an empirical population of growing-finishing barrows and gilts; (ii) simulate the growth and excretion of nitrogen and phosphorus of the average pig offered either a commercial two-phase feeding plan, or a precision feeding plan with daily adjustments; and (iii) simulate the growth and excretion of nitrogen and phosphorus across the pig population under two scenarios: a two-phase feeding plan formulated to meet the nutrient requirements of the average pig or a precision feeding plan with daily adjustments for each and every animal in the population. The distributions of mature bodyweight and ratio of lipid to protein weights at maturity had median (IQR) values of 203 (47.8) kg and 2.23 (0.814) kg/kg, respectively; these estimates were obtained without any prior assumptions concerning correlations between the traits. Overall, it was found that a proposed precision feeding strategy could result in considerable reductions in excretion of nitrogen and phosphorus (average pig: 8.07 and 9.17% reduction, respectively; heterogenous pig population: 22.5 and 22.9% reduction, respectively) during the growing-finishing period from 35 to 120 kg bodyweight. This precision feeding modelling framework is anticipated to be a starting point toward more accurate estimation of individual level nutrient requirements, with the general aim of improving the economic and environmental sustainability of future pig production systems.

Keywords: Bayesian inference, body composifion, individual traits, nitrogen excretion, phosphorus excretion, precision feeding, pigs

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INTRODUCTION

To address economic and environmental concerns about standard feeding practises in commercial pig production (1–3), precision feeding strategies have been suggested as a way forward (4–6). Precision feeding strategies aim to accurately match nutrient supply to the demand of animals by formulating feeds that account for the dynamic changes in nutrient requirements, preferably at the individual level (7, 8). This is in contrast to standard feeding practises, which typically neglect variation in nutrient requirements among individuals, as they involve formulating feeds that satisfy the estimated nutrient requirements of a nominal average pig in a population, at a given static reference point specified by bodyweight (BW) or age (9). Initial evaluations of precision feeding strategies against standard population level feeding regimes in growing-finishing pigs have been encouraging based on reports of considerable reduction in excretion of nitrogen (N) and phosphorus (P), without any apparent loss in growth performance (10–12).

A successful implementation of precision feeding requires the development of methods for estimating the nutrient requirements of individual pigs, which in turn requires estimating their growth potential and body composition. There are notable issues associated with this challenge, which concern: (1) estimation of unobserved traits from data; and (2) characterisation of the distributions and correlations of these unobserved traits. Regarding the first issue, while body composition is a major determinant of nutrient requirements, real-time data on e.g., protein or lipid retention are either rare or unavailable due to technological and logistical limitations (13–15), and consist of tissue scan proxies with limited correlation to body amounts (16). Consequently, these traits are often estimated from data on BW and feed consumption by making assumptions whose validity could be restrictive. This limits the ability to formulate optimal feeding rations. For example, a typical approach to obtain information on lean tissue growth and requirements for precision feeding of growing pigs assumes an isometric relationship relating protein retention and BW gain, and that the isometric parameters are the same across pigs (5). However, this approach neglects individual variation in protein growth among animals as well as the non-proportionality between these variables during growth (17, 18). Alternative models, including a polynomial regression relating body protein weight to BW have been recently developed (19), but their validity is still largely unascertained.

Regarding the issue of the distributions and correlations of the unobserved traits of individuals, a typical approach relies on an explicit specification of their multivariate distribution (20–26). Within this setting, it is necessary to either assume or estimate multiple mean and variance-covariance parameters from data, which carries uncertainty (27) and can be challenging in practise (9). To avoid these challenges, a potential alternative approach to model trait variation, based on separately inferring individual level distributions within a Bayesian framework, has been recently suggested by Filipe and Kyriazakis (27). This framework is yet to be comprehensively tested in the context of the estimation of traits that are typically required for precision feeding purposes.

The aims of this chapter were to develop alternative data-driven approaches to estimate uncertain traits in individual pigs and incorporate this information in a proposed precision feeding modelling framework. This modelling framework was applied to evaluate feeding strategies in their effectiveness to minimise excess excretion of N and P when compared to standard feeding practises. These evaluations were conducted by considering the average of the individual responses in a population and the response of the average pig in the population, to gain a better insight into possible impacts of phenotypic heterogeneity on nutrient excretion. The specific objectives were to: (1) determine the empirical average and distribution of individual traits characterising growth potential and body composition in a pig population; (2) simulate the growth and excretion of N and P of the average pig offered either a commercial two-phase feeding plan, or a precision feeding plan with daily adjustments; and (3) simulate the growth and excretion of N and P across the pig population under two scenarios: a two-phase feeding plan formulated to meet the nutrient requirements of the average pig or a precision feeding plan with daily adjustments for each and every animal in the population.

MATERIALS AND METHODS

There was no requirement for ethical approval, since the data used originated from a previous experiment, which was granted ethical approval on behalf of the original trial investigators.

Data

Empirical sequential data on individual daily feed intake DFI_t (kg/d) and BW_t (kg), at ages t (d), of 32 barrows and gilts [(Large White × Landrace) × Pietrain] were obtained from a trial conducted by the INRAE at the UE3P unit (Pig Physiology and Phenotyping Experimental Facility, https://doi.org/10.15454/1.5573932732039927E12), Saint Gilles, France. Pigs were kept in near-commercial conditions (*ad-libitum* access to water and feeds, group housing, ambient room temperature of 20–24°C) for a period of 81 d from an initial mean BW of 35.2 (SD: 4.70) kg until a final mean BW of 118 (SD: 8.87) kg. The pigs were given access to two feeds in succession formulated to meet or exceed the expected population level average nutritional requirements. The change in feeds occurred when animals reached ∼65.0 kg.

Approach to Estimate Individual Level Variation in Growth Potential and Body Composition

Model Description

The Gompertz growth model (28), comprehensively reviewed by Filipe et al. (29), was used to describe the evolution of BW_t of each individual pig over time:

$$BW_t = BW_m \times \exp\left(-\ln\left(\frac{BW_m}{BW_{in}}\right) \times \exp\left(-\frac{t-t_0}{B}\right)\right) \text{(kg)} \quad (1.1)$$

where t and t_0 were the current and initial times (d), BW_{in} (kg) was the observed initial bodyweight at the start of the data collection period, and BW_m (kg) and B (d) were unknown parameters (traits) estimated for each pig. The unknown model traits correspond to the weight at maturity and the inverse of the growth rate controlling how fast the weight at maturity is reached.

After accounting for gut fill to derive the empty BW, eBW_t (30), this eBW_t was expressed as a sum of the four main body chemical components (31): protein [$N^* = 6.25 \times \times$ N, where N is nitrogen (kg)], lipid (L) (kg), water (W) (kg) and ash (Ash) (kg):

$$eBW_t = \alpha \times BW_t = N^*_t + L_t + W_t + Ash_t(\text{kg}) \quad (1.2)$$

where α was assumed to be a constant proportion over the growth period under consideration, equal to 95% of BW_t (32, 33) and to be the same across animals.

The growth of these four body chemical components was represented by the following allometric relationships (29, 34–36):

$$N^*_t = N^*_m \times \left(\frac{BW_t}{BW_m}\right)^{\frac{\log\left(N^*_m/N^*_{in}\right)}{\log(BW_m/BW_{in})}} (\text{kg}) \quad (1.3)$$

$$L_t = L_m \times \left(\frac{BW_t}{BW_m}\right)^{\frac{\log(L_m/L_{in})}{\log(BW_m/BW_{in})}} (\text{kg}) \quad (1.4)$$

$$W_t = 3.04 \times \left(\frac{N^*_t}{N^*_m}\right)^{0.855} (\text{kg}) \quad (1.5)$$

$$Ash_t = 0.190 \times \left(\frac{N^*_t}{N^*_m}\right) (\text{kg}) \quad (1.6)$$

where N^*_m and L_m are mature weights, and N^*_{in} and L_{in} are initial weights of protein and lipid, respectively; these traits were unknown in advance and had to be estimated from individual data from each pig in the population.

Fitting to the Data

To estimate the traits characterising each individual pig in the population, Equations (1.1–1.6) describing the dynamic evolution of BW_t, N^*_t, L_t, W_t, and Ash_t were fitted to the data of each individual pig one at a time.

To account for the uncertainty and correlations between individual trait estimates, a Bayesian inference approach was utilised, which outputs estimated distributions rather than point estimates of the traits (37). Samples of trait estimates were obtained using the Markov Chain Monte Carlo (MCMC) methods (38) and more specifically, the Metropolis-Hastings algorithm (39). The posterior inferences on the traits were based on samples generated using the MCMC engine *rjags* (40). Prior distributions for the traits are given in the **Supplementary Material**, together with a justification for their choice. Four independent MCMC chains, each containing 100,000 samples and initialised with different random starting parameter values, were generated, from which the first ten percent samples were discarded as burn-in (41, 42). The posterior inferences were carried out on the remaining 90,000 samples from each chain; no thinning was applied (43). Four MCMC chains, rather than one, were used as a way of assessing differences among the sampled trait distributions and thus, was a first convergence diagnostic (44). The convergence of each sample chain was also assessed by investigating trace plots (after burn-in) for each trait and by calculating the potential scale reduction factor, $\hat{R}$ (45, 46). Values of $\hat{R}. > 1.01$ were considered to indicate poor convergence (47). The posterior distribution of sampled traits used for inference comprised every chain that converged; for example, when the four chains converged, it comprised $N_s = 4 \times 90{,}000 = 360{,}000$ sampled trait values.

Data-Based Estimation of the Average Pig in the Population

The average pig in the population was estimated by minimising the following metric across the pigs in the population:

$$D_i = \sum_{j=1}^{4} \left| \frac{\hat{Y}_{ij} - \bar{Y}_j}{\bar{Y}_j} \right| \quad (1.7)$$

Where $\hat{Y}_{ij}$ are obtained estimates of the traits BW_m, B, N^*_m and L_m for pig i ($i = 1, \ldots, 32$) in the population, and $\hat{Y}_j$ are the median values of these trait estimates calculated across the population. The pig whose set of estimates $\hat{Y}_j$ had the lowest value of D was chosen to characterise the average pig as its traits were regarded as central in the population. This specific approach to multidimensional estimation of the average pig was chosen because it preserves the individual level correlations between traits which were estimated jointly for each animal in the population (27).

Estimation of Nutrient Requirements

Daily requirements for N^*, P and energy of the estimated average pig and of each pig in the pig population (whose individual traits were estimated) were expressed as a sum of requirements for maintenance and growth using the equations in **Table 1**; inputs to these equations were the data-driven trait estimates that are the parameters of Equations (1.1–1.6).

Maintenance requirements for N^*, P and energy at t were related to the estimated N^*_t and N^*_m, rather than BW_t and BW_m, to account for any potential variation in these requirements due to differences in body composition among animals (30). It was assumed that there were no inefficiencies in utilising these nutrients for maintenance purposes (48, 49).

Growth requirements for N^*, P and energy at t were related to the maximum daily retention of N^* (kg/d) and P (kg/d) and to the desired (normal) retention of L (kg/d), which were estimated as:

$$N^{*'}_{max}(t) = \frac{1}{B} \times N^*_t \times \log\left(\frac{N^*_m}{N^*_t}\right) \quad (1.8)$$

$$P'_{max}(t) = 0.0337 \times \frac{1}{B} \times N^*_t \times \log\left(\frac{N^*_m}{N^*_t}\right) \quad (1.9)$$

TABLE 1 | Equations to estimate individual daily requirements for maintenance and growth in terms of effective energy (*E*), digestible protein (*N**) and digestible phosphorus (P).

Quantity	Abbreviation	Equation	Unit	Efficiency value	Source
Energy	$E_{maint}(t)$	$\frac{1}{e_{E_m}} \times \left(1.63 \times \frac{N^*(t)}{N_m^{*\,0.27}}\right)$	(MJ/d)	$e_{E_m} = 1.00$	(31)
Protein	$N^{*'}_{maint}(t)$	$\frac{1}{e_{N^{*'}m}} \times \left(0.004 \times \frac{N^*(t)}{N_m^{*\,0.27}}\right)$	(kg/d)	$e_{N^{*'}m} = 1.00$	(30)
Phosphorus	$P'_{maint}(t)$	$\frac{1}{e_{P'_m}} \times \left(0.0001293 \times \frac{N^*(t)}{N_m^{*\,0.27}}\right)$	(kg/d)	$e_{P'_m} = 1.00$	(30)
Energy	$E_{growth}(t)$	$e_{E_{g_{N^{*'}}}} \times N^{*'}_{max}(t) + e_{E_{g_{L'}}} \times L'_{max}(t)$	(g/kg)	$e_{E_{g_{N^{*'}}}} = 50.0; e_{E_{g_{L'}}} = 56.0$	(30)
Protein	$N^{*'}_{growth_{max}}(t)$	$\frac{1}{e_{N^{*'}_g}} \times N^{*'}_{max}(t)$	(kg/d)	$e_{N^{*'}_g} = 0.763$	(48)
Phosphorus	$P'_{growth_{max}}(t)$	$\frac{1}{e_{P'_g}} \times P'_{max}(t)$	(kg/d)	$e_{P'_g} = 0.940$	(49)

$N^{*'}_{max}(t) = \frac{1}{B} \times N^*_t \times log\left(\frac{N^*_m}{N^*_t}\right)$ is the daily maximum retention of N^* at time t; $P'_{max}(t) = 0.0337 \times \frac{1}{B} \times N^*_t \times log\left(\frac{N^*_m}{N^*_t}\right)$ is the daily maximum retention of P at time t; $L'_{max}(t) = \frac{1}{B} \times L_t \times log\left(\frac{L_m}{L_t}\right)$. is the daily desired (normal) retention of lipid at time t.

$$L'_{max}(t) = \frac{1}{B} \times L_t \times \log\left(\frac{L_m}{L_t}\right) \quad (1.10)$$

To calculate growth requirements, equations (1.8–1.10) were multiplied by coefficients that account for the metabolic inefficiencies in the utilisation of nutrients for retention processes (50–52) and thus, to derive requirements expressed on digestible N^* (30) (kg/d), digestible P (kg/d) (49) and effective energy basis (MJ/d), which is the difference between digestible energy and losses associated with feed consumption (53).

Simulated Feeding Scenarios

Four feeding scenarios were considered to quantify the effects on N and P excretion of the within- and between- animal variation in growth potential and body composition. The first two scenarios were designed to predict differences in growth performance, and N and P excretion of the average pig offered either a "static" feeding strategy that targeted its nutrient requirements at pre-specified reference points, or a precision feeding strategy that adapted to the dynamic evolution of the performance of this animal. These two scenarios are equivalent to investigating responses of the homogeneous pig population. The remaining two scenarios were designed to quantify differences in growth performance, and in N and P excretion across the heterogenous pig population offered either a "static" feeding strategy that targeted nutrient requirements of the average animal or a precision feeding strategy that adapted to real-time performance of each individual pig within the population.

Scenario 1: Two-Phase Feeding Strategy for the Average Pig

The first scenario (S1) simulated the growth of the average pig from 35.0 to 120 kg when given *ad-libitum* access to two feeds (Feed 1 and Feed 2), offered in succession with a switch from Feed 1 to Feed 2 at ∼65.0 kg. The nutritional composition of Feed 1 and Feed 2, in terms of crude N^* (g/kg), digestible N^* (g/kg), total P (g/kg), digestible P (g/kg), and effective energy (MJ/kg) were inputs into the growth model. The following contents were calculated by dividing the estimated nutrient requirements for maintenance and growth of the average pig at reference points t_i by the median DFI_t at the same point from the collected data across the thirty-two pigs (section Data):

$$X_{t_i} = \frac{X_{maint}(t_i) + X_{growth}(t_i)}{DFI_{t_i}} \quad (1.11)$$

where X = [digestible N^*, digestible P, effective energy] and t_i = (1,2) are the reference points where BW_{t_1} = 50.0 kg (Feed 1) or BW_{t_2} = 92.5 kg (Feed 2), which are based on Symeou et al. (54). Crude N^* contents were calculated according to Wellock et al. (55) by dividing digestible N^* contents in each feed by the product of the digestibility coefficient, 0.800, and the biological value [a common measure of N^* quality in the feed (56)], 0.750, reflective of typical commercial feeds (57). Total P contents were calculated by dividing digestible P contents in each feed by the digestibility coefficient, equal to 0.500 (58) to derive total P values consistent with the typical commercial feeds (26). As nutrient requirements for the average pig were conditional on the estimates under section Estimation of nutrient requirements, estimated nutritional composition of Feed 1 and Feed 2 is given in section Estimation of nutrient requirements and feed composition.

Scenario 2: Precision Feeding Strategy for the Average Pig

The second scenario (S2) simulated the growth of the average pig given *ad-libitum* access to feeds adjusted daily for a time period (d) equal to the length of S1. Daily adjustments to the nutritional composition of the feeds, in terms of digestible N^*, digestible P and effective energy were calculated as the ratio of the estimated daily nutrient requirements for maintenance and growth of the average pig to the estimated target DFI_t of this pig. The target DFI_t was estimated using:

$$DFI_t = \theta_1(CG_t^{\theta_2} - CG_{t-1}^{\theta_2}), \quad (1.12)$$

where CG is the cumulative gain, θ_1 and θ_2 are parameters estimated from the animal's past BW and feed consumption. Crude N^* feed contents were calculated according to Wellock et al. (55) by dividing digestible N^* contents in each feed by the product of the digestibility coefficient, equal to 0.800 and the biological value, equal to 0.750. Total P contents in Feed 1 and Feed 2 were calculated by dividing digestible P contents in each feed by the digestibility coefficient, equal to 0.500 to derive total P values. It is assumed that the usual practise of blending high-nutrient and low-nutrient basal feeds would not alter nutrient composition of these feeds. However, blend feeding was not explicitly considered in this study.

Scenario 3: Two-Phase Feeding Strategy for the Heterogenous Pig Population

The third scenario (S3) extended S1 to the heterogenous pig population by simulating the growth of each pig in the population for a time period from the population average BW of 35.0–120 kg. Each pig in the population was given *ad-libitum* access to Feed 1 and Feed 2, with a change in feeds when the population average BW reached 65.0 kg.

Scenario 4: Precision Feeding Strategy for the Heterogenous Pig Population

The fourth scenario (S4) extended S2 in the context of the heterogenous pig population. This scenario simulated the growth of each pig in the population offered *ad-libitum* access to the individualised precision feeding plan, adjusted daily to adapt to real-time performance of each pig, for a time period equal to the length of S3. For each pig, daily adjustments to the nutritional composition of the feeds were calculated using the approach described in section Scenario 2: precision feeding strategy for the average pig but accounting for the individualised daily nutrient requirements for maintenance and growth, and target DFI_t.

Estimation of Growth and Nutrient Excretion

In S1 and S3, which described the commercial two-phase feeding strategy, Feed 1 and Feed 2 were assumed to result in periods of nutrient under-supplementation or over-supplementation for a number of pigs (54). When undersupplied with nutrients, pigs were assumed to consume excess amounts of feeds when either N^*or energy was the most deficient (20, 59), as an attempt to eat for the first limiting feed resource in the feed, but not when P was the most deficient (49, 60). In the cases of P deficiencies, feed intake was assumed to be controlled only by the energy needed to support the potential growth. In S2 and S4, which described the precision feeding strategy, the individualised feeds were assumed to provide the precise quantities of nutrients to support maintenance and growth requirements of each pig.

Daily feed consumption, DFI_t was predicted using the following equation:

$$DFI_t = \begin{cases} \frac{E_{maint}(t)+E_{growth}(t)}{E_{feed}}, & \text{energy or P limiting} \\ \frac{N^{*'}_{maint}(t)+N^{*'}_{growth_{max}}(t)}{N^*_{feed}}, & \text{protein limiting} \end{cases} \quad (1.13)$$

where terms in the numerator of this equation are given in **Table 1**, and E_{feed} and N^*_{feed} are effective energy feed content (MJ/kg) and digestible N^* (g/kg), respectively. There were no additional constraints (such as bulkiness of the feed) imposed on the actual feed consumption and pigs were assumed to be kept in a thermoneutral housing environment (61). The predicted DFI_t was utilised to inform the actual growth, which could differ from the potential growth. The actual retention of protein ($N^{*'}(t)$) and retention of P ($P'(t)$) were determined by the actual DFI_t function used but these quantities were assumed to not exceed $N^{*'}_{max}(t)$ or $P'_{max}(t)$, respectively. Any excess N^* consumed was assumed to be deaminated and excreted as urea (53); any excess energy was assumed to be retained as excess L (62). The actual L retention was calculated as follows:

$$L'(t) = \frac{DFI_t \times E_{feed} - E_{maint}(t) - E_N \times N^{*'}(t)}{E_L} \quad (1.14)$$

where E_N and E_L are the energy used (and expressed in effective energy scale) per kg of N^* and L retained, respectively. The retention of Ash and W were related to $N^{*'}$ and implemented as in Wellock et al. (30) and Symeou et al. (49).

Daily excretion of N ($N_{out}(t)$) (kg/d) and P ($P_{out}(t)$) (kg/d) were calculated as follows:

$$N_{out}(t) = \frac{\left(\left(DFI_t \times \frac{\text{crude } N^*}{1000}\right) - N^{*'}_{maint}(t) - N^{*'}(t)\right)}{6.25} \quad (1.15)$$

$$P_{out}(t) = \left(DFI_t - \frac{\text{total } P}{1000}\right) - P'_{maint}(t) - P'(t) \quad (1.16)$$

where crude N^* and total P denote the feed levels of these quantities per kg of feed.

Simulated Outputs

The following outputs were generated to assess growth performance and nutrient excretion of either the average pig (S1 and S2) or of the heterogenous pig population (S3 and S4): (1) average daily feed intake (ADFI; kg/d/pig); (2) average daily gain (ADG; kg/d/pig); (3) feed conversion ratio (FCR; kg/kg/pig); (4) average daily N^* retention (kg/d/pig); (5) average daily L retention (kg/d/pig); (6) final N^* weight at end of each simulation (kg/pig); (7) final L weight at end of each simulation (kg/pig); (8) cumulative N and P balances [intake, retention, excretion (kg/pig)]. For S1 and S2, the outputs were expressed in terms of mean values; for S3 and S4 the outputs were expressed in terms of mean (SD) values.

RESULTS

Data-Based Estimation of the Average Pig and the Heterogenous Pig Population

Estimated traits for each pig in the heterogenous population are visualised in **Figure 1** and are summarised by the descriptive statistics calculated across the individuals in **Table 2**. Within the population: (1) BW_m ranged from 124 to 580 kg; (2) B ranged from 50.1 to 127 d; (3) L_m/N^*_m ranged from 0.683 to 4.41 (kg/kg); (4) N^*_m ranged from 16.6 to 93.5 kg; (5) L_m ranged from 35.7 to 184 kg; (6) N^*_{in} ranged from 3.86 to 8.24 kg; (7) L_{in} ranged from 2.17 to 5.72 kg. There were three pigs that were notably different from the remaining animals in the population, namely: (i) two pigs were notably larger at maturity than the

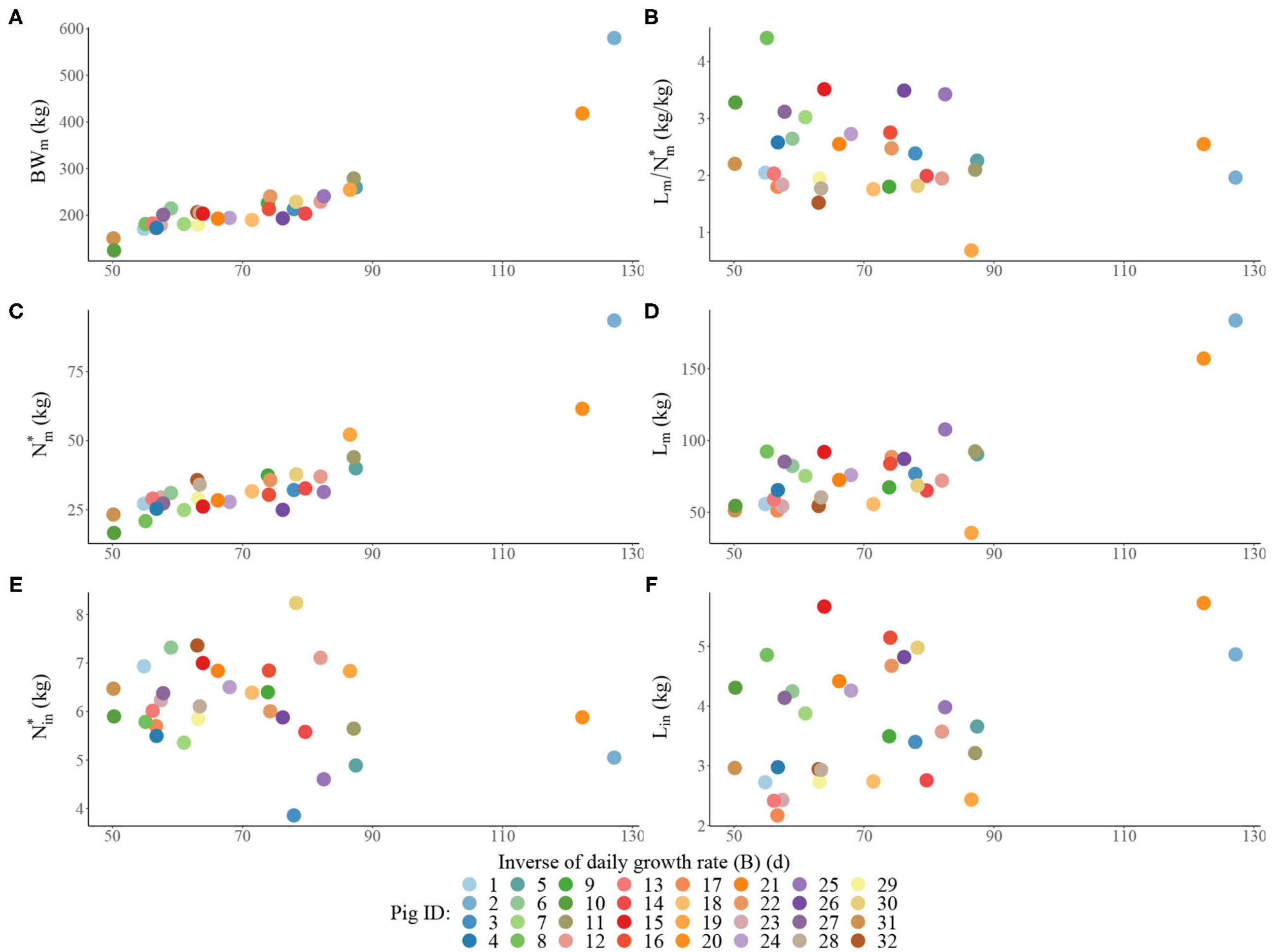

FIGURE 1 | Scatterplots of the estimated traits for each individual pig in the population: **(A)** mature bodyweight (BW_m) vs. inverse of daily growth rate BB; **(B)** ratio of lipid to protein weights at maturity (L_m/N_m^*) vs. B; **(C)** mature protein weight (N_m^*) vs. B; **(D)** mature lipid weight (L_m) vs. B; **(E)** initial protein weight (N_{in}^*) vs. B; **(F)** initial lipid weight (L_{in}) vs. B.

rest, with the estimated BW_m exceeding 400 kg (**Figure 1A**); (ii) one pig was notably leaner than the rest, with the estimated L_m/N_m^* below one (**Figure 1B**). Despite these differences, these three potential outlier pigs were kept in further analyses as their inclusion or exclusion did not influence the overall comparisons of different feeding strategies (see **Supplementary Material** for results produced in the context of pig population which excluded the three aforementioned pigs). Estimated traits of the average pig in the population were: $BW_m = 205$ kg; $B = 65.0$ days; $L_m/N_m^* = 2.31$ (kg/kg); $N_m^* = 31.0$ kg; $L_m = 71.6$ kg; $N_{in}^* = 7.33$ kg; $L_{in} = 3.34$ kg.

Estimation of Nutrient Requirements and Feed Composition

For the two-phase feeding strategies under consideration (S1 and S3), the kg of Feed 1 was estimated to contain 181 g of crude N^*, 109 g of digestible N^*, 6.01 g of total P, 3.01 g of digestible P and 11.8 MJ of effective energy, in order to meet precisely the requirements of this pig at the mid-point of the period under consideration. Subsequently, the kg of Feed 2 was estimated to contain 122 g of crude N^*, 72.9 g of digestible N^*, 4.06 g of total P, 2.03 g of digestible P and 11.8 MJ of effective energy.

Estimated nutritional composition of the feeds (in terms of digestible N^*, digestible P and effective energy) in S1 and S2, together with the accompanying estimated daily nutrient requirements for maintenance and growth of the average pig is given in **Figure 2**. In the context of the average pig, the precision feeding strategy (S2) resulted in gradual decreases in digestible N^* and digestible P feed contents over time; the effective energy content of the feeds remained largely unchanged over time. On the first day of S2, the kg of feed was estimated to contain 205 g of crude N^*, 123 g of digestible N^*, 6.78 g of total P, 3.39 g of digestible P and 11.9 MJ of effective energy. On the last day, the kg of feed was estimated to contain 96.3 g of crude N^*, 57.8 g of digestible N^*, 3.25 g of total P, 1.62 g of digestible P and 11.8 MJ of effective energy.

Estimated nutritional composition of the feeds in S3 and S4 (in terms of digestible N^*, digestible P and effective energy), together with accompanying estimated daily nutrients requirements for

TABLE 2 | Summary statistics of the estimated traits across the thirty-two pigs in the population.

Trait	Min	Median	IQR	Mean	SD	Mode	Max
BW_m (kg)	124	203	47.8	221	82.2	226	580
B (days)	50.1	67.1	20.9	71.4	17.9	73.9	127
L_m/N_m^* (kg/kg)	0.683	2.23	0.814	2.39	0.737	1.80	4.41
N_m^* (kg)	16.6	30.8	8.78	34.0	13.9	37.4	93.5
L_m (kg)	35.7	72.3	31.0	77.2	29.5	67.4	184
N_{in}^* (kg)	3.86	6.06	1.115	6.14	0.880	6.40	8.24
L_{in} (kg)	2.17	3.61	1.59	3.73	1.01	3.49	5.72

BW_m, mature bodyweight; B, inverse of daily growth rate; L_m/N_m^*, ratio of lipid to protein weights at maturity; N_m^*, mature protein weight; L_m, mature lipid weight; N_{in}^*, initial protein weight; L_{in}, initial lipid weight.

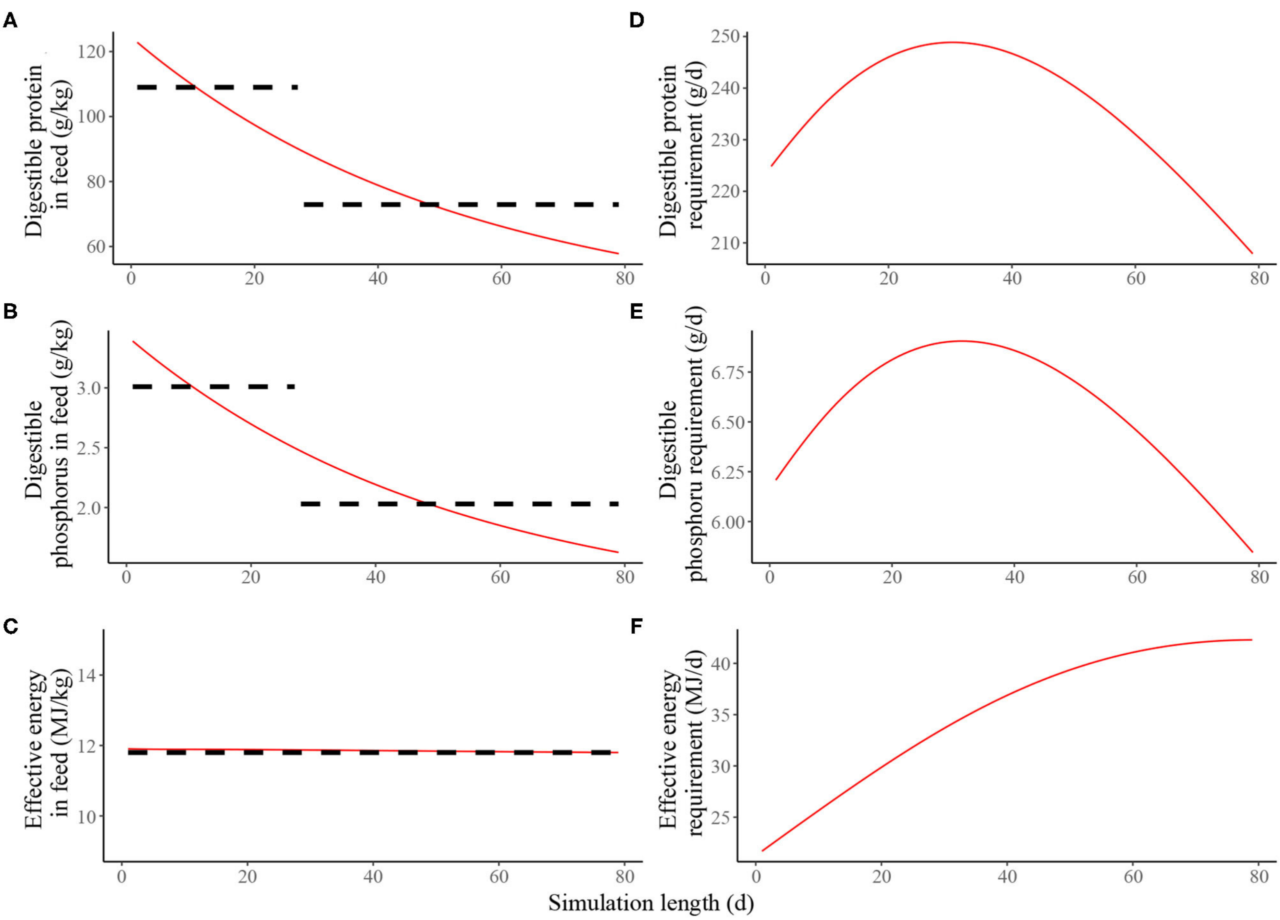

FIGURE 2 | Estimated nutritional composition of the feeds in either two-phase feeding strategy (dashed black line, Scenario 1) or precision feeding strategy (solid red line; Scenario 2) offered to the average pig in terms of: **(A)** digestible protein (g/kg); **(B)** digestible phosphorus (g/kg); **(C)** effective energy (MJ/kg); estimated daily nutrient requirement for maintenance and growth of the average pig in terms of: **(D)** digestible protein (g/d); **(E)** digestible phosphorus (g/d); **(F)** effective energy (MJ/d). For a detailed description of the simulated scenarios, see section Simulated Feeding Scenarios.

maintenance and growth of each pig in the heterogenous population is given in **Figure 3**. In the context of the pig population, the precision feeding strategy (S4) also resulted in gradual decreases in digestible N^* and digestible P feed contents over time for each pig; the effective energy content also remained largely unchanged over time for each pig. There were notable differences in nutrient requirements of individual pigs, which were reflected in the differences in the estimated nutritional composition of the individualised feeds. For example, on the first day, the kg of feed offered to the pig with the lowest nutrient

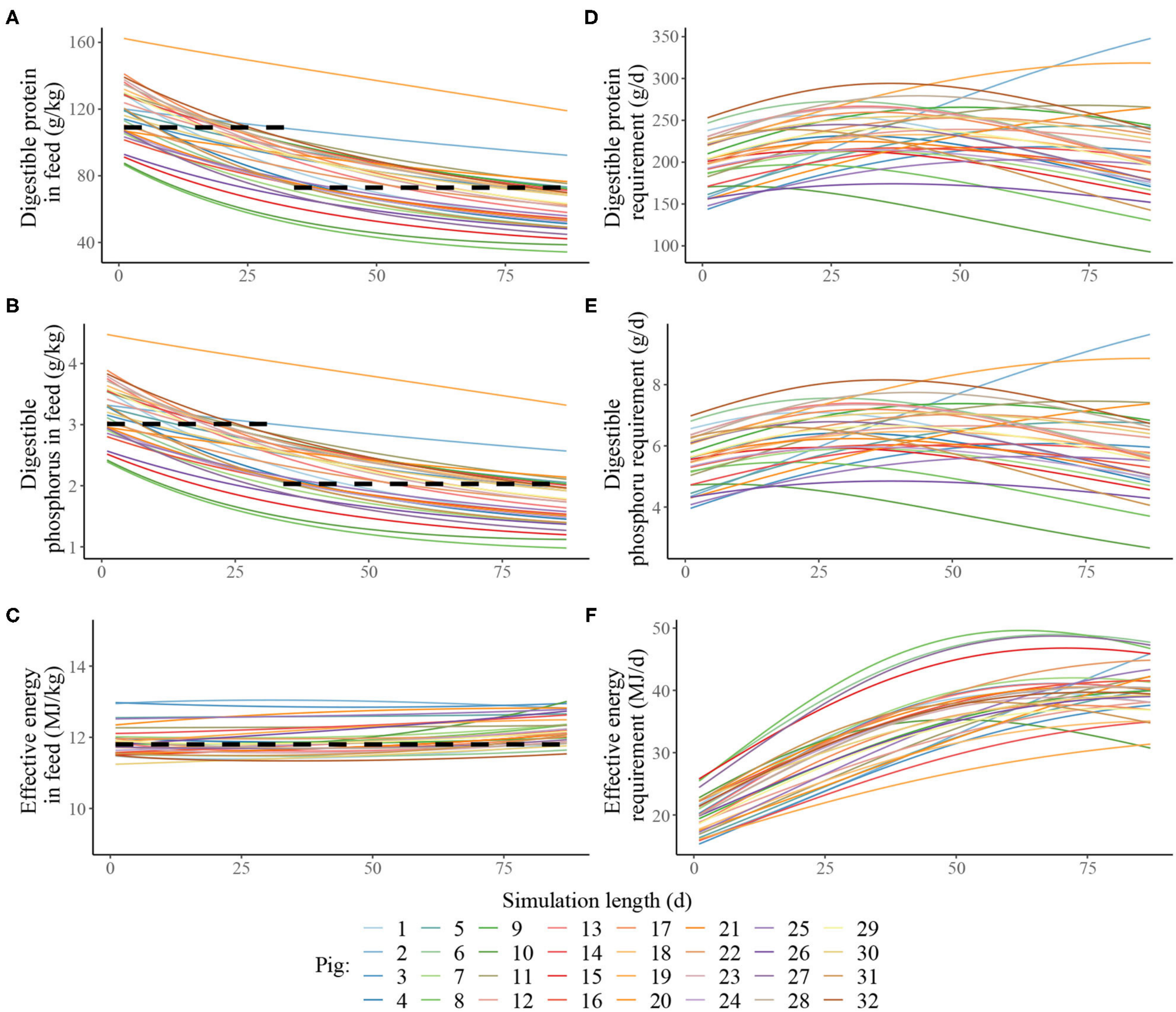

FIGURE 3 | Estimated nutritional composition of the feeds in either two-phase feeding strategy (dashed black line, Scenario 3) or precision feeding strategy (solid lines; Scenario 4) given to a pig population in terms of: **(A)** digestible protein (g/kg); **(B)** digestible phosphorus (g/kg); **(C)** effective energy (MJ/kg); estimated daily nutrient requirement for maintenance and growth of each of the thirty-two pigs in the population in terms of: **(D)** digestible protein (g/d); **(E)** digestible phosphorus (g/d); **(F)** effective energy (MJ/d). For a detailed description of the simulated scenarios, see section Simulated Feeding Scenarios.

requirements was estimated to contain 145 g of crude N^*, 86.7 of digestible N^*, 4.78 g of total P, 2.39 g of digestible P and 11.2 MJ of effective energy, while the kg of feed offered to the pig with the highest nutrient requirements pig was estimated to contain 271 g of crude N^*, 162 of digestible N^*, 8.96 g of total P, 4.48 g of digestible P and 13.0 MJ of effective energy.

Comparison of Growth Performance and Nutrient Excretion

A summary of the growth performance indicators calculated in the context of S1–S4 is given in **Table 3**. Relative to S1, S2 resulted in: 0.270% decrease in ADFI; 0.570% increase in ADG; 0.834% decrease in FCR; 1.19% increase in average daily N^* retention; 0.964% decrease in daily L retention; 0.902% increase in final N^* weight; and 0.949% decrease in final L weight. Relative to S3, S4 resulted in [mean (SD)]: 1.31 (3.38)% decrease in ADFI; 1.76 (3.32)% increase in ADG; 3.64 (7.04)% decrease in FCR; 3.12 (5.36)% increase in average daily N^* retention; 2.43 (4.54)% decrease in daily L retention; 2.13 (3.80)% increase in final N^* weight; and 2.19 (3.91)% decrease in final L weight.

Summary of the daily excretion of N and P over time for pigs considered in S1–S4 is given in **Figure 4**. Cumulative N and P balances calculated in the context of S1–S4 are given in **Table 4**. Relative to S1, S2 resulted in: 4.04% decrease in N intake; 0.858% increase in N retention; 8.25% decrease in N excretion; 3.93% decrease in total P intake; 1.04% increase in

TABLE 3 | Summary statistics of average daily feed intake (ADFI); average daily gain (ADG); feed conversion ratio (FCR); protein (*N**) retention; lipid (*L*) retention; final protein (*N**) weight; and final lipid (*L*) weight in each of the four simulated scenarios in terms of mean values (S1 and S2 for the average pig) and mean (SD) values (S3 and S4 for the population of pigs).

	Simulated Scenario			
Trait	S1	S2	S3	S4
ADFI (kg/pig)	2.97	2.96	2.84 (0.359)	2.81 (0.403)
ADG (kg/pig)	1.03	1.04	0.982 (0.0949)	1.00 (0.0944)
FCR (kg/kg/pig)	2.88	2.86	2.89 (0.277)	2.82 (0.356)
*N** retention (kg/d/pig)	161	163	147 (18.6)	153 (21.4)
L retention (kg/d/pig)	325	322	323 (67.1)	317 (72.4)
Final *N** weight (kg/pig)	19.9	20.1	18.8 (2.02)	19.3 (2.24)
Final *L* weight (kg/pig)	28.6	28.4	31.5 (6.36)	31.0 (6.82)

For a detailed description of the simulated scenarios, see section Simulated Feeding Scenarios.

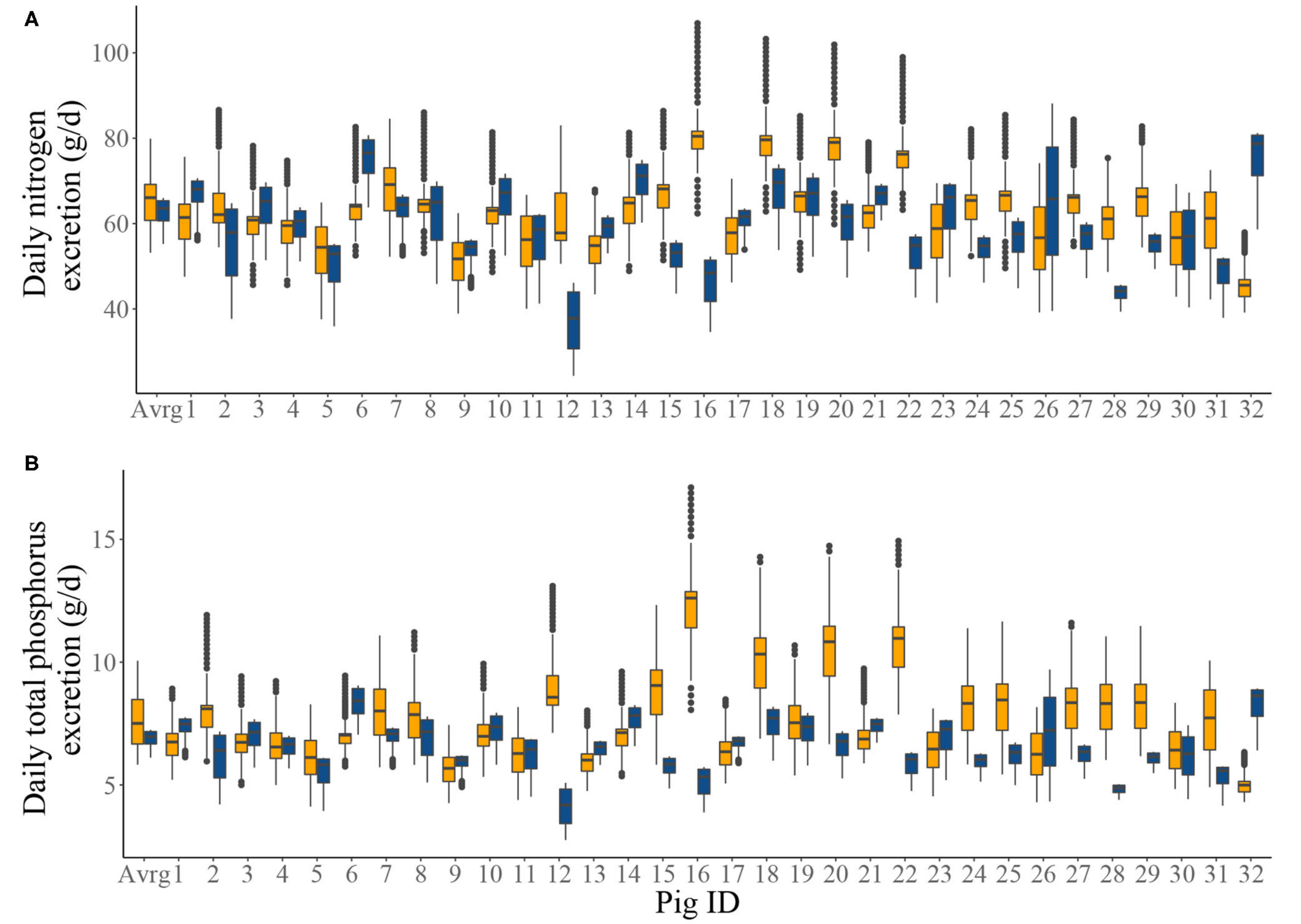

FIGURE 4 | Boxplots of: **(A)** the individual daily nitrogen excretion (g/d); and **(B)** the individual daily total phosphorus excretion from two-phase feeding strategy or precision feeding strategy offered either to the average pig (Avrg) or each of the thirty-two pigs in the population (1–32). For a detailed description of the simulated scenarios, see section Simulated Feeding Scenarios.

P retention; and 9.17% decrease in total P excretion. Relative to S3, S4 resulted in [mean (SD)]: 10.3 (23.9)% decrease in N intake; 2.98 (5.05)% increase in N retention; 22.5 (42.6)% decrease in N excretion; 10.0 (23.6)% decrease in total P intake; 4.35 (7.65)% increase in P retention; and 22.9 (40.2)% decrease in total P excretion.

TABLE 4 | Calculated nitrogen (*N*) and phosphorus (P) balances in each of the four simulated scenarios in terms of mean values (S1 and S2 for the average pig) and mean (SD) values (S3 and S4 for the population of pigs).

Trait	Simulated Scenario			
	S1	S2	S3	S4
Cumulative N intake (kg/pig)	5.16	4.95	5.51 (0.710)	5.12 (0.751)
Cumulative N retention (kg/pig)	2.31	2.33	2.33 (0.273)	2.41 (0.307)
Cumulative N output (kg/pig)	2.85	2.62	3.18 (0.710)	2.71 (0.460)
Cumulative total P intake (kg/pig)	1.08	1.03	1.15 (0.148)	1.07 (0.154)
Cumulative P retention (kg/pig)	0.481	0.486	0.481 (0.0541)	0.506 (0.716)
Cumulative total P output (kg/pig)	0.599	0.544	0.669 (0.142)	0.564 (0.0828)

For a detailed description of the simulated scenarios, see section Simulated Feeding Scenarios.

DISCUSSION

Estimation of the Unobserved Traits From Data

In practise, it is not possible to collect individual sequential measurements of the traits that determine growth potential and body composition, such as the growth of protein or lipid in growing-finishing pig systems (13–15). Yet, estimates of these traits are required to accurately estimate individual nutrient requirements (63). In this context, there is substantial research interest in developing mathematical models that utilise sequential data on individual bodyweight and feed consumption from electronic feeding and weighing stations to estimate these unobserved traits (4, 8, 64). To date, approaches to estimate the growth of protein have been developed, but the growth of the remaining main body chemical components (i.e., lipid, water, ash) has been largely overlooked, which could impact the estimation of the nutrient requirements needed to deliver tailored feeding strategies. In this chapter, an inferential approach utilising the concepts of inverse modelling (13, 65) was developed to estimate altogether the growth of the four main body chemical components (protein, water, lipid, and ash) from sequential bodyweight data that is typically available for precision feeding purposes. Joint estimation is preferred, as it ensures that all parameters that estimated traits are mutually consistent with the observed individual data (29). Accordingly, the estimates obtained via this approach could be used to formulate data-driven feeding strategies that more optimally match nutrient supply to the demand of pigs.

One of the main building blocks of the developed approach concerned a mathematical description of the relationship between protein weight and bodyweight, which consequently informs protein deposition. There is a considerable body of evidence suggesting that the relationship between these traits is approximately allometric (66–72). In light of this empirical evidence, the allometric model was chosen to describe the relationship between protein weight and bodyweight. This is in contrast with previous precision feeding studies, which suggest alternative ways of relating these traits, including isometric, quadratic and Gompertz relationships (5, 19). However, these models are inconsistent with the aforementioned empirical evidence and thus, were not considered further in this chapter. The remaining body elements were related to protein, based on similar well-established allometric scaling rules supported by the view that lipid-free dry matter is considered to be one of the best indicators of the growth progress (29, 34–36). While these rules seem plausible for pigs kept in high-standard livestock production systems, modifications to the allometric body composition models could be needed if there is evidence suggesting that the data originated from pigs faced with severe limitations in the availability of nutrient resources.

Characterisation of the Distributions and Correlations of Unobserved Traits

In the context of pig production data, population and individual level trait estimation is typically carried out within a framework based on hierarchical regression models (19, 73). Under this framework, the overall quality of inferences could be negatively impacted by having to directly estimate multiple variance-covariance parameters (27), which can be challenging due to data limitations (9). The technical difficulties associated with this estimation procedure are the main reason why several studies make various working assumptions that neglect trait correlations (20, 54, 61). However, as highlighted by Pomar et al. (22), this is undesirable as it could lead to an overestimation of the trait variation in a population. In an attempt to alleviate these concerns, the developed approach to estimate population and individual level traits described in this paper, shifted away from hierarchical regression modelling in favour of an alternative framework based on separately inferring individual level trait distributions, which were then scaled up to obtain population level traits. This alternative framework does not necessitate an explicit specification of the aforementioned variance-covariance parameters (27). Thus, reducing the number of assumptions and the number of parameters that need to be estimated should increase the ability to adequately characterise the traits of individual pigs, which should lead to a greater understanding of the impact of such differences on the estimation of population averages (74).

Overall, the developed approach to characterise unobserved traits from bodyweight data on growing (Large White × Landrace) × Pietrain barrows and gilts converged to biologically plausible estimates for most pigs in the population (65, 75). There was considerable variation in the estimated traits among pigs, but very few individuals were identified as potential outliers. However, it is difficult to ascertain if these potential outliers are a result of genetics, environment, feeding and management practises, a combination of some of these factors (76), or data limitations (27). Moreover, it is also important to note that since the parameters on body composition were estimated conditional on the bodyweight parameter estimates and without any additional data, some of these estimates could carry increased uncertainty and have limited biological interpretability (29).

As highlighted by Gauthier et al. (77), mathematical models applied in the context of precision feeding should be able to process both more extensive "historical" data, covering longer timescales and less extensive "real-time" data, covering shorter timescales. When dealing with the latter type of data, it is likely that there will be additional uncertainty in the estimates of body composition and thus, in the estimates of nutrient requirements. The purpose of the current study was to hindcast the nutrient excretion of growing-finishing pigs under differing hypothesised feeding strategies to quantify the differences in the nutrient excretion between these different feeding strategies. Consequently, the developed approach was not tested in the context of "real-time" data, but such evaluations could be an area of future research.

Mechanism of Feed Intake Regulation and Consequences on Nutrient Intake

Recent advances in engineering enable the delivery of feeds, that can be tailored to the needs of individual pigs at a particular point in time (78, 79). It is expected that there would be improvement in feed and nutrient utilisation efficiency if such individualised, data-driven feeding strategies are implemented. In this study, simulation modelling was utilised to illustrate how the estimated variation in individual growth potential and body composition traits could be incorporated in a proposed precision feeding strategy. Specifically, simulations were carried out to assess growth performance and nutrient excretion in the context of a precision feeding plan with daily adjustments and a commercial two-phase feeding plan that did not adapt to real-time animal performance. However, before describing the outcomes of these simulations, it is important to highlight some of the key assumptions concerning how growth was simulated, as these assumptions predetermine the consequent assessments.

In the simulations, the actual growth of pigs was allowed to differ from the estimated potential growth. These differences were largely conditional upon feed composition. Specifically, it was assumed that when the feeds were deficient in either energy or protein, the pigs would attempt to increase their feed consumption according with the previous empirical evidence (59, 80–83). For the purposes of this study, no constraint was assumed to prevent the pigs from meeting their requirements for these two nutrient resources. In reality, however, it is likely that some constraints would operate and prevent the animals from achieving these goals (84, 85). This potential compensatory feed consumption was assumed to be absent in cases when P was the most deficient nutrient to reflect the current knowledge of feed intake regulation in the context of this nutrient (49, 60). In those cases, it was assumed that feed intake could be predicted solely from the estimated energy requirements and energy content of feeds. If the nutritional deficiency triggers attempts to eat for the most deficient nutrient resource, then a possible consequence of this feed intake mechanism would be the excess consumption of the remaining nutrients, leading to their excess excretion (62). In scenarios when feeds were no longer deficient, there was no attempt to correct for any potential imbalances in the body composition as a result of uncertainty surrounding the phenomenon of compensatory growth, especially in relation to the correction of the lipid to protein ratio in the body (86–88).

Comparison of Feeding Strategies Regarding Growth Performance and Nutrient Excretion

The aforementioned simulations were structured to assess the potential advantages (or disadvantages) of precision feeding strategies as measured by the average of individual responses in a population and by the response of an assumed average pig in a population, as it is appreciated that there could be notable differences between these two responses (20). These differences are conditional upon the levels of heterogeneity in the population (24). Note that the present simulations serve mainly as an illustration of the developed approach to estimate individual level variation in unobserved traits and assess deviations from the population average. Additional simulations could be carried out as sensitivity analysis or to evaluate different feeding scenarios.

In scenarios simulating the average pig in the population, which would only represent a population if it were homogenous, the precision feeding strategy led to an approximate ten percent decrease in N and P excretion compared to the typical two-phase feeding strategy. In this case, the higher nutrient excretion from the typical two-phase feeding strategy could be attributed to periods of over-supplementation. Extending the comparisons to the heterogeneous pig population demonstrated an even greater decrease in N and P excretion in the precision feeding strategy compared to the two-phase feeding strategy that targeted nutrient requirements of the average animal (∼20% reduction). These estimates are consistent with previous studies evaluating precision feeding strategies, which reported an average reduction in N and P excretion ranging from approximately ten to forty percent (10, 12, 89), although those studies focussed on evaluating individualised feeding strategies against three-phase feeding sequences. The additional decrease in nutrient excretion observed in the context of the heterogenous pig population could be explained by what happens to the pigs whose nutrient requirements differed from those of the average pig. When offered the phase feeding strategy, the pigs with lower nutrient requirements were oversupplied, leading to notable periods of excess excretion that was mitigated by the precision feeding strategy. The converse was also true implying that the pigs with higher nutrient requirements were excreting more nutrients when offered the precision feeding strategy due to the inefficiencies associated with higher nutrient intakes. Both feeding strategies resulted in comparable growth performance, which is consistent with the previous literature (10, 12, 89). However, there were some differences between growth performance in the context of the two feeding strategies under consideration. Specifically, the precision feeding strategy led to small increases in average daily gain and protein retention, and small decreases in average daily feed intake, feed conversion ratio and lipid retention when compared to the typical two-phase feeding strategy. Again, these differences were magnified in the context of the heterogenous pig population due to the individual level variation in nutrient requirements, which led to more severe periods of both under-supplementation and over-supplementation for some animals.

Note that in the precision feeding strategies under consideration, two feed components (protein and phosphorus) were subject to considerable adjustments over time. It was assumed that the current practise of blending high-nutrient and low-nutrient basal feeds would be largely compatible with such adjustments, although this is not fully guaranteed. To ensure universality, blending three basal feeds is likely to be needed (90).

CONCLUSIONS

Alternative data-driven approaches to estimate individual level variation in unobserved traits using the available data on *BW* were developed. The key advantages of these alternative approaches relate to the improvements made in terms of characterisation of the traits of individual pigs, which should also lead to a greater understanding of the impact of such differences on the estimation of population averages. This was achieved through: (1) a more comprehensive description of the growth potential and body composition; and (2) a reduction in the number of parameters needed to be estimated compared to the typical hierarchical regression models. Consequently, these alternatives approaches were incorporated in a proposed precision feeding modelling framework to quantify the differences in the nutrient excretion between individualised feeding strategies and standard feeding strategies. It was found that the implementation of individualised feeding strategies could notably reduce nutrient excretion in pig populations, which supports the earlier findings by other researchers. The main outstanding challenge relates to whether the developed approaches are applicable in the context of 'real-time' data on bodyweight of pigs that has been collected over shorter periods of time, than those examined in this study which covered the entire growing-finishing phase of growth. Overall, the outcomes of this study should increase the ability to accurately match nutrient supply to the demand of animals by building a more comprehensive picture of their individual nutrient requirements. Moreover, the proposed methodology could also be relevant in the context of selective breeding focussing on improving feed efficiency, such as in the case of residual feed intake-based genetic selection.

DATA AVAILABILITY STATEMENT

The data analysed in this study was obtained from the National Research Institute for Agriculture, Food and Environment (INRAE) Pig Physiology and Phenotyping Experimental Facility (UE3P) in Saint-Gilles (France) (https://doi.org/10.15454/1.5573932732039927E12). Requests to access these datasets should be directed to Dr Ludovic Brossard, ludic.brossard@inrae.fr.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the empirical data used in this paper were not generated in this study. The data originated from animals treated under normal husbandry procedures and for this reason no Institutional or other relevant ethics board approval was required for its collection.

AUTHOR CONTRIBUTIONS

MM led the development and implementation of the approaches in R and drafted the first version of the manuscript. JF conceptualised the algorithms for the estimation of unobserved traits. IK managed both the BBSRC and Feed-a-Gene projects which supported financially the paper development and MM studies. This paper is a part of MM doctoral thesis. All authors contributed equally to the inception of the study, its development, interpretation and conclusions, and contributed equally to the development and finalisation of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.689206/full#supplementary-material

REFERENCES

1. Cole D, Todd L, Wing S. Concentrated swine feeding operations and public health: a review of occupational and community health effects. *Environ. Health Perspect.* (2000) 108:685–99. doi: 10.1289/ehp.00108685
2. Moran D, Wall E. Livestock production and greenhouse gas emissions: defining the problem and specifying solutions. *Anim Front.* (2011) 1:19–25. doi: 10.2527/af.2011-0012
3. Garcia-Launay F, Van Der Werf HMG, Nguyen TTH, Le Tutour L, Dourmad JY. Evaluation of the environmental implications of the incorporation of feed-use amino acids in pig production using life cycle assessment. *Livest Sci.* (2014) 161:158–75. doi: 10.1016/j.livsci.2013.11.027
4. Pomar C, Hauschild L, Zhang GH, Pomar J, Lovatto PA. Applying precision feeding techniques in growing-finishing pig operations. *R Bras Zootec.* (2009) 38:226–37. doi: 10.1590/S1516-35982009001300023
5. Hauschild L, Lovatto PA, Pomar J, Pomar C. Development of sustainable precision farming systems for swine: estimating realtime individual amino acid requirements in growing-finishing pigs. *J Anim Sci.* (2012) 90:2255–63. doi: 10.2527/jas.2011-4252
6. Zuidhof MJ. Precision livestock feeding: matching nutrient supply with nutrient requirements of individual animals. *J Appl Poult Res.* (2020) 29:11–4. doi: 10.1016/j.japr.2019.12.009
7. Pomar C, Remus A. Precision pig feeding: a breakthrough toward sustainability. *Anim Front.* (2019) 9:52–9. doi: 10.1093/af/vfz006
8. Pomar C, Van Milgen J, Remus A. Precision livestock feeding, principle and practice. In: Hendriks WH. Verstegen MWA, Babinszky L, editors. *Poultry And Pig Nutrition: Challenges Of The 21st Century*. Wageningen: Wageningen Academic Publishers (2019). doi: 10.3920/978-90-8686-884-1_18
9. Brossard L, Dourmad JY, Garcia-Launay F, Van Milgen J. Modelling nutrient requirements for pigs to optimize feed efficiency. *Achieving Sustainable Production of Pig Meat*. Cambridge: Burleigh Dodds Science Publishing Limited (2017).
10. Andretta I, Pomar C, Rivest J, Pomar J, Lovatto PA, Radünz Neto J. The impact of feeding growing–finishing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition1. *J Anim Sci.* (2014) 92:3925–36. doi: 10.2527/jas.2014-7643
11. Pomar C, Pomar J, Dubeau F, Joannopoulos E, Dussault JP. The impact of daily multiphase feeding on animal performance, body composition, nitrogen and phosphorus excretions, and feed costs in growing–finishing pigs. *Animal.* (2014) 8:704–13. doi: 10.1017/S1751731114000408
12. Andretta I, Pomar C, Rivest J, Pomar J, Radünz J. Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. *Animal.* (2016) 10:1137–47. doi: 10.1017/S1751731115003067
13. Knap PW, Roehe R, Kolstad K, Pomar C, Luiting P. Characterization of pig genotypes for growth modeling. *J Anim Sci.* (2003) 81:E187–95. doi.org/10.2527/2003.8114_suppl_2E187x
14. Scholz AM, Bünger L, Kongsro J, Baulain U, Mitchell AD. Non-Invasive methods for the determination of body and carcass composition in livestock: dual-energy x-ray absorptiometry, computed tomography, magnetic resonance imaging and ultrasound: invited review. *Animal.* (2015) 9:1250–64. doi: 10.1017/S1751731115000336
15. Fernandes AFA, Dórea JRR, Valente BD, Fitzgerald R, Herring W, Rosa GJM. Comparison of data analytics strategies in computer vision systems to predict pig body composition traits from 3d images. *J Anim Sci.* (2020) 98:178. doi: 10.1093/jas/skaa278.327
16. Quiniou N, Noblet J. Prediction of tissular body composition from protein and lipid deposition in growing pigs. *J Anim Sci.* (1995) 73:1567–75. doi: 10.2527/1995.7361567x
17. Cloutier L, Pomar C, Létourneau Montminy MP, Bernier JF, Pomar J. Evaluation of a method estimating real-time individual lysine requirements in two lines of growing–finishing pigs. *Animal.* (2014) 9:561–8. doi: 10.1017/S1751731114003073
18. Remus A, Hauschild L, Corrent E, Létourneau-Montminy MP, Pomar C. Pigs receiving daily tailored diets using precision-feeding techniques have different threonine requirements than pigs fed in conventional phase-feeding systems. *J Anim Sci Biotechnol.* (2019) 10:16. doi: 10.1186/s40104-019-0328-7
19. Remus A, Hauschild L, Methot S, Pomar C. Precision livestock farming: real-time estimation of daily protein deposition in growing–finishing pigs. *Animal.* (2020) 14:S360–70. doi: 10.1017/S1751731120001469
20. Ferguson NS, Gous RM, Emmans GC. Predicting the effects of animal variation on growth and food intake in growing pigs using simulation modelling. *Anim Sci.* (1997) 64:513–22. doi: 10.1017/S1357729800016143
21. Knap PW. *Variation in Maintenance Requirements Of Growing Pigs in Relation to Body Composition: A Simulation Study*. Ph.D. Thesis (Doctoral Thesis), Wageningen University and Research (2000).
22. Pomar C, Kyriazakis I, Emmans GC, Knap PW. Modeling stochasticity: dealing with populations rather than individual pigs. *J Anim Sci.* (2003) 81:E178–86. doi.org/10.2527/2003.8114_suppl_2E178x
23. Wellock IJ, Emmans GC, Kyriazakis I. Modeling the effects of stressors on the performance of populations of pigs. *J Anim Sci.* (2004) 82:2442–50. doi: 10.2527/2004.8282442x
24. Brossard L, Dourmad JY, Rivest J, Van Milgen J. Modelling the variation in performance of a population of growing pig as affected by lysine supply and feeding strategy. *Animal.* (2009) 3:1114–23. doi: 10.1017/S1751731109004546
25. Morel PCH, Alexander DLJ, Sherriff RL, Sirisatien D, Wood GR. A new development in pig growth modelling. In: Sauvant D, Van Milgen J, Faverdin Friggens PN, editors *Modelling Nutrient Digestion And Utilisation in Farm Animals*. Wageningen: Wageningen Academic Publishers (2011).
26. Symeou V, Leinonen I, Kyriazakis I. The consequences of introducing stochasticity in nutrient utilisation models: the case of phosphorus utilisation by pigs. *Brit J Nutr.* (2015) 115:389–98. doi: 10.1017/S0007114515004523
27. Filipe JAN, Kyriazakis I. Bayesian, likelihood-free modelling of phenotypic plasticity and variability in individuals and populations. *Front. Genet.* (2019) 10:727. doi: 10.3389/fgene.2019.00727
28. Winsor CP. The gompertz curve as a growth curve. *Proc Natl Acad Sci USA.* (1932) 18:1. doi: 10.1073/pnas.18.1.1
29. Filipe JAN, Leinonen I, Kyriazakis I. The quantitative principles of animal growth. In: Moughan PJ. Hendriks WH, editors. *Feed Evaluation Science*, *1 Edn.* Wageningen: Wageningen Academic Publishers (2018).
30. Wellock IJ, Emmans GC, Kyriazakis I. Modelling the effects of thermal environment and dietary composition on pig performance: model logic and concepts. *Anim Sci.* (2003) 77:255–66. doi: 10.1017/S1357729800058999
31. Emmans GC, Fisher C. Problems in nutritional theory. In: Emmans, Fisher GC, editors. *Nutrient Requirements of Poultry and Nutritional Research*. London: Butterworths and Co (Publishers) Ltd. (1986).
32. Moughan PJ, Smith WC, Pearson G. Description and validation of a model simulating growth in the pig (20–90 kg liveweight). *New Zeal J Agr Res.* (1987) 30:481–9. doi: 10.1080/00288233.1987.10417960
33. Whittemore EC, Emmans GC, Kyriazakis I. The problem of predicting food intake during the period of adaptation to a new food: a model. *Br J Nutr.* (2003) 89:383–99. doi: 10.1079/BJN2002781
34. Emmans GC, Kyriazakis I. A general method for predicting the weight of water in the empty bodies of pigs. *Animal Sci.* (1995) 61:103–8. doi: 10.1017/S1357729800013576
35. Emmans GC. A method to predict the food intake of domestic animals from birth to maturity as a function of time. *J Theor Biol.* (1997) 186:189–99. doi: 10.1006/jtbi.1996.0357
36. Emmans GC, Kyriazakis I. Models of pig growth: problems and proposed solutions. *Livest Prod Sci.* (1997) 51:119–29. doi: 10.1016/S0301-6226(97)00061-4
37. Gelman A, Carlin JB, Stern HS, Dunson DB, Vehtari A, Rubin DB. *Bayesian Data Analysis*. Boca Raton, FL: Crc Press (2013). doi: 10.1201/b16018
38. Gamerman D, Lopes HF. *Markov Chain Monte Carlo: Stochastic Simulation for Bayesian Inference*, Boca Raton, Us: Chapman And *Hall*/Crc (2006). doi: 10.1201/9781482296426
39. Chib S, Greenberg E. Understanding the metropolis-hastings algorithm. *Am Stat.* (1995) 49:327–35. doi: 10.1080/00031305.1995.10476177
40. Plummer M, Stukalov A, Denwood M. *Rjags: Bayesian Graphical Models Using Mcmc*. 4–10 Edn. Vienna, Austria (2019).
41. Raftery AE, Lewis SM. Implementing Mcmc. In: Gilks WR. Richardson, Spiegelhalter SD J, editors. Markov Chain Monte Carlo In Practice. Boca Raton, Us: Chapman and Hall/Crc (1996).
42. Plummer M, Best N, Cowles K, Vines K. Coda: convergence diagnosis and output analysis for Mcmc. *R News.* (2006) 6:7–11. Available online at: http://cran.r-project.org/doc/Rnews/Rnews_2006-1.pdf#page=7

43. Link WA, Eaton MJ. On thinning of chains in MCMC. *Methods Ecol Evol.* (2012) 3:112–5. doi: 10.1111/j.2041-210X.2011.00131.x
44. Toft N, Innocent GT, Gettinby G, Reid SWJ. Assessing the convergence of markov chain monte carlo methods: an example from evaluation of diagnostic tests in absence of a gold standard. *Prev Vet Med.* (2007) 79:244–56. doi: 10.1016/j.prevetmed.2007.01.003
45. Gelman A, Rubin DB. Inference from iterative simulation using multiple sequences. *Stat Sci.* (1992) 7:457–72. doi: 10.1214/ss/1177011136
46. Brooks SP, Gelman A. General methods for monitoring convergence of iterative simulations. *J Comput Graph Stat.* (1998) 7:434–55. doi: 10.1080/10618600.1998.10474787
47. Vehtari A, Gelman A, Simpson D, Carpenter B, Bürkner PC. *Rank-Normalization, Folding, and Localization: An Improved R∧ for Assessing Convergence of MCMC*. (2020). p. 1–26. doi: 10.1214/20-BA1221
48. Sandberg FB, Emmans GC, Kyriazakis I. Partitioning of limiting protein and energy in the growing pig: testing quantitative rules against experimental data. *Br J Nutr.* (2005) 93:213–24. doi: 10.1079/BJN20041322
49. Symeou V, Leinonen I, Kyriazakis I. Modelling phosphorus intake, digestion, retention and excretion in growing and finishing pigs: model description. *Animal.* (2014) 8:1612–21. doi: 10.1017/S1751731114001402
50. Kyriazakis I, Dotas D, Emmans GC. The effect of breed on the relationship between feed composition and the efficiency of protein utilization in pigs. *Br J Nutr.* (1994) 71:849–59. doi: 10.1079/BJN19940191
51. Van Milgen J, Dourmad JY. Concept and application of ideal protein for pigs. *J Anim Sci Biotechnol.* (2015) 6:15. doi: 10.1186/s40104-015-0016-1
52. Van Milgen J, Noblet J. Partitioning of energy intake to heat, protein, and fat in growing pigs. *J Anim Sci.* (2003) 81:E86–93. doi: 10.2527/2003.8114_suppl_2E86x
53. Emmans GC. Effective energy: a concept of energy utilization applied across species. *Br J Nutr.* (1994) 71:801–21. doi: 10.1079/BJN19940188
54. Symeou V, Leinonen I, Kyriazakis I. Quantifying the consequences of nutritional strategies aimed at decreasing phosphorus excretion from pig populations: a modeling approach. *Animal.* (2015) 10:578–91. doi: 10.1017/S1751731115002293
55. Wellock IJ, Emmans GC, Kyriazakis I. Modelling the effects of thermal environment and dietary composition on pig performance: model testing and evaluation. *Anim Sci.* (2003) 77:267–76. doi: 10.1017/S1357729800059002
56. Hoffman JR, Falvo MJ. Protein - which is best? *J Sports Sci Med.* (2004) 3:118–30.
57. Boisen S, Hvelplund T, Weisbjerg MR. Ideal amino acid profiles as a basis for feed protein evaluation. *Livest Prod Sci.* (2000) 64:239–51. doi: 10.1016/S0301-6226(99)00146-3
58. Fan MZ, Archbold T, Sauer WC, Lackeyram D, Rideout T, Gao Y, et al. Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. *J Nutr.* (2001) 131:2388–96. doi: 10.1093/jn/131.9.2388
59. Schiavon S, Dalla Bona M, Carcò G, Carraro L, Bunger L, Gallo L. Effects of feed allowance and indispensable amino acid reduction on feed intake, growth performance and carcass characteristics of growing pigs. *PLoS ONE.* (2018) 13:E0195645. doi: 10.1371/journal.pone.0195645
60. Misiura MM, Filipe JAN, Walk CL, Kyriazakis I. How do pigs deal with dietary phosphorus deficiency? *Br J Nutr.* (2020) 124:256–72. doi: 10.1017/S0007114520000975
61. Wellock IJ. *Modelling the Effects of Enviornmental Stressors on Pig Perfromance.* Ph.D. Thesis (Doctoral Thesis), Edinburgh University (2003).
62. Ferguson NS, Gous RM, Emmans GC. Preferred components for the construction of a new simulation model of growth, feed intake and nutrient requirements of growing pigs. *S Afr J Anim Sci.* (1994) 24:10–17.
63. Remus A, Hauschild L, Pomar C. Simulated amino acid requirements of growing pigs differ between current factorial methods. *Animal.* (2020) 14:725–30. doi: 10.1017/S1751731119002660
64. Gaillard C, Brossard L, Dourmad JY. Improvement of feed and nutrient efficiency in pig production through precision feeding. *Anim Feed Sci Technol.* (2020) 268:114611. doi: 10.1016/j.anifeedsci.2020.114611
65. Doeschl-Wilson AB, Knap PW, Kinghorn BP, Van Der Steen HAM. Using mechanistic animal growth models to estimate genetic parameters of biological traits. *Animal.* (2007) 1:489–99. doi: 10.1017/S1751731107691848
66. Needham J. Heterogony and the chemical ground-plan of animal growth. *Nature.* (1932) 130:845–6. doi: 10.1038/130845b0
67. Fortin A, Simpfendorfer S, Reid JT, Ayala HJ, Anrique R, Kertz AF. Effect of level of energy intake and influence of breed and sex on the chemical composition of cattle. *J Anim Sci.* (1980) 51:604–14. doi: 10.2527/jas1980.513604x
68. Whittemore CT, Tullis JB, Emmans GC. Protein growth in pigs. *Anim Prod.* (1988) 46:437–45. doi: 10.1017/S0003356100019048
69. Moughan PJ, Smith WC, Stevens EVJ. Allometric growth of chemical body components and several organs in the pig (20–90 kg liveweight). *N Z J Agric Res.* (1990) 33:77–84. doi: 10.1080/00288233.1990.10430663
70. Landgraf S, Susenbeth A, Knap PW, Looft H, Plastow GS, Kalm E, et al. Developments of carcass cuts, organs, body tissues and chemical body composition during growth of pigs. *Anim Sci.* (2007) 82:889–99. doi: 10.1017/ASC2006097
71. Schinckel AP, Mahan DC, Wiseman TG, Einstein ME. Growth of protein, moisture, lipid, and ash of two genetic lines of barrows and gilts from twenty to one hundred twenty-five kilograms of body weight. *J Anim Sci.* (2008) 86:460–71. doi: 10.2527/jas.2007-0625
72. Nieto R, Lara L, Barea R, García-Valverde R, Aguinaga MA, Conde-Aguilera JA, et al. Response analysis of the iberian pig growing from birth to 150 kg body weight to changes in protein and energy supply. *J Anim Sci.* (2012) 90:3809–20. doi: 10.2527/jas.2011-5027
73. Strathe AB, Danfær A, Sørensen H, Kebreab E. A multilevel nonlinear mixed-effects approach to model growth in pigs. *J Anim Sci.* (2010) 88:638–49. doi: 10.2527/jas.2009-1822
74. Vincenzi S, Mangel M, Crivelli AJ, Munch S, Skaug HJ. Determining individual variation in growth and its implication for life-history and population processes using the empirical bayes method. *Plos Comput Biol.* (2014) 10:E1003828. doi: 10.1371/journal.pcbi.1003828
75. Ferguson NS. Basic concepts describing animal growth and feed intake. In: Gous R, Morris, Fisher TC, editors. *Mechanistic Modelling in Pig and Poultry Production*. Trowbridge: Cabi (2006). p. 22–53.
76. Magowan E, Mccann MEE, Beattie VE, Mccracken KJ, Henry W, Smyth S, et al. Investigation of growth rate variation between commercial pig herds. *Animal.* (2007) 1:1219–26. doi: 10.1017/S1751731107000572
77. Gauthier R, Largouët C, Gaillard C, Cloutier L, Guay F, Dourmad JY. Dynamic modeling of nutrient use and individual requirements of lactating sows. *J Anim Sci.* (2019) 97:2822–36. doi: 10.1093/jas/skz167
78. Pomar J, López V, Pomar C. Agent-based simulation framework for virtual prototyping of advanced livestock precision feeding systems. *Comput Electron Agric.* (2011) 78:88–97. doi: 10.1016/j.compag.2011.06.004
79. Zuidhof MJ, Fedorak MV, Kirchen CC, Lou EHM, Ouellette CA, Wenger II. *System and Method for Feeding Animals*. USA Patent Application Us15/283 (2019).
80. Henry Y. Dietary factors involved in feed intake regulation in growing pigs: a review. *Livest Prod Sci*. (1985) 12:339–54. doi: 10.1016/0301-6226(85)90133-2
81. Bradford MMV, Gous RM. The response of growing pigs to a choice of diets differing in protein content. *Anim Sci.* (1991) 52:185–92. doi: 10.1017/S0003356100005821
82. Kyriazakis I, Emmans GC, Whittemore CT. The ability of pigs to control their protein intake when fed in three different ways. *Physiol Behav.* (1991) 50:1197–203. doi: 10.1016/0031-9384(91)90582-9
83. Ferguson NS, Gous RM. The influence of heat production on voluntary food intake in growing pigs given protein-deficient diets. *Anim Sci.* (1997) 64:365–78. doi: 10.1017/S1357729800015939
84. Kyriazakis I, Emmans GC. The voluntary feed intake of pigs given feeds based on wheat bran, dried citrus pulp and grass meal, in relation to measurements of feed bulk. *Br J Nutr.* (1995) 73:191–207. doi: 10.1079/BJN19950023
85. Kyriazakis I, Tolkamp BJ, Emmans GC. Diet selection and animal state: an integrative framework. *Proc Nutr Soc.* (1999) 58:765–72. doi: 10.1017/S0029665199001044

86. Kyriazakis I, Emmans GC. The growth of mammals following a period of nutritional limitation. *J Theor Biol.* (1992) 156:485–98. doi: 10.1016/S0022-5193(05)80639-3
87. Skiba G. Physiological aspects of compensatory growth in pigs. *J Anim Feed Sci.* (2005) 14:191–203. doi: 10.22358/jafs/70362/2005
88. Menegat MB, Dritz SS, Tokach MD, Woodworth JC, Derouchey JM, Goodband RD. A review of compensatory growth following lysine restriction in grow-finish pigs. *Transl Anim Sci.* (2020) 4:531–47. doi: 10.1093/tas/txaa014
89. Pomar C, Hauschild L, Zhang GH, Pomar J, Lovatto PA. Precision feeding can significantly reduce feeding cost and nutrient excretion in growing animals. In: Sauvant D, Van Milgen J, Faverdin, Friggens PN, editors. *Modelling Nutrient Digestion And Utilisation In Farm Animals*. Wageningen: Wageningen Academic Publishers (2011). p. 327–34. doi: 10.3920/978-90-8686-712-7_36
90. Gaillard C, Quiniou N, Gauthier R, Cloutier L, Dourmad JY. Evaluation of a decision support system for precision feeding of gestating sows. *J Anim Sci.* (2020) 98:skaa255. doi: 10.1093/jas/skaa255

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Reduction of Energy Intensity in Broiler Facilities: Methodology and Strategies

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Broiler facilities consume a lot of energy resulting in natural source depletion and greater greenhouse gas emissions. A way to assess the energy performance of a broiler facility is through an energy audit. In the present paper, an energy protocol for an energy audit is presented covering both phases of data collection and data elaboration. The operational rating phase is analytically and extendedly described while a complete mathematical model is proposed for the asset rating phase. The developed energy audit procedure was applied to poultry chambers located in lowland and mountainous areas of Epirus Greece for chambers of various sizes and technology levels. The energy intensity indices varied from 46 to 89 kWh/m^2 of chamber area 0.25–0.48 kWh/kg of produced meat or 0.36–1.3 kWh/bird depending on the chamber technology level (insulation, automation, etc.) and the location where the unit was installed. The biggest energy consumer was heating followed by energy consumption for ventilation and cooling. An advanced technology level can improve energy performance by $\sim$ 27%−31%. Proper insulation (4–7 cm) can offer a reduction of thermal energy consumption between 10 and 35%. In adequately insulated chambers, the basic heat losses are due to ventilation. Further energy savings can be achieved with more precise ventilation control. Automation can offer additional electrical energy saving for cooling and ventilation (15–20%). Energy-efficient lights can offer energy saving up to 5%. The use of photovoltaic (PV) technology is suggested mainly in areas where net-metering holds. The use of wind turbines is feasible only when adequate wind potential is available. Solar thermal energy is recommended in combination with a heat pump if the unit's heating and cooling systems use hot/cold water or air. Finally, the local production of biogas with anaerobic fermentation for producing thermal or electrical energy, or cogenerating both, is a choice that should be studied individually for each farm.

Keywords: energy audit, energy save, renewable energy sources, poultry houses, broiler chicken farms

INTRODUCTION

The European Union has set the goal of reducing energy consumption and CO_2 emissions due to high energy prices and the need to achieve sustainable development (1). Broiler houses consume a lot of energy which on the one hand leads to natural source depletion and on the other hand is responsible for greater greenhouse gas emissions (GHGs). Furthermore, GHGs emitted by livestock operations (including broiler houses) along with emitted air pollutants represent potential risks to farmers' health, livestock, and residents in the vicinity. The energy consumption in livestock buildings is expected to increase in the coming years due to increasing levels of mechanization and automation and due to the intensification of livestock production to meet the enlarged nutritional needs of a growing population. On the other hand, the reduction of energy intensity in livestock facilities can help the European Union achieve sustainable development in the near future, introducing green and eco-labeled products into the European market.

The annual energy consumption in livestock buildings concerns (a) the control of internal microclimate (temperature, humidity, air quality, and lighting), (b) the animals' feeding (provision food, medicines, and water), (c) both animal and facility hygiene, and (d) applications related with the production process. In broiler facilities, the basic energy needs are limited to the first two categories. In fact, a broiler house is an enclosed building in which there is complete mechanical control of the microclimate.

Energy crises in the '70s induced in the food sector the concepts of primary energy and life cycle analysis (2). The relevant work of the '70s and '80s is summarized in a review paper (3) in 1989. High energy prices, the upgrade of used equipment, and environmental issues raised by the food production at the beginning of the 21st century resulted in several activities including an evaluation of energy consumption in broiler facilities. In this context, the issue of energy consumption in broiler farms has been addressed in some publications (4–8) which address different locations on earth. According to (9) and (10), in a broiler house, the energy consumption varies between 12 and 16 MJ/t of bird or 60–80 kWh/m^2.

Energy audits are processes that reveal the most energy-intensive operations and devices of a production unit as well as the energy efficiency of the examined processes and equipment. Thus, energy audits guide veterinarians, engineers, and farmers to choose the most effective energy measures to reduce energy consumption, leading to reduction of natural resource depletion and GHGs. The energy audit concept was initially developed in the US being adopted by Europe, 20 years ago, in many applications. Methodologies have been developed for conducting energy audits in industry and buildings under relevant European Union Directives (from 93/76/EC to 2018/844/EU) (11–15). For conventional buildings, the energy audit methodology is based on the 2002/91/EC (12) directive supported by numerous European and International norms (ENs and ISOs). In the last two decades, the issue of an energy audit in the industry has been addressed by many research and development projects (like FP7, Intelligent Energy and Horizon 2020), however, without any specific directive unless 93/76/EC (12). The issue of energy audit in livestock facilities has not been addressed in Europe at the level of directives as a separate subject. For this reason, it is treated utilizing a combination of methods concerning buildings and industry. In this endeavor, the NRCS/USDA recommended valuable practices, based on energy audits conducted by experts in the USA (16). However, energy efficiency issues of individual processes such as heating and cooling, cogeneration, and energy label and eco-design are covered by relevant EU directives (17–19). In (10), a methodology for energy audits in broiler facilities is presented. However, the process of an energy audit in broiler farms has not been presented in detail yet at a theoretical level with a complete description of the mathematical model used.

Renewable energy source (RES) utilization in broiler farms usually focuses on the utilization of biomass. In this work, in addition to biomass, the use of wind, solar, and geothermal energy will be examined.

The use of wind energy in a poultry house of 22,000 birds in Turkey is examined in (20) according to the yearly electrical energy consumption profile and the available wind potential focusing on electrical energy consumed for lighting and ventilation.

Greater interest has been developed in the use of photovoltaic (PV) to cover electrical loads given the large available area on the roof of broiler farms. (21, 22) examined the use of PV as a stand-alone and interconnected system to meet the needs of a poultry farm with or without storage of electricity in batteries. The same subject is analyzed in (23) using a different approach. In (24), it is proved that the use of PVs in the roof of poultry chambers only slightly aggravates the microclimate inside the chamber. Finally, in (23), a feasibility study for the use of PV in poultry farms is presented.

The use of solar thermal energy for heating in poultry farms requires sophisticated heating systems beyond the usual ones used, such as heated walls, floors, ceilings, and heat exchangers for air heating. Thus, at a research level, passive solar systems with a heated roof (25) or solar walls (26) have been proposed. The most common is the investigation of the utilization of heat that can be abducted from the rear surface of a PV since the incident radiation only by a percentage of 12–18% is converted into electricity while the rest is reemitted as thermal radiation. Thus, the heat utilization by photovoltaic/thermal (PV/T) hybrid systems (26, 27) has been considered. This heat can be used directly or indirectly through a heat exchanger to heat the air of a poultry house. Other thermal solar systems, such as concentrating solar collectors and vacuum solar thermal collectors, are still very abstract and practical progress has been much less (26). Finally, the use of thermal solar energy in collaboration with a heat pump (26) is examined for poultry heating as well as for the enhancement of the operation of anaerobic digestion systems.

Abbreviations: PV, photovoltaic; GHGs, greenhouse gas emissions; RES, renewable energy sources; PV/T, photovoltaic/thermal; EM, electromechanical; HDD, heating degree days; CDH, cooling degree hours; CHP, combined heat and power.

Instead, the use of geothermal energy is considered to meet the thermal needs of poultry chambers (28). Apparently, this can be applied only to new units, as in existing facilities it is needed to reconstruct the main buildings. It is important to note that in the case of a geothermal system, the cooling needs during the summer can also be met. Finally, (29) examined the effect of using a geothermal system on bird health.

In this paper, an analysis of broiler houses' energy performance is presented and it is accompanied by energy-saving measures that are suggested according to the findings of this analysis. For the energy analysis, the method of the energy audit is used. For that, a protocol for energy audit in broiler houses is developed and presented for the first time analytically, fully documented, and with a full description of the mathematical model. This protocol is applied in several broiler houses of various technology levels and topographic relief (e.g., mountainous and lowland stations). The findings of these energy audits are presented and analyzed followed by suggested energy-saving measures and renewable energy solutions for the different types of broiler facilities. In addition, some strategies to reduce energy intensity in broiler facilities are suggested according to the type of broiler facility evaluated.

METHODOLOGY

The basic methodology used was the assessment of broiler facilities' energy performance through the procedure of energy audits. An energy audit is a systematic process that aims to (a) form a comprehensive view on the energy consumption profile of a building or system by identifying the factors that affect it, (b) consider energy-saving options taking into account the total cost of the product, and (c) provide a comprehensive proposal with the energy-saving measures that could be implemented. In livestock, the building, its operating strategies, and the electromechanical (EM) systems are examined at the same time.

Energy audit procedures for broiler houses have been suggested and presented by authors in (10, 30). An energy audit consists of two discrete phases. The first concerns an operational rating approach using the data of energy bills and the production data to calculate the energy consumption. From the first phase, the auditor acquires a general perspective about the broiler facility energy performance but analysis is required to be able to (a) distribute the consumed energy among the chambers of broiler facility units or different procedures inside a chamber, (b) allocate the most energy-consuming activities, (c) assess the efficiency of various procedures, and finally (d) suggest energy performance improvement measures. This analysis is realized in the second phase which is an asset rating approach. In **Figure 1**, a flowchart of the energy audit procedure is presented. It should be noted that the proposed energy audit procedure concerns only the energy consumption and/or production inside the farm.

Data Acquisition for the Energy Audit

The data acquisition procedure consists of the following: (a) site visit, (b) data collection, and (c) measurements and recording.

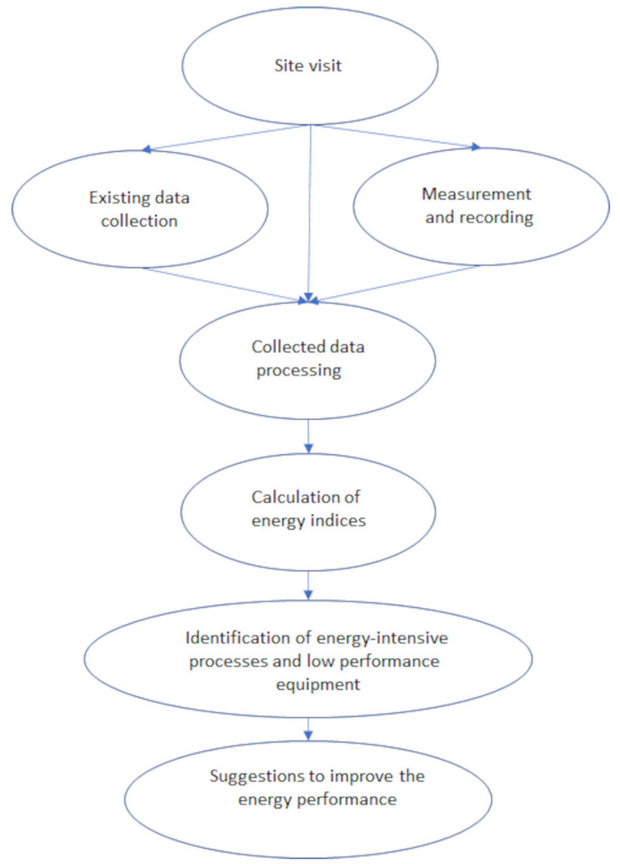

FIGURE 1 | Flowchart of the energy audit procedure.

Site Visit Procedure

In the first step (site visit), the auditor (i) records the installed equipment which consumes energy, (ii) records the construction characteristics, (iii) records the basic characteristics of the surrounding area, and (iv) interviews the unit manager.

The energy demand in a broiler is for (i) food and water supply (terminal motors for the operation of food lines, auger motors for the transfer of food from silos to the chamber, drilling pumps, water pumps), (ii) lighting (lighting fixtures in the chambers, in the vestibule, outdoor), (iii) heating (radiant brooders, local space air heaters, gas boiler), (iv) ventilation (axial exhaust fans, axial recirculation fans, motors for the operation of ventilation slots), (v) cooling (evaporative pads' pumps, evaporative pads' flaps' motor, mist pumps, heat pumps), and (vi) farm management (compressor, power generator, vehicles, air conditions, incinerator, etc.). For the installed energy-consuming equipment, the auditor records the kind of equipment, the nominal power, the number of identical devices, the efficiency performance coefficients, the position where it is sited inside the farm, and finally the operational characteristics.

The construction elements that can be found in a broiler house are walls (exterior or interior), evaporative pads (as part of the buildings' shell), roof, floor, space divider (plastic curtain), and openings (ventilation openings, ventilation windows, security windows, doors, and fans as part of buildings' shell). For each of these elements, except for openings, the following information should be recorded: (i) kind of construction element, (ii) name, (iii) position in the building, (iv) orientation, (v) length, (vi) height or width, (vii) color, and (vii) composition. The composition concerns the different layers of which the construction material is composed. For each layer, the material and the thickness should be recorded. For openings, the following information should be recorded: (i) kind of opening, (ii) name, (iii) construction elements where it belongs, (iv) height from the floor and distance from the beginning of the construction element where it belongs, (v) orientation, (vi) length and height of the opening or diameter, (vii) material, and (viii) color.

Regarding the surrounding area, the auditor should first get the geographical coordinates of the farm position. Then he should record the relative position of the chambers as well as the location of the chambers inside the broiler farm site. For each element that could cause shading (other buildings, parts of the same building, cantilevers, shades, or natural elements, e.g., mountains), the following information should be recorded: (i) type of shading element, (ii) construction element that shades, (iii) dimensions (length, height, or width), and (iv) distance from the construction element that shades. Furthermore, the existence of an element that may alter the local microclimate to what prevails in the general area (e.g., water elements, or elements that block the passage of wind, etc.) and local wind regime should be recorded. Finally, the availability of water and electricity networks should be examined.

Finally, the first step is completed with an interview with the owner or manager of the broiler unit. In this interview, data should be recorded about the (i) owner/manager name and contact info as well as his position in the farm, (ii) data about the poultry farm establishment like the year of construction and renovation, capacity of chambers in birds, and existence of unit's plans, (iii) energy consumption information for the last 3 years, e.g., electricity and gas invoices, (iv) production information for the same period, e.g., number and weight of birds per year, (v) existence of equipment manuals, (vi) operational strategy, (vii) renovation that has taken place, and (viii) interventions that are planned. Specifically about the operational strategy, information should be collected about (i) breeding duration, (ii) time interval between two consecutive breedings, (iii) lighting operation schedule, (iv) heating operating conditions (design temperature each day of the breeding), (v) schedule and operating conditions of feeding and water supply equipment, (vi) fans' operation schedule, (vii) schedule (operating conditions) of cooling equipment, (viii) schedule (operating conditions) of window motors, and (ix) schedule of operation of other machines.

Data Collection

In the second step, the auditor should collect data that cannot be recorded by farm inspection. Most of them are collected and delivered by the owner/manager after the interview or during it. These data include (i) construction plans of the chambers and plans of the area, (ii) manuals and technical characteristic specifications of the equipment, (iii) existing energy consumption measurements, (iv) energy consumption invoices, (v) production data in breeding and annual base for the last 3 years (initial number of birds, final number of birds, final weight of birds), and (vi) local climatic data. Energy consumption invoices can have covered the financial data. As far as electricity consumption is concerned, the following info should be gathered on a monthly basis: (i) periods that cover the invoice, (ii) energy consumption, (iii) agreed electrical power, (iv) electrical power demand, and (v) power factor. For the fossil fuel consumption, the auditor records the date of purchase (or the period between two invoices), the purchase quantities in kg (and/or liters), and the specific volume of the fuel. The local climatic data can be collected either by existing measurements from local weather stations or by national databases.

Measurement and Recording

The data acquisition phase is completed with measurements. The measurements can be instantaneous or specific in duration. Measurements may give information about the equipment's efficient operation, the materials' properties, and the consumed energy and check whether the equipment used and the applied breeding strategy ensure the required microclimate. Instantaneous measurements for the assessment of equipment efficient operation may concern (i) the burners' efficiency with exhaust gas analysis, (ii) the exhaust fans' operation concerning the airflow rate and the pressure drop with differential manometer and/or pitot tube and/or hotwire anemometer, (iii) evaporative pad operation with differential manometer, and (iv) heat losses from tubes with infrared laser thermometers. Long time measurements may concern (i) the thermal transmittance of a construction element with a combination of heat flux meters and differential thermometers, (ii) the electrical energy consumption of the farm or a specific device and electrical power quality with an electricity analyzer, (iii) the fuel consumption with a flow meter, etc. Measurements concerning the quality of achieved microclimate may include (i) lighting level at the bird height with a lux meter, (ii) air quality (temperature, relative humidity, and CO_2 concentration) with an air quality meter, (iii) airspeed at the birds' level and at the fans' level with a hotwire anemometer, (iv) surfaces' temperatures with an infrared camera or with infrared laser thermometer or with contact thermometers, and (v) noise levels. Finally, external area climatic conditions during the measurements should be recorded.

Data Analysis

The data analysis phase is constituted by four steps: (a) processing of the collected data, (b) calculation of energy indices, (c) identifying energy-intensive processes and low-performing equipment, and (d) suggestions for energy performance improvement.

Collected Data Processing

The processing of the collected data includes (i) collection of installed power data, (ii) distribution of installed power by type of consumption and chambers, (iii) elaboration of time series of energy consumption and production, (iii) calculation of operating hours of the individual devices, and (iv) distribution of energy consumption per type of consumption and chamber.

The installed power data are organized in tables according to the chamber of the farm where they belong and according to the type of consumption. The installed power is distinguished among thermal and electrical power. The thermal power is distinguished among thermal power for heating and thermal power for motion (vehicles' operation). The electrical power is distinguished to (i) feeding and water supply, (ii) lighting, (iii) heating, (iv) ventilation, (v) cooling, and (vi) other equipment.

The production data are used for the creation of time series of production. The energy consumption data are also used for the creation of time series on a monthly basis. After elaboration of 3 years of data, the basic yearly pattern is determined, as shown in **Figure 2** where the monthly energy consumption is presented. This pattern along with analytical calculations about the theoretical energy consumption is used for the determination of operation hours of each device.

For the calculation of operating hours, an asset-rating approach with several assumptions is used. The basic assumption is that the equipment operates in its nominal capacity and succeeds to achieve the desired internal microclimate conditions. In the pattern shown in **Figure 2**, a base load and two peaks (winter and summer) are recognized.

$$\mathbf{E_{bl,m} = E_{f+w,m} + E_{l,m}} \tag{1}$$

where $E_{bl,m}$ (kWh/m) is the average monthly lower energy consumption. Two difficulties exist in the calculation of the base load. The first is that the breeding is not continuous and the second is that in a broiler farm the breedings among the different chambers are not synchronized. $E_{l,m}$ (kWh/m) is the average monthly energy consumption for lighting, and $E_{f+w,m}$ is the average monthly energy consumption for feeding and water supply.

The total yearly energy consumption for lighting, E_l (kWh), can be calculated directly from the installed power and the standard daily lighting schedule. According to the schedule, the energy consumption is calculated from Equation (2).

$$\mathbf{E_l = n_{yb}\left(\sum_{i} t_i P_l + \sum_{i,aux} t_{i,aux} P_{l,aux}\right)} \tag{2}$$

where n_{yb} (27) is the number of breedings during the year, i is the number of breeding days, t_i [h] is the time of lighting operation during the i day, P_l is the installed lighting power inside the broiler chamber, $t_{i,aux}$ is the daily time of operation of auxiliary lighting (lobby and exterior lighting), and $P_{l,aux}$ is the installed power of auxiliary lighting. The average monthly energy consumption for lighting is calculated from the following equation.

$$\mathbf{E_{l,m} = \frac{E_l}{\left(\frac{n_{yb} n_b}{30}\right)}} \tag{3}$$

where n_b [days] is the duration of each breeding in days. The monthly average energy for feeding and water is calculated from the following equation.

$$\mathbf{E_{f+w,m} = E_{f,m} + E_{w,m}} \tag{4}$$

where $E_{f,m}$ is the monthly energy consumption for the operation of feeding equipment and $E_{w,m}$ is the monthly average energy consumption for water supply. It is assumed that the feeding equipment operates automatically securing food and water at demand. It is assumed that it operates for 6 h per day (31). Thus, the monthly energy consumption for feeding is calculated as

$$\mathbf{E_{f,d} = t_f P_f} \tag{5}$$

where t_f [h] is the monthly hours of operation of feeding equipment and P_f (25) is the installed power of feeding equipment. From the combination of Equations 1–5, the average daily energy consumption for water, E_{wd} (kWh/d), supply can be derived. Finally, the daily and yearly hours of operation of water supply equipment can be calculated as follows:

$$\mathbf{t_{w,d} = \frac{E_{w,d}}{P_w}} \tag{6}$$

where $t_{w,d}$ (h) is the monthly average hours of operation of water supply equipment and Pw (25) is the installed power of water supply equipment.

The difference between the base load and the winter peak corresponds to the energy consumed for heating and ventilation.

$$\mathbf{E_{w,p,m} - E_{bl,m} = E_{v,m} + E_{h,m}} \tag{7}$$

where $E_{w,p,m}$ (kWh/m) is the monthly average energy consumption peak during the winter, $E_{v,m}$ (kWh/m) is the monthly energy consumption for ventilation, and $E_{h,m}$ is the monthly average energy consumption for heating.

The yearly thermal energy consumption $E_{h,th,y}$ (kWh) for heating can be calculated analytically with an hourly step according to ISO 13790 (32) with the following assumptions: (a) the effect of dynamic phenomena related to heat storage is ignored since the ratio area/volume is small and the heat capacity of the construction materials is low, (b) thermal gains are considered fully exploitable by setting their utilization heat gain coefficient unity, (c) direct solar gains are not taken into account since during the operation of the broiler house the openings are closed while the existence of insulation prevents the indirect solar thermal gains, and (d) thermal gains due to equipment operation are not taken into account. This requires the calculation of (i) thermophysical properties of construction elements (33), (ii) the average heat transfer coefficient, U_m, (iii) the hourly variation of external temperature during a typical day of breeding (one for each of the five breedings per year), (iv) chickens' thermophysical properties and emission for each day of the breeding (34, 35),

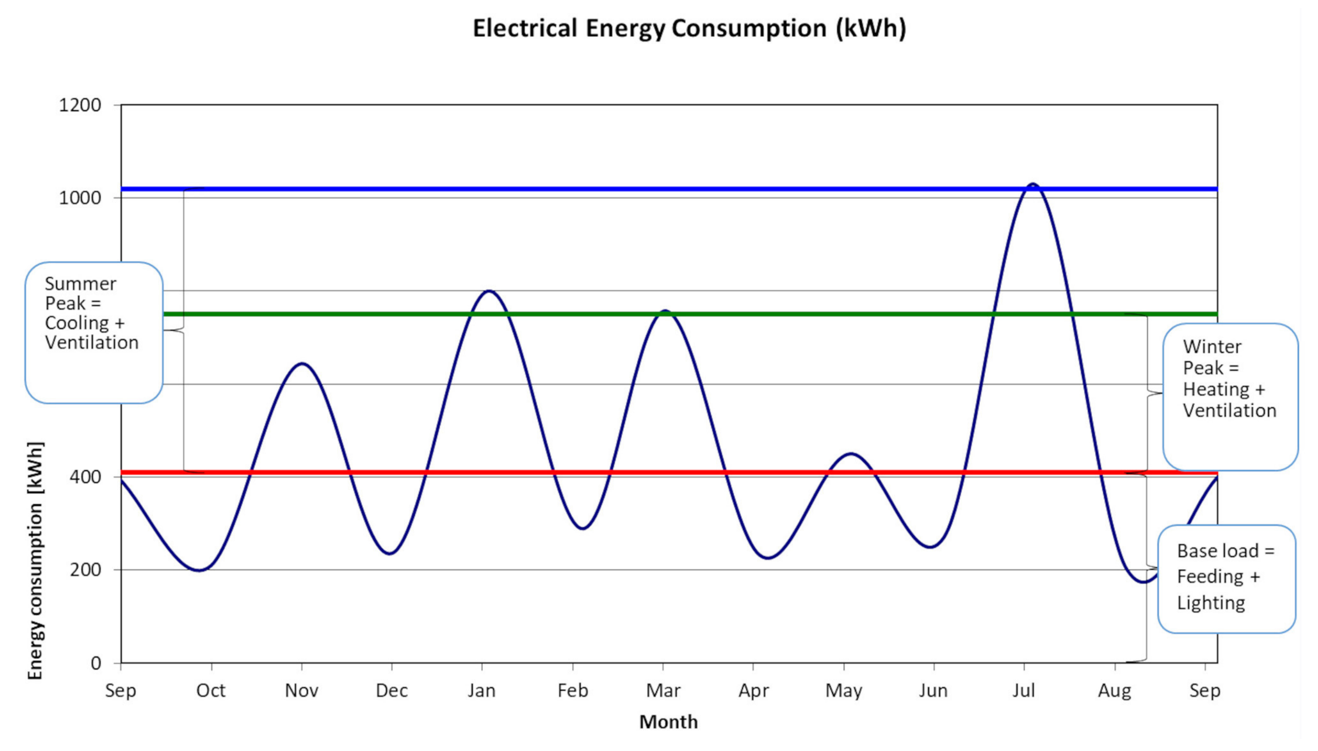

FIGURE 2 | Yearly pattern of electrical energy consumption in broiler farms.

and (v) ventilation needs (10, 30). Finally, the yearly hours of operation of the heating equipment, $t_{h,y}$ (h), is calculated.

$$\mathbf{t_{h,y}} = \frac{\mathbf{E_{h,th,y}}}{\mathbf{P_{h,th}}} \tag{8}$$

where $P_{h,th}$ (25) is the thermal installed power for heating. The corresponding yearly electrical energy consumption can be calculated from the following relationship:

$$\mathbf{E_{h,y}} = \mathbf{t_{h,y}P_{h,e}} \tag{9}$$

For the calculation of the monthly average energy consumption for heating, the heating period, $t_{h,p}$ [months], should be calculated according to ISO 13790:

$$\mathbf{E_{h,m}} = \frac{\mathbf{E_{h,y}}}{\mathbf{t_{h,p}}} \tag{10}$$

Then the monthly average energy consumption for ventilation can be calculated from Equation (7). Then, the monthly operation hours of ventilation equipment, $t_{v,m}$ (h), can be calculated by

$$\mathbf{t_{v,m}} = \frac{\mathbf{E_{v,m}}}{\mathbf{P_{v,m}}} \tag{11}$$

This can be compared with the info taken from the interview about the ventilation operation strategy. If important discrepancies are observed, then it should be calculated whether the installed equipment is adequate for the supply of necessary fresh air. According to the conclusions of the results, the auditor will calibrate the operational hours either of ventilation or of heating.

The difference between the base load and the summer peak corresponds to the energy consumed for cooling and ventilation.

$$\mathbf{E_{s,p,m}} - \mathbf{E_{bl,m}} = \mathbf{E_{v,m}} + \mathbf{E_{c,m}} \tag{12}$$

where $E_{s,p,m}$ (kWh/m) is the monthly average energy consumption peak during the summer, $E_{v,m}$ (kWh/m) is the monthly energy consumption for ventilation, and $E_{c,m}$ is the monthly average energy consumption for cooling. From Equation (12), the monthly average energy consumption for cooling can be calculated. Then, the cooling period $t_{c,p}$ (months) will be calculated according to ISO 13790. Finally, the yearly energy consumption for cooling will be calculated according to

$$\mathbf{E_{c,y}} = \mathbf{t_{c,p}E_{c,m}} \tag{13}$$

This can be compared with the theoretical energy consumption for cooling. Differences may be due to the inability to meet the requirements of the indoor microclimate. Care should be taken when final energy consumption is calculated through energy demand, and the relevant efficiency coefficients should be taken into account.

When the distribution of electricity consumption is done between cooling, feeding and water supply, heating, lighting, and ventilation, there is always a difficulty in classifying the operation of the fans. We know that fans supply fresh air but at the same time for important periods they are also used for cooling. Based on the cooling base temperature and the climatic data of the areas, it can be considered that the fans operate by 35% for cooling and by 65% for ventilation. Alternatively, the auditor may distribute electricity consumption according to the appliances being consumed and not according to the use being served.

Energy Audit Results' Presentation

Since the energy consumption in the level of individual chambers and application has been calculated, the results are presented in terms of (i) energy distribution pies and (ii) energy indices.

The total energy consumption may be distributed among the farms' different chambers. Chambers may also be grouped according to their technology level, their average heat transfer coefficient, U_m, and age. Then thermal and electrical energy consumption may be distributed separately among chambers and/or among groups of them and/or among different uses. These distributions usually are presented in the form of pies.

An additional expression of the results is the calculation of energy indices (e.g., energy consumption per selected unit). Energy indices may concern the total energy consumption in the whole farm and/or on grouped chambers, separately the thermal and electrical energy in the whole farm and/or in grouped chambers and/or in specific uses, and finally primary energy consumption. The unit for which the energy indices are calculated may be the chambers area square meter, the number of the birds, and the weight of the birds.

Identification of Energy-Inefficient Processes and Equipment

From the above analysis, the high energy-consuming processes are revealed. These processes will attract our interest in the planning of proposed interventions. Furthermore, information about the individual equipment operation may be drawn from the measurements. Finally, the calculated energy indices may be assessed by comparison to each other or according to international literature values. This will reveal the inefficient processes and inefficient equipment.

Suggestions to Improve Energy Performance

The energy audit is completed with the preparation of proposals for the improvement of the energy performance of the broiler unit. Improvement proposals should be categorized into three levels: (i) low cost, (ii) medium cost, and (iii) high cost. They should be accompanied by calculations—assessment of energy improvement—so that their effectiveness can be costed.

For poultries, optimization suggestions may have three general directions:

- In the case of high thermal energy consumption, chamber insulation is recommended mainly if the roof is not insulated or it is poorly insulated.
- In the case of well-insulated chambers, the following interventions should be considered: (a) correct dimensioning of electromechanical Equipment, (b) system efficiency coefficients, and (c) application of automation systems.
- The operation strategy should be considered in collaboration with a specialized zoo technician in terms of breeding seasons and internal microclimate design conditions.

STUDY CASE

The above-described energy audit protocol was applied for the energy performance assessment of eight broiler farms (with 25 chambers) of various sizes, ages, and technology levels located in lowland and mountainous areas in West Greece. The examined farms belong to two of the biggest broiler cooperatives in Greece. An attempt was made to select units that cover all types of units based on size and technology used in lowland and mountainous areas. Specifically, the following were examined: (a) two (2) large (of equal capacity) farms, one lowland and one mountainous, with seven (7) chambers each (three chambers of new technology and four chambers of old technology for the lowland, four chambers of new and three chambers of old technology for the mountain), (b) two small farms, one lowland with one chamber and one mountainous with three chambers, (c) one mountainous farm with only one chamber old technology, (d) two farms with chambers of only new technology, one mountainous and one lowland with one chamber each, and (e) one mountainous farm with three chambers of mixed technology. **Table 1** describes the basic characteristics of examined farms.

The lowland area is at sea level with an average latitude of 39°, where the heating degree days are 1,313 (HDD with a base temperature 18.3°C) and the cooling degree hours are 3,399 (CDH with base temperature of 26°C). The mountainous area is considered with an average elevation of more than 500 m at almost the same latitude, with HDD = 2,037 and CDH = 1,694 (36). This means that mountainous areas have almost twice the need for heating and half the need for cooling compared to the lowland areas. Available total solar radiation at the horizontal plane varies from 56.2 to 219.1 kWh/m^2 for the lowland areas with a clearness index of 0.54 and from 45.1 to 212 kWh/m^2 for mountainous areas with a clearness index of 0.49 (36). Climatic data are summarized in **Table 2**. In all the examined units, food and water are supplied automatically "at demand." Units characterized as "new technology" have chambers with sufficient insulation (with average heat transfer coefficient, U_m, smaller than 0.71 W/m^{2}K for the lowland chambers and 0.58 W/m^{2}K for the mountainous chambers) and operation for heating and cooling automated according to the desired internal climate conditions. Old technology is characterized as units with no or insufficient insulation (with average values of U_m in the order of 1 W/m^{2}K) and operation of heating and cooling without taking into account the internal climatic conditions.

In **Table 3**, the installed power is given in terms of total power per square area of chambers and per bird capacity for lowland and mountainous farms and separately for thermal and electrical power.

From the above, it is clear that the major contribution in installed power comes from thermal power since it represents 91 and 94% for the lowland and mountainous farms, respectively. The thermal power is analyzed to heating and vehicles, as shown

TABLE 1 | Description of the audited broiler facilities.

Farm type	Location	Capacity (number of birds)	Chamber's area (m^2)	Number of chambers (-)	Yearly production (birds/year)	Yearly production (kg/year)
Big farm	Lowland*	115,170	7,723	7	532,733	1,472,816
Small farm	Lowland	61,000	3,740	3	324,100	776,544
Only new technology**	Lowland	25,000	1,404	1	119,218	273,009
Big farm	Mountainous*	88,000	6,345	7	347,666	828,140
Mixing old and new technology	Mountainous	24,000	1,641	3	128,253	326,071
Only old technology***	Mountainous	30,000	1,933	2	157,331	384,486
Small farm	Mountainous	20,000	1,253	1	102,994	251,277
Only new technology	Mountainous	20,000	1,264	1	101,509	249,619

*Lowland and mountainous locations are explained in **Table 2** and in the study case description.*
***New technology refers to well-insulated chambers with automatic control of internal microclimate.*
****Old technology refers to purely insulated chambers without automatic control of internal microclimate.*

TABLE 2 | Climatic data summary for lowland and mountainous farms.

Climatic parameter	Lowland farms	Mountainous farms
Elevation (m)	0 (sea level)	>500
Heating degree days with base temperature 18°C	1,313	2,037
Cooling degree hours with base temperature 26°C	3,399	1,694
Total solar radiation at horizontal plane per month [kWh/m^2mo]	56–219.1	45.1–212
Clearness index (-)	0.54	0.49

in **Figure 3**. In both lowland and mountainous farms, heating represents the highest ration of installed thermal power.

The distribution of installed electrical power is presented in **Figure 4**. In both lowland and mountainous farms, fans represent almost half of the installed electrical power. It should be noted that fans are used not only for ventilation but also for cooling. The rest of the cooling equipment represents 11% in lowland and 23% in mountainous electrical installed power. Feeding requirements cover 20%, and the rest of the installed power concerns lighting and other machines (e.g., compressor). Nevertheless, the installed electrical power is bigger in lowland farms than in mountainous farms due to the increased needs for cooling.

Indicative time series of propane and electrical energy consumption for the lowland and mountainous big farms have been presented in (10).

Installed Equipment for Food and Water Supply

In all the examined units, the feeding of the birds is done automatically, depending on the level of food in the feeders, through screws that lead the food to the feeders following a path along with the chamber. Depending on the width of the chamber, there are three or four screws driven by motors mounted on one end of the chamber—terminal motors with a power of 0.23–1.12 kW. For the transfer of food from the storage silos (outside the chambers) into the chambers, other screws are used that also work with motors—silo motors, usually one in each chamber with a power of 0.55–2 kW.

Installed Equipment for Lighting

The energy consumption for lighting mainly concerns the necessary level of lighting inside the chambers to ensure the growth of the birds. For this reason, 11–24-W energy-saving lamps, with >60 lm/W efficiency, or 11–72-W fluorescent lamps are mainly used. Secondarily, lighting is used in the antechambers, when they exist, for auxiliary work. There, a variety of luminaires are used from energy-saving lamps 11–14 W, incandescent lamps 60–160 W, and halogen lamps 125 W. The chamber lighting operates either manually or with a timer, while the auxiliary lighting operates always manually on demand.

Installed Equipment for Heating

Three types of heating devices were found: (a) fan heaters with thermal power from 50 to 120 kW, (b) brooders with thermal power from 10 to 14 kW, and (c) at one case a gas air boiler of 217 kW. In all cases, the main energy source is propane while in the case of fan heaters and gas boiler there are small electrical consumptions of 0.15–1 kW.

Installed Equipment for Ventilation

All the fans found to be used for ventilation were axial and can be divided into three categories: (a) exhaust fans mounted on the small side of the chamber, opposite of the evaporative pads (when they exist), (b) exhaust fans mounted in the large side of the chamber, and (c) recirculation fans inside the chamber. Exhaust fans are of 0.55–1.12 kW with diameter varying from 0.5 to 1.25 m and flow rate from 3,000 to 36,000 m^3/h. Recirculating fans are 0.1–0.37 kW. In addition to the fans, in high-technology units, for the operation of ventilation, there are also small motors that open and close the ventilation openings with electrical power from 0.12 to 0.8 kW.

Installed Equipment for Cooling

The basic technology used for air conditioning, in the examined broiler farms, is that of evaporative cooling

TABLE 3 | Installed power in the broiler facilities examined.

Power	Installed power/area (kW/m^2)			Installed power/birds capacity (kW/bird)		
	Min	Max	Average	Min	Max	Average
Lowland*						
Total power	0.18	0.38	0.27	0.011	0.022	0.016
Thermal power	0.15	0.37	0.24	0.009	0.021	0.015
Electrical power	0.01	0.03	0.03	0.001	0.002	0.001
Mountainous*						
Total power	0.17	0.32	0.25	0.011	0.020	0.017
Thermal power	0.16	0.30	0.24	0.01	0.019	0.016
Electrical power	0.01	0.03	0.01	0.001	0.002	0.001

Lowland and mountainous locations are explained in **Table 2 and in the study case description.*

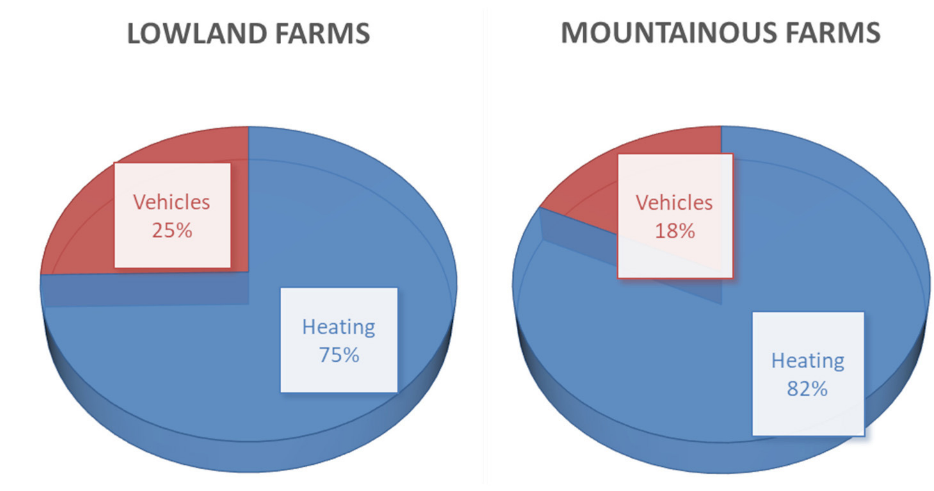

FIGURE 3 | Installed thermal power distribution for lowland and mountainous farms.

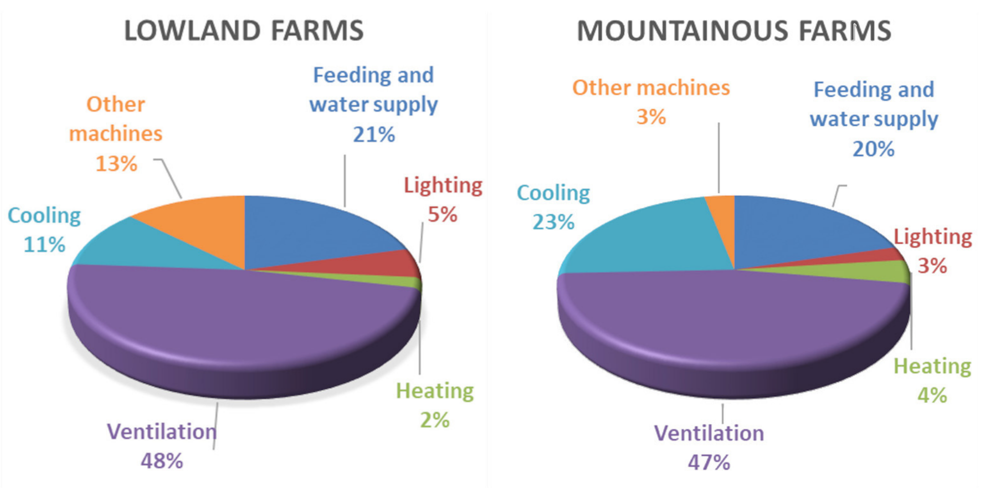

FIGURE 4 | Installed electrical power distribution in lowland and mountainous farms.

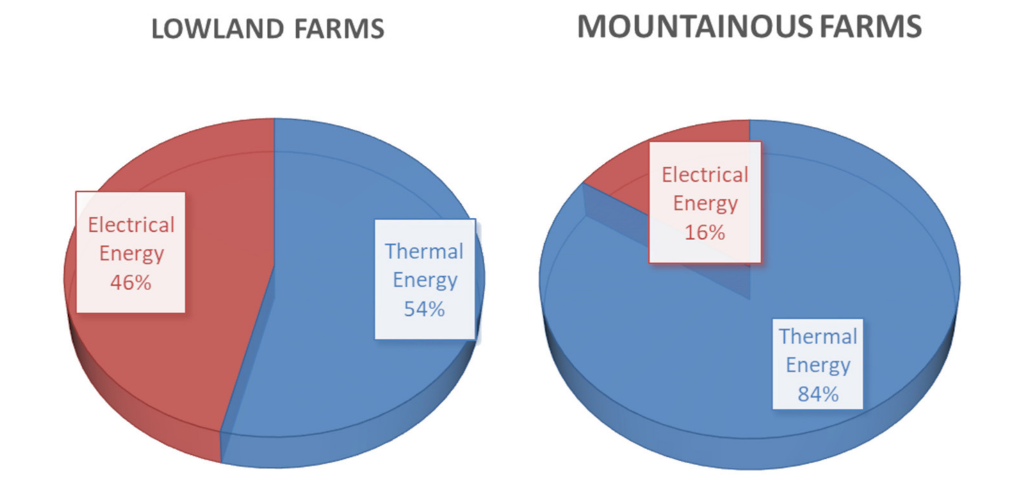

FIGURE 5 | Distribution of total final energy consumption in lowland and mountainous farms.

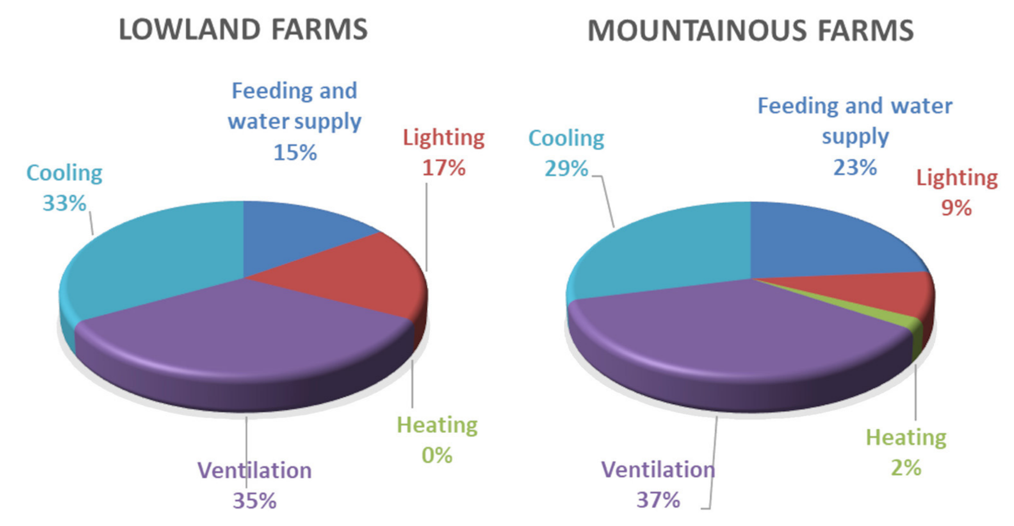

FIGURE 6 | Distribution of electrical energy consumption in lowland and mountainous farms.

and is carried out either with evaporative pads or with evaporator air coolers. For the operation of evaporative pads, pumps of electrical power from 0.4 to 1.5 kW are used to circulate water, which is the basic energy consumption. In addition, lower consumptions concern the movement of the evaporative pads' flaps made with motors of 0.12–0.55 kW. Evaporator coolers as compact devices were of 2.2–2.5 kW electrical power.

RESULTS

After the elaboration of energy audit data according to the described methodology, the energy consumption is calculated at the chamber and process levels.

Energy Consumption Distribution

In **Figure 5**, the distribution among thermal and electrical energy is given for lowland and mountainous farms. Although in both lowland and mountainous farms the percentage of electrical power was small, the final energy consumption pattern reveals two different responses. In lowland farms, the electrical energy is 46% of the final energy consumption while in the mountainous farms the percentage of electrical energy is only 16%. It is obvious that in mountainous farms the high energy demand is related to heating needs while in lowland farms cooling needs are equally important.

In **Figure 6**, the distribution of electrical energy among the served processes is given for lowland and mountainous farms. The pattern of distribution is similar with small differences in the percentages of cooling and feeding energy consumption.

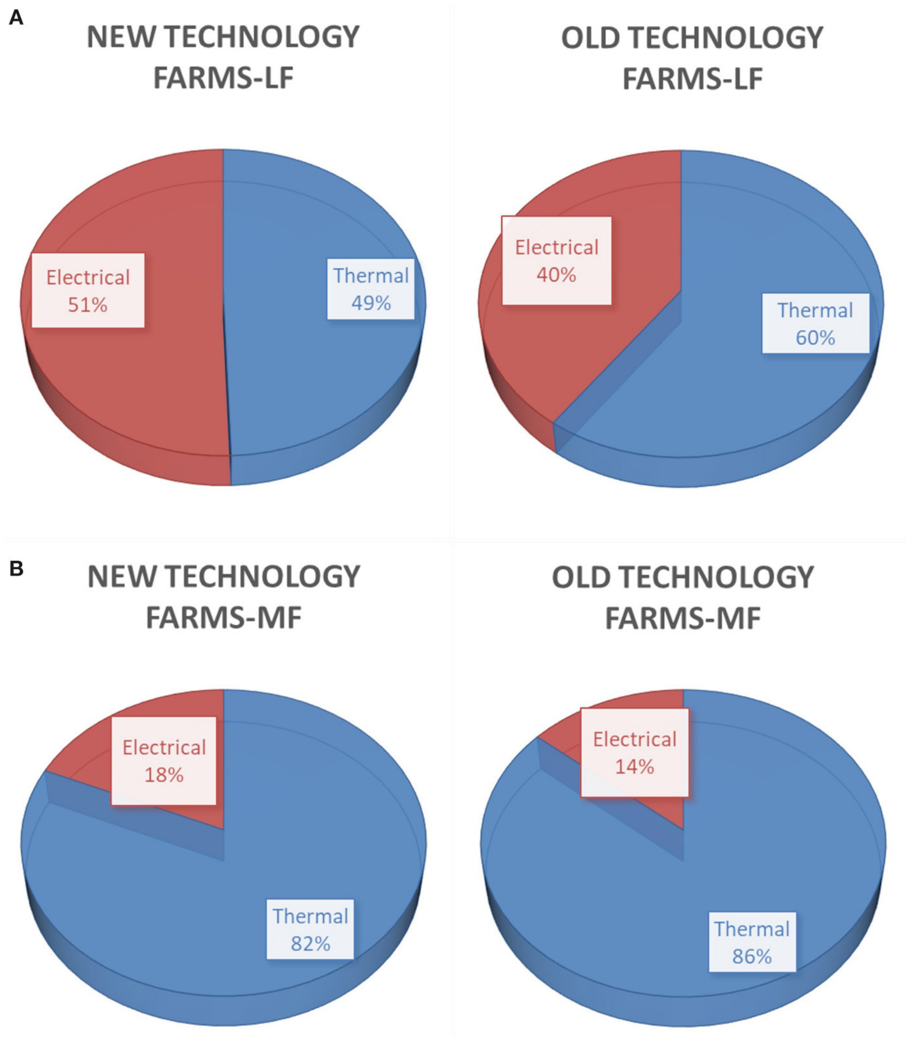

FIGURE 7 | Distribution of total final energy consumption **(A)** in lowland farms and **(B)** in mountainous farms, for new and old technology chambers.

For cooling, about 30% of the electrical energy is consumed. Ventilation represents the biggest consumer since it operates during the whole year. Feeding is the third consumer followed by lighting.

In **Figure 7A**, the distribution among thermal and electrical energy in lowland farms is given for new and old technology chambers. In the lowland farms, where the cooling loads are important, inefficient cooling technologies lead to increased electrical energy consumption.

In **Figure 7B**, the distribution of thermal and electrical energy in mountainous farms is given for new and old technology chambers. In mountainous farms, the big consumer is heating. Small differences are observed in the distribution among old and new technology chambers attributed to poorer electromechanical equipment.

In **Figure 8A**, the distribution of electrical energy among the served processes in lowland farms is given for new and old technology chambers. In new technology chambers, cooling is comparable with ventilation. In old technology level, ventilation share is much more important than cooling share since ventilation is widely used for temperature control. In new technology chambers, the use of energy-efficient lights leads to important energy saving.

In **Figure 8B**, the distribution of electrical energy among the served processes in mountainous farms is given for new and old technology chambers. The electrical energy distribution profile for new technology chambers in mountainous farms is almost the same as the distribution in lowland farms except the appearance of a small share of electrical energy consumption for heating. As far as the old technology chambers are concerned, the increased share of cooling is attributed to low efficient equipment.

Energy Indices

In **Table 4**, the energy indices concerning final energy consumption are presented for lowland and mountainous

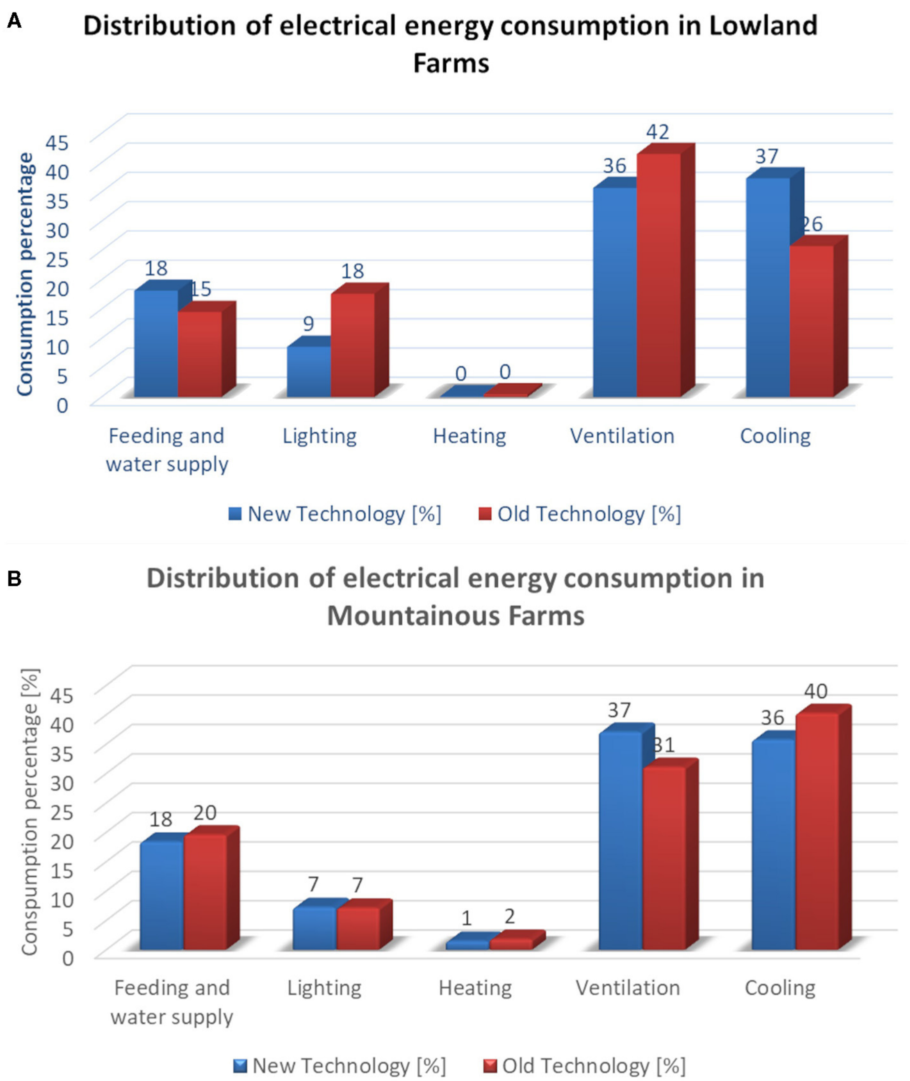

FIGURE 8 | Distribution of electrical energy consumption **(A)** in lowland farms and **(B)** in mountainous farms, for new and old technology chambers.

farms, for old and new technology chambers. The presented energy indices are (i) final total energy consumption per chamber area, (ii) final total energy consumption per bird, and (iii) final total energy consumption per produced meat weight. For each index, three values are given: the average, the minimum, and the maximum. The lower energy consumption is achieved to lowland farms using new technology while the worst performance is met in the mountainous farms with old technology. This is expected since the higher energy consumer is the heating and mountainous farms with insufficient insulation have a big energy demand. Nevertheless, lowland farms with old technology have comparable energy indices with mountainous farms with new technology. This means that there is energy-saving potential in electrical consumption as well.

Before proceeding to the discussion of energy audit findings, another issue should be considered. This is related to the quality of consumed energy. Electrical energy is expensive energy in terms of "primary energy" consumption. In (10), authors had presented energy indices, concerning energy consumption per produced meat weight and per broiler house area, split into thermal and electrical energy and finally energy indices according to primary energy consumption. The energy indices concerning the consumed energy per bird for lowland and mountainous units with old and new technology are presented in **Table 5**. Nevertheless, when this index is given, it should be accompanied by information about birds' final weight. In the examined cases, final weight varies between 2.4 and 2.8 kg per bird, depending on the time period and the broiler house location.

Finally, in **Table 6**, energy indices are presented in terms of final use for the examined cases for final energy consumption and primary energy consumption. There are four uses of energy consumption: (a) feeding, (b) lighting, (c) heating, and (d) cooling and ventilation. It should be noted that energy for heating

TABLE 4 | Energy indices according to the final energy consumption of the examined broiler facilities.

Energy index	Old technology***–lowland*			Old technology–mountainous*			New technology***–lowland			New technology–mountainous		
	Max	Min	Av	Max	Min	Av	Max	Min	Av	Max	Min	Av
Final energy consumption/area (kWh/m^2)	89.4	52.48	67.54	131.56	74.63	96.59	61	30.15	46.38	106.26	54.64	70.72
Final energy consumption/bird (kWh/bird)	1.30	0.79	0.99	1.27	0.68	1.05	0.89	0.36	0.73	1.29	0.83	0.99
Final energy consumption/weight (kWh/kg)	0.47	0.29	0.37	0.52	0.27	0.43	0.32	0.15	0.25	0.53	0.35	0.41

*Lowland and mountainous locations are explained in **Table 2** and in the study case description.
**New technology refers to well-insulated chambers with automatic control of internal microclimate.
***Old technology refers to purely insulated chambers without automatic control of internal microclimate.

TABLE 5 | Energy indices according to the primary energy consumption of the examined broiler facilities.

Chamber location/technology level	Energy index	Final energy per bird (kWh/bird)	Primary energy per bird (kWh/bird)
Lowland*-new technology**	Thermal energy	0.36	
	Electrical energy	0.37	
	Total energy	0.73	1.45
Lowland–old technology***	Thermal energy	0.60	
	Electrical energy	0.39	
	Total energy	0.99	1.76
Mountainous*-new technology	Thermal energy	0.80	
	Electrical energy	0.19	
	Total energy	0.99	1.39
Mountainous–old technology	Thermal energy	0.90	
	Electrical energy	0.15	
	Total energy	1.05	1.38

*Lowland and mountainous locations are explained in **Table 2** and in the study case description.
**New technology refers to well-insulated chambers with automatic control of internal microclimate.
***Old technology refers to purely insulated chambers without automatic control of internal microclimate.

is different than thermal energy presented earlier since it contains both thermal and electrical energy consumed for heating.

As expected, feeding energy indices are the same for all examined cases since they are not dependent on position and technology level (even old technology level units have automated feeding systems). The same remark holds for lighting, which represents a very small percentage of energy consumption. Energy for heating decreases from mountainous old technology level units to mountainous new technology units due to thermal insulation and higher efficiency equipment. A lower energy index for cooling and ventilation is observed in mountainous old technology chambers, due to reduced needs for cooling and to the absence of relative equipment. The highest values appear in lowland old technology chambers due to increased needs for cooling and low efficiency used equipment. Cooling and ventilation represent the second bigger energy consumption in terms of final energy and the higher energy consumption in terms of primary energy in lowland units.

In conclusion, according to the above-described tables, the best behavior is achieved in the lowland new technology chambers, while the worst behavior is met in the lowland old technology chambers since they consume too much electrical energy in a non-efficient way. Mountainous chambers with old technology have a primary energy index per housing area very close to mountainous chambers with new technology. This is attributed to the fact that old technology chambers have lower electrical-powered equipment at the expense of the final product. The last is proved by the energy index per produced weight.

DISCUSSION

Energy Audit Results

In order to evaluate our results, the results of other relevant works were investigated. According to a review paper (3) in 1989, 71% of the total energy consumption was used for heating, 18% was used for feed and water distribution and for manure removal, 7%

TABLE 6 | Energy indices according to the energy usage of the examined broiler facilities.

Chamber location/technology level	Energy index	Final energy per kg (kWh/kg)	Final energy per area (kWh/m^2)	Primary energy per kg (kWh/kg)	Primary energy per area (m^2)
Lowland–new technology	Feeding	0.02	3.48	0.06	10.09
	Lighting	0.01	1.65	0.03	4.88
	Heating	0.14	26.25	0.18	32.84
	Cooling and ventilation	0.08	15	0.23	43.5
Lowland–old technology	Feeding	0.02	3.61	0.06	10.47
	Lighting	0.02	4.4	0.07	12.76
	Heating	0.23	42.84	0.27	54.06
	Cooling and ventilation	0.1	16.69	0.29	48.4
Mountainous–new technology	Feeding	0.02	3.62	0.06	10.5
	Lighting	0.01	1.43	0.03	4.15
	Heating	0.28	63.14	0.27	57.27
	Cooling and ventilation	0.07	14.62	0.21	42.4
Mountainous–old technology	Feeding	0.02	0.04	2.81	8.15
	Lighting	0.01	0.02	1.02	2.97
	Heating	0.39	0.41	75.32	76.31
	Cooling and ventilation	0.06	0.15	10.22	29.64

was used for lighting, and only 4% was used for ventilation. In this review, an increase in energy needs in two broiler farms of 10,000 birds in Saskatchewan of Canada is reported. The annual LPG consumption for a well-insulated broiler house was 188,000 kWh and became 214,000 kWh for a poorer-insulated chamber, with the electrical annual energy consumption being 24,000 and 20,000 kWh respectively.

Later, in the 21st century, in 2007, the feasibility of an expensive renovation was examined, concluding that this depends on farm location, energy costs, and management strategy (4). According to measurements in Sweden in 2008 (5), the electricity consumption per bird was 0.13 kWh/bird. Another 0.78 kWh/bird must be added for heating and manure handling. In 2009, Liang et al. (6) measured the electrical energy consumption in a renovated chamber to 0.102 kWh/kg in Northwest Arkansas. In 2012 (7), the total energy consumption in an insulated broiler house in Finland was measured to 1.83 kWh/kg. This measurement corresponds to electrical energy consumption for lighting (0.009 kWh/kg), for ventilation (0.021 kWh/kg), and for heating (1.8 kWh/kg). It is obvious that the mechanization of broiler houses increased the percentage of electrical energy consumed for ventilation compared to the '80s. In 2016, in surveying concerning 29 broiler farms in Turkey, the machinery energy consumption was 0.078 kWh/bird (8).

The calculated electrical energy consumption, from our work, is of the order of 0.12–0.16 kWh/kg for lowland farms with increased cooling loads and of 0.07 kWh/kg for mountainous farms. This is in agreement with other researchers' findings' who refer consumption of 0.102 kWh/kg in Arkansas in 2009 (6) and 0.078 kWh/kg in Turkey in 2016 (8), while being at odds with predictions of 0.03 kWh/kg in Finland in 2012 (7) with characteristically low cooling demand (0.021 kWh/kg in Finland for ventilation and 0.06 to 0.1 kWh/kg for cooling and ventilation in Greece). Nevertheless, the energy consumption for lighting in the examined cases was 0.01 kWh/kg which agrees with the Finland predictions (7) of 0.009 kWh/kg. The total final energy consumption per bird varies from 0.66 kWh/bird (lowland–new technology farms) to 1.05 kWh/bird (mountainous–old technology farms), which coincides with measurements of 0.91 kWh/bird consumption in Sweden (5) at 2008, although this depends on the final birds' weight.

From the results, it is obvious that the bigger energy consumer is the heating, especially for mountainous farms. This is in line with other researchers' findings in [Michigan 1989 71% (3), Sweden 2008 85% (5), Finland 2012 98% (7)], especially in the case of mountain chambers where heating represents 84% of energy consumption. Heating is usually provided with thermal energy. However, electrical energy consumption is also important, especially in lowland farms. The biggest percentage of electrical consumption is due to ventilation and cooling (73% for the new technology farms and 67% for the old technology farms); this is also in line with the findings of (7) where they found that 70% of the electrical energy is consumed for ventilation. In electrical energy consumption, the bigger share belongs to ventilation and cooling. Feeding represents a standard consumption. Lighting energy consumption can be significantly reduced with the use of energy-efficient lights as is proved in the new technology chambers.

As far as final energy consumption in the lowland farm is concerned, new technology offers a 31% energy save compared with old technology chambers. For mountainous farms, this save is restricted to 27% which is yet important. The final energy consumption in lowland farms is 30–34% lower than in mountainous farms. The energy saving for heating due to insulation of the energy consumption is of the order of 30% higher than the 10% predicted for Canada in 1988 (3).

In terms of primary energy, new technology offers a 27% energy save in lowland farms. In mountainous farms, the primary energy reduction achieved with new technology is 24%. Since in lowland farms the share of electrical energy is big, the achieved reduction of primary energy consumption in lowland farms in comparison with the mountainous varies from 2 to 7%.

Finally, the CO_2 emissions can be calculated from the split energy consumption presented in **Table 4**. Thus, lowland new technology chambers present a 26% reduction in CO_2 emission compared to old technology. Mountainous new technology chambers reduce CO_2 emissions by 22%. However, the emitted CO_2 by the lowland farms is 7–11% higher than the mountainous farms' emissions.

Proposals to Improve the Energy Performance of Broiler Facilities

Energy-Saving Measures

The energy consumed in broiler units for heating varies from 55% in lowland farms to 85% in mountainous farms. Heat losses in a broiler house have two basic sources. The first is the heat losses through the chamber shell due to conduction–convection. These losses are directly affected by the building insulation. The second source of loss is ventilation since the necessary fresh air that is supplied to the building must be air conditioned (e.g., heated or cooled). The reduction of these energy losses can be achieved either with precise control of the supplied fresh air either with heat recovery from the exhaust air.

According to the analysis presented in (32), an insulation thickness of 4–5 cm is appropriate for small and big chambers since thicker insulation cannot offer further significant benefits. For the mountainous chamber, a little bit thicker insulation of 6–7 cm to achieve proper insulation levels is proposed. The smaller the size of the chamber, the greater the role of insulation. A chamber without any insulation can have up to three times the thermal needs of an elementally insulated one, especially when the insulation concerns the roof. In mountainous farms, the losses through the walls are comparable to the losses due to ventilation and therefore the cost of insulation as a function of energy costs determines the optimal thickness.

When an adequate Um value has been achieved, the ventilation losses become the big source of heat losses. In an insulated lowland chamber, the ventilation heat losses are three times the shell heat losses, while in an insulated mountainous chamber the ventilation heat losses are twice the shell heat losses. In practice, this is much bigger since farmers used to supply much more than the necessary fresh air in the chambers.

Thus, the next proposed measure for energy saving is the precise control of the supplied fresh air according to the real needs of birds. For this, the existence of a net for the measurement of internal microclimate inside the chamber is necessary. These measurements contain temperature, humidity, airspeed, and NH_3 concentration. Since the existence of such a net is expensive, the measurements can concern only a few sensors provided that software will be used to assess the real microclimate in the whole chamber and that these few sensors are located in the appropriate positions inside the chamber. A system for the precise control of ventilation also includes inverter-equipped fans controlled by a central unit.

The use of automation in heating and cooling also can offer significant energy saving as shown by the comparison of new and old technology chambers. Automation in feeding and water supply equipment is already commonplace in all types of broiler farms. Further energy consumption reduction can be achieved with the use of motors equipped with inverters.

The use of energy-efficient lights can offer energy save of the order of 5% as proved by the energy audit results. Two other general measures are the correct sizing of the energy-consuming electromechanical equipment, as there is usually a tendency to oversize and the use of electromechanical equipment with high efficiency. Finally, a general measure for energy saving is the proper maintenance of the equipment that will allow it to work at the optimum degree of efficiency.

Local Energy Production

Broiler units can also be energy producers. The local energy production can improve the units' energy balance, reducing the energy intensity of the breeding. In order to size RES systems, the time profile of consumption must be known.

The use of photovoltaics for local energy production is a very attractive choice for a broiler house since large roof areas are available. In fact, in a broiler house, the entire roof is available regardless of orientation due to the small angle of the pitched roof. The cost of produced kWh from PV depends on the installed power and available solar potential. Thus, in Greece, this cost ranges from 0.13 €/kWh for a small installation of the order of 3 kW to 0.073 €/kWh for an installation of 20 kW and up to 0.063 €/kWh for an installation of 100 kW. An auditor can examine three scenarios: (i) power production for sale to the grid, (ii) power production for net-metering (which is a billing mechanism that credits solar energy system owners for the electricity they add to the grid), and (iii) stand-alone PV installation with batteries for energy autonomy.

The first scenario can be examined for the cases in which the price of sale of kWh to the grid is higher than the cost of produced energy.

The second scenario, in the countries where the net-metering holds, usually is the preferred scenario since the cost of energy production by PV should be compared with the cost of purchasing the energy from the grid. In the case of net-metering, the annual energy production from PV is calculated and compared with the annual demand. The PV configuration that gives the minimum possible negative annual balance value is chosen as optimal. For the solar potential of Greece, this may lead to an installed power of (i) 8 kW for a small mountainous chamber (600 m^2), (ii) 13 kW for a small lowland chamber (600 m^2), (iii) 16 kW for a big mountainous chamber (1,200 m^2), and (iv) 25 kW for a big lowland chamber (1,200 m^2).

Finally, the cost of kWh for a stand-alone system is usually higher from 0.28 to 0.35 €/kWh according to (37). Thus, the stand-alone PV system may be attractive only for isolated units.

For a low wind potential, with a yearly average wind velocity of the order of 3.5 m/s at a height of 10 m and according to the yearly time profile of electrical power consumption, a wind turbine of (i) 10 kW for a small mountainous chamber (600 m^2), (ii) 15 kW for a small lowland chamber (600 m^2), (iii) 20 kW for a big mountainous chamber (1,200 m^2), and (iv) 25 kW for a big lowland chamber (1,200 m^2) will be needed. For such low wind potential, the chosen wind turbine is required to have a rated wind velocity of the order of 7 m/s and a cut-in wind velocity of the order of 2 m/s. However, it is not very easy to find wind turbines to cover these requirements. The cost of energy production varies from 0.18 to 0.28 €/kWh. Thus, the use of wind turbines in a low wind potential could be attractive only if the cost of purchase of electricity from the grid is higher or is in isolated areas. However, if the wind potential is important the cost of energy production may decrease to 0.05 €/kWh.

Solar thermal energy can be used to cover the self-consumption for biogas production. Since initial heating of the biogas reactor requires high temperatures that must be achieved in a short time, it will be considered that these will be covered by burning biogas and only the heat losses of the reactors will be covered by thermal solar systems. Underfloor heating may be considered only for new chambers. Nevertheless, this requires important modification to the chamber basic construction since it requires replacement of the bedding with flooring with special specifications that allow the birds to live safely, have special consideration for manure management, and do not impede heat transfer. Another way to utilize thermal solar energy is in combination with heat pumps provided that the appropriate air duct heating system has been selected. Since there is no need for a high water temperature in the above applications, the proposed type is the flat selective collector. The use of concentrating solar collectors in these applications would not offer an advantage.

Shallow geothermal systems combined with (38) both heat pumps and soil heating applications to agricultural activities (e.g., asparagus) proved advantageous, resulting in a discounted thermal energy unit cost of <45 €/MWhth contributing an internal rate of return on investment up to 24%. Nevertheless, in existing poultry facilities, the use of shallow geothermal energy would require the use of an underfloor heating system or the collaboration with a heat pump. The cost of replacement of existing heating/cooling systems only for improving the energy efficiency is considered prohibitive.

The basic method that is suggested for the utilization of the produced biomass of broiler farms is anaerobic fermentation (39–41). The raw material used as biomass is bird manure mixed with the litter since in this type of unit no separation can be done. According to the literature, anaerobic fermentation leads to biogas production (with 50–60% CH_4). This method is well established in some livestock facilities (e.g., pigsties, cowsheds); however, in the case of broiler farms, some particular problems are faced in the application of this method: (i) discontinuous feeding of the reactor with biomass, (ii) requirement to purchase necessary additives (to set required C:N ratio), (iii) water management, (iv) self-consumption for the reactor operation, and (v) energy utilization of discontinuously produced biogas (the biogas will be produced when the breading is over and so it should be stored).

Unlike in other livestock facilities where manure is collected on a daily basis or at a fixed time step and has a constant supply over time, in broiler farms manure can only be collected at the end of the breading (five times a year).

If the bedding is straw before feeding it to in the reactor, pretreatment should be done to reduce the size of the straw pieces. Regardless of the type of litter (straw or rice husks) before the introduction of the mixture into the reactor, additional material from agricultural residues should be added for the mixture to obtain the necessary organic load (set to required C:N ratio 20–40). Of course, the amount and characteristics of the additives depend on the type of litter since a different litter means a different chemical composition of the collected biomass.

In any case, water should be added if the humidity of the specific livestock waste is very low (required dry matter for the case of horizontal reactor 15–20%, while for the case of vertical reactor 10–15%). The management of the water used becomes a major problem since it must be cleaned of nitrogen before being reused or disposed of in the environment.

The produced biogas must be purified from H_2S before its use. The biogas can be used either to generate electricity by supplying an internal combustion engine that drives an electric generator, or to generate heat by combustion in a gas boiler, or to simultaneously generate electricity and heat in a combined heat and power (CHP) unit.

Here are some general guidelines for the technology used. The use of a vertical reactor (300–1,500 m^3) with a batch operation is suggested. Initial heating for 1 h at 70°C and fermentation in the mesophilic area (35°C), with residence time in the reactor, is 35–40 days.

No further general guidelines can be given, and each case should be studied individually according to the size of the chambers, the type of used litter, the type of used heating, and cooling systems and mainly the timing of breeding between the different chambers of a unit. In a multichamber broiler farm, proper synchronization of breeding between chambers can reduce the problem of discontinuous biomass production. In addition, in the case where several units use a common biogas unit, the same can be achieved by synchronizing the breeding between the different farms, provided, of course, that the timing of the breeding does not affect other more important parameters of the breeding.

CONCLUSIONS

The consumed energy at poultry facilities varies from 46 to 89 kWh/m^2 of chamber area or from 0.25 to 0.48 kWh/kg of produced meat depending on the chamber technology level (insulation, automation, etc.) and the location where the unit is installed. However, in terms of primary energy, the above energy indices become 91–126 kWh/m^2 and 0.5–0.69 kWh/kg. The bigger energy consumer is heating followed by energy consumption for ventilation and cooling. Advanced technology levels can improve energy performance up to 27–31%.

Proper insulation (4–7 cm depending on the location) can offer a reduction of thermal energy consumption between 10 and 35%. In adequately insulated chambers, the basic heat losses are due to ventilation. Thus, further energy saving can be achieved with precise control of ventilation according to the real needs of birds. The use of automation can offer an additional save of electrical energy consumption for cooling and ventilation

(15–20%). The use of energy-efficient lights can offer energy savings up to 5%.

Energy intensity in broiler facilities can be reduced through local energy production. The use of PV is suggested mainly in areas where net-metering holds. The use of wind turbines is feasible only when adequate wind potential is available to reduce the cost of producing energy lower than the cost of purchasing energy from the grid or for isolated areas. A thermal solar system is suggested in combination with a heat pump if adequate systems for heating and cooling are used.

Finally, the local production of biogas with anaerobic fermentation for producing thermal or electrical energy, or cogenerating both, is a choice that should be studied individually for each farm depending on the type of the litter, the synchronization among the breading of different farms, and the availability of additives. In any case, special attention must be paid to the management of the water that will be used to add to the biomass for the necessary moisture.

The presented energy audit protocol can be a useful tool to reduce the energy and environmental footprint of broiler farms.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CB and DF: conceptualization, methodology and writing–original draft preparation. CB, DF, IG, EB, and IS: validation, investigation, data curation, writing–overview, and editing. IS: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. European Forum (2009). *Livestock housing for the future* [Online]. European Forum. Available online at: https://docplayer.net/134373164-Livestock-housing-for-the-future.html (accessed 10th October 2013).
2. Southwell PH, Rothwell TM. "*Report on analysis of output/input energy ratios of food production in Ontario*". Guelph, Ontario: School of Engineering, University of Guelph. (1977)
3. Barber EM, Classen HL, Thacker PA. Energy use in the production and housing of poultry and swine–an overview. *Can J Anim Sci.* (1989) 69 :7–21. doi: 10.4141/cjas89-002
4. Tabler GT. Applied broiler research farm report: Production results before and after renovation. *Avian Advice*. (2008) 9:2.
5. Hörndahl T. *Energy Use in Farm Buildings – A Study of 16 Farms With Different Enterprises*. Swedish University of Agriculture Sciences, Report ISSN 1654–5427, Alnarp (2008).
6. Liang Y, Tom Tabler G, Watkins E, Susan Xin H, Berry L, Ivan. *Energy Use Analysis of Open-Curtain vs. Totally Enclosed Broiler Houses in Northwest Arkansas. 2008 Providence*. Rhode Island: St. Joseph, MI. (2008). doi: 10.13031/2013.24756
7. Rajaniemi M, Ahokas J. A case study of energy consumption measurement system in broiler production. *Agronomy Res.* (2012) 10:195–204.
8. Kilic I. Analysis of the energy efficiency of poultry houses in the Bursa region of Turkey. *J Applied Animal Res.* (2016) 44:165–72. doi: 10.1080/09712119.2015.1021813
9. De Vries M, De Boer IJM. Comparing environmental impacts for livestock products: A review of life cycle assessments. *Livestock Sci.* (2010) 128:1–11. doi: 10.1016/j.livsci.2009.11.007
10. Baxevanou C, Fidaros D, Bartzanas T, Kittas C. *Energy consumption in poultries – energy audits in Greece*. Zurich, Switcherland: International Conference of Agricultural Engineering (AgEng 2014). (2014)
11. European Council, (1993), Council Directive 93/76/EEC to limit carbon dioxide emissions by improving energy efficiency, Brussels, European Council
12. European Council. *Directive 2002/91/EC of the European Parliament and of the Council on the energy performance of buildings*. Brussels: European Council (2002)
13. European Council. *Directive 2012/27/EU of the European Parliament and of the Council on energy efficiency*. Brussels: European Council (2012)
14. European Council. *Directive 2018/844 of the European Parliament and of the Council amending Directive 2010/31/EU on the energy performance of buildings and Directive 2012/27/EU on energy efficiency*. Brussels: European Council (2018)
15. European Council. *Directive 2010/31/EU of the European Parliament and of the Council on the energy performance of buildings*. Brussels: European Council (2010)
16. Moris M, Hill H, Mumma T, Kurki A. *Farm Energy Calculators: Evaluations and Recommendations*. National Center of Appropriate Technology Report, Butte, Montana (2009).
17. European Council. *Commission Delegated Regulation 2019/826 amending Annexes VIII and IX to Directive 2012/27/EU on the contents of comprehensive assessments of the potential for efficient heating and cooling*. Brussels: European Council (2019)
18. European Council. *Regulation 2017/1369 of the European Parliament and of the Council on setting a framework for energy labeling and repealing Directive 2010/30/EU*. Brussels: European Council (2017)
19. European Council. *Directive 2009/125/EC of the European Parliament and of the Council on establishing a framework for the setting of ecodesign requirements for energy-related products*. Brussels: European Council (2009)
20. Batir U. *Wind energy proposal for a poultry farm* [Online]. (2014). Available online at: https://www.academia.edu/12010128/Wind_Energy_Proposal_for_a_Poultry_Farm (accessed 18 October 2020).
21. Morgan R. *Harnessing Solar Energy for Broiler Production* [Online]. University of Delaware. (2008). Available online at: https://docplayer.net/11750540-Harnessing-solar-energy-for-broiler-production.html (accessed 12 October 2014).

22. Byrne J, Glover L, Hegedus S, Van Wicklen G. *The Potential of Solar Electrical Applications for Delaware's Poultry Farms'*. University of Delaware: Center for Energy and Environmental Policy Report, Newark, Delaware (2005).
23. Bazen FE, Brown AM. Feasibility of solar technology (photovoltaic) adoption: A case study on Tennessee's poultry industry. *Renew Energy*. (2009) 34:748–54. doi: 10.1016/j.renene.2008.04.003
24. Baxevanou C, Fidaros D, Bartzanas T, Katsoulas N. Effect of roof PV panels on poultry house microclimate. In: *11th National Conference for Renewable Energy Sources*, editor. *I.O.S. Energy: IHT*. p. 663–74.
25. Okonkwo WI, Akubuo CO. Thermal analysis and evaluation of heat requirement of a passive solar energy poultry chick brooder in Nigeria. *Nigerian J Renew Energy*. (2001) 9:83–87.
26. Cui Y, Theo E, Gurler T, Su Y, Saffa R. A comprehensive review on renewable and sustainable heating systems for poultry farming. *Int J Low-Carbon Technolog*. (2019) 15:121–42. doi: 10.1093/ijlct/ctz048
27. Gad S, El-Shazly MA, Wasfy KI, Awny A. Utilization of solar energy and climate control systems for enhancing poultry houses productivity. *Renew Energy*. (2020) 154:278–89. doi: 10.1016/j.renene.2020.02.088
28. Busoul M, Elayyan M. Utilization of geothermal energy in poultry farming. *J Energy Technolog Policy*. (2014) 4:25–33.
29. Choi HC, Salim HM, Akter N, Na JC, Kang HK, Kim MJ, et al. Effect of heating system using a geothermal heat pump on the production performance and housing environment of broiler chickens. *Poultry Sci*. (2012) 91:275–81. doi: 10.3382/ps.2011-01666
30. Baxevanou C, Fidaros D, Bartzanas T, Kittas C. Energy Consumption and Energy Saving Measures in Poultry. *Int J Energy Environ Eng*. (2017) 5:29–36. doi: 10.13189/eee.2017.050201
31. Ibarra M. *Farm energy audit report to: John Sample* [Online]. (2009). Available online at: https://www.ensave.com/energy-audit (accessed 2 March 2014).
32. International Organization for Standardization. *EN ISO 13790 - Energy performance of buildings - Calculation of energy use for space heating and cooling*. Geneva, Switzerland (2008)
33. International Organization for Standardization. *EN ISO 6946 - Building components and building elements - Thermal resistance and thermal transmittance – Calculation method*. Geneva, Switzerland (2007)
34. Czarick M, Fairchild B. Poultry Housing Tips: Oxygene. The University of Georgia, College of Agricultural and Environmental Science/Athens: Georgia. (2013)
35. Barott HG, Fritz JC, Pringle EM, Titus HW. Heat Production and Gaseous Metabolism of Young Male Chickens: Eight Figures. *J Nutrit*. (1938) 15:145–67. doi: 10.1093/jn/15.2.145
36. Technical Chamber of Greece. *Technical Guidance of Technical Chamber of Greece TOTEE 20701-3-2010 – Climatic Data*. Athens Greece: Technical Chamber of Greece. (2010)
37. Baxevanou C, Fidaros D, Tsintzos P, Schinas C, Bartzanas T, Kittas C. Design and Performance of Hybrid Power Systems. 5th International Conference on Renewable Sources & Energy Efficiency New Challenges. Nicosia, Cyprus. (2016)
38. Dalampakis P, Gelegenis J, Ilias A, Ladas A, Kolios P. Technical and economic assessment of geothermal soil heating systems in row covered protected crops: A case study from Greece. *Appl Energy*. (2017) 203:201–18. doi: 10.1016/j.apenergy.2017.06.029
39. Bujoczek G, Oleszkiewicz J, Sparling R, Cenkowski S. High solid anaerobic digestion of chicken manure. *J Agri Engineer Res*. (2000) 76:51–60. doi: 10.1006/jaer.2000.0529
40. Zhang T, Yang Y, Liu L, Han Y, Ren G, Yang G. Improved biogas production from chicken manure anaerobic digestion using cereal residues as co-substrates. *Energy & Fuels*. (2014) 28:2490–95. doi: 10.1021/ef500262m
41. Sträuber H, Bühligen F, Kleinsteuber S, Nikolausz M, Porsch K. Improved anaerobic fermentation of wheat straw by alkaline pre-treatment and addition of alkali-tolerant microorganisms. *Bioengineering (Basel, Switzerland)*. (2015) 2:66–93. doi: 10.3390/bioengineering20 20066

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β-Mannanase Supplementation as an Eco-Friendly Feed Strategy to Reduce the Environmental Impacts of Pig and Poultry Feeding Programs

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Little is still known about the environmental impacts of exogenous enzyme supplementation in pig and poultry feeding programs. Thus, this study aimed to assess the potential environmental impacts of producing feeds for pigs and broilers by simulating the effects of β-mannanase *Hemicell*™ *HT* supplementation through energy savings during diet formulation. Life-cycle assessment standards were applied to simulate a cradle-to-feed mill gate scope. The functional units used were the production of 1 kg of the enzyme and 1 kg of feed at a feed mill gate located in Concórdia, Santa Catarina, Brazil. Climate change, eutrophication, and acidification were the chosen environmental impact categories. Energy savings through β-mannanase supplementation were assessed by different metabolizable energy (ME) matrices (45 or 90 kcal of ME/kg of feed) during diet formulation in different grain production scenarios (Southern and/or Central-West origin). A total of 28 feeds were formulated based on the nutritional requirements and feeding programs described in the Brazilian Tables for Poultry and Swine. The least-cost formulation method was used based on real price averages practiced in a local industry over 12 months. The production of 1 kg of β-mannanase was associated with the emission of 1,800 g of CO_2-eq, 4.53 g of PO_4-eq, and 7.89 g of SO_2-eq. For pig feeds, β-mannanase supplementation mitigated both climate change and eutrophication impacts up to 8.5 and 1.4% (45 kcal of ME/kg of feed) or up to 16.2 and 2.7% (90 kcal of ME/kg of feed) compared to control diets formulated without the enzyme. For broiler feeds, these impacts were mitigated up to 5.6 and 1.1% (45 kcal of ME/kg of feed), respectively. On the other hand, the effect of using β-mannanase on the acidification impact was not consistent among feeds/species. Overall, β-mannanase supplementation reduced the amount of soybean oil in feed formulas, which is associated with high environmental impacts.

Consequently, the potential impacts of climate change and eutrophication associated with producing feeds for pigs and broilers were substantially mitigated. These results suggest that β-mannanase supplementation is an eco-friendly feed strategy to reduce the environmental impacts of pig and poultry feeding programs.

Keywords: swine, broiler, environment, feed, enzyme, climate change, sustainability, life-cycle assessment

INTRODUCTION

Pig and poultry feeding programs require a huge amount of feed resources, with several studies indicating feeding as a major source of environmental impact (1–3). A systematic review recently developed by Andretta et al. (4) on the use of life cycle analysis confirmed the importance of feeding processes as the largest source of environmental impact associated with pig and poultry production. In their review, the relative participation of feed production in the overall greenhouse gas emissions varied from 31 to 76% or 28 to 82% for the pig and poultry databases, respectively (4). Regardless of the exact amount of impact attributed to feeding, practically all studies indicated feeding as the most important environmental impact source. These results support the hypothesis that novel feeding strategies could be used as eco-friendly strategies to mitigate the environmental impacts of pig and poultry production.

The use of exogenous enzymes has been highlighted as a promising alternative to mitigate the environmental impacts of livestock (5, 6). Nonetheless, pigs and poultry lack some enzymes, such as β-mannanase, to completely digest β-mannans commonly present in a great variety of feedstuffs, including soybean, corn DDG, sunflower, copra, and palm kernel meal-based diets. This may reduce growth performance once β-mannans are associated with increased intestinal viscosity and decreased nutrient digestibility, following an inflammatory process initiated in response to the β-mannans presence (7–9). *Hemicell*™ *HT* is a source of β-mannanase, an energy-sparing enzyme that hydrolyzes β-mannans, avoiding the inflammatory reaction (10). β-mannanase supplementation can then potentially improve the nutrient digestibility and growth performance of pigs and broilers. In addition, when an energy matrix is attributed to the enzyme during feed formulation, some resources are saved, leading to an increase in energy-use efficiency.

Despite the importance of both pig and poultry sectors in developing countries, most studies that assessed the environmental impacts of exogenous enzyme supplementation had been developed based on European and North American conditions, with limited applicability to other major pig and poultry production regions. In addition, little is still known about the environmental impacts of using β-mannanase in feeding programs. Therefore, this study aimed to assess the potential environmental impacts of producing feeds for pigs and broilers by simulating the effects of β-mannanase *Hemicell*™ *HT* supplementation through energy savings during diet formulation.

Abbreviations: CO_2-eq, carbon dioxide equivalent; CW, Central-West region; CW-CW, the scenario in which only grains from the Central-West region were used to produce the feed; CW-SO, the scenario in which soybeans from the Central-West region and corn from the Southern region were used to produce the feed; DE: digestible energy; LCA, life-cycle assessment; ME, metabolizable energy; SO_2-eq, sulfur dioxide equivalent; SO, Southern region; SO-SO, the scenario in which only grains from the Southern region were used to produce the feed; PO_4-eq, phosphate equivalent.

MATERIALS AND METHODS

Environmental impacts were assessed according to life-cycle assessment (LCA) standards based on four interrelated steps, described by Guinée (11) as (i) goal and scope definition, (ii) life cycle inventory, (iii) life cycle impact assessment, and (iv) interpretation of results. Brazil was chosen because it is a large producer and exporter of pork and chicken meat. For this study, in a cradle-to-feed mill gate scope, the major stages considered in the model were the production of β-mannanase *Hemicell*™ *HT*, the production of feed ingredients from plant sources (corn and soybean meal), and the production of the other feed ingredients (including amino acids, limestone, dicalcium phosphate, salt, and vitamin-mineral premix). Drying and processing in the feed industry as well as transportation were also considered, as illustrated in **Figure 1**. The functional unit used to study the environmental cost of feedstuff (especially for enzyme or grain production) was 1 kg of each ingredient at the feed factory. The functional unit used to study potential environmental impacts associated with feeds was 1 kg of feed manufactured and ready to be delivered to the farm (at the feed mill gate). The animal phase was not included in the scope due to limitations in data availability, mainly on the impact of β-mannanase supplementation (e.g., enteric fermentation). In addition, previous evidence showed no differences in performance (i.e., feed efficiency and nutrient metabolism) when supplementing β-mannanase with an energy matrix attributed during diet formulation (12).

Description of the Pig and Poultry Production Systems Evaluated

An inventory for β-mannanase *Hemicell*™ *HT* production was developed using detailed information provided by the manufacturer company (Elanco Animal Health, Greenfield, IN, US). Energy requirements (electricity, heating, and cooling) and emissions of CO_2 during enzyme production were adapted from Gilpin et al. (13). Simulations considered enzyme production in the industrial plant at Greenfield (Delphos, US), followed by road transportation using trucks and marine transportation using cargo ships until arriving at the feed factory in Brazil.

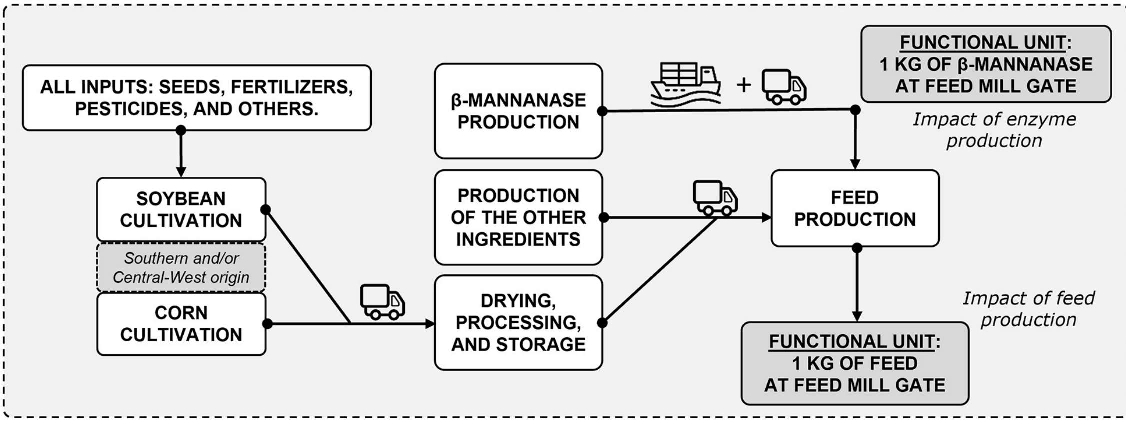

FIGURE 1 | Flowchart of the pig and poultry feeding programs being assessed through life-cycle assessment standards. Crop inputs, crop production, β-mannanase production, production of the other feed ingredients, drying, processing, storage, transportation, and feed production were the main processes considered, with system boundaries including all sub-processes.

TABLE 1 | Composition of nursery pig feeds[a].

	Pre-starter				Starter	
	Complex		Simple			
	Control	β-mannanase[b]	Control	β-mannanase	Control	β-mannanase
Ingredient (as-fed basis), %						
Corn	57.08	58.25	54.30	55.47	55.08	56.25
Soybean meal	12.00	11.88	25.00	24.88	37.87	37.75
Soybean oil	1.61	0.52	2.52	1.44	3.35	2.27
Meat and bone meal	5.00	5.00	5.00	5.00	-	-
Soybean isolate protein	8.13	8.13	4.30	4.30	-	-
Spray-dried plasma	5.00	5.00	2.50	2.50	-	-
Whey	10.00	10.00	5.00	5.00	-	-
L-lysine HCL	0.11	0.11	0.17	0.17	0.29	0.29
DL-methionine	0.10	0.10	0.11	0.11	0.13	0.13
L-threonine	0.05	0.05	0.09	0.09	0.12	0.12
L-valine	0.02	0.02	0.02	0.02	0.05	0.05
Salt	0.22	0.22	0.21	0.21	0.19	0.19
Limestone	0.41	0.41	0.42	0.42	1.05	1.05
Dicalcium phosphate	-	-	-	-	1.13	1.13
Vitamin-mineral premix	0.50	0.50	0.50	0.50	0.50	0.50
β-mannanase	-	0.03	-	0.03	-	0.03
Calculated chemical composition[c]						
Crude protein, %	23.81	23.81	24.08	24.08	21.88	21.88
SID[d] lysine, %	1.35	1.35	1.35	1.35	1.28	1.28
Metabolizable energy, kcal/kg	3,375	3,375	3,375	3,375	3,350	3,350
Digestible phosphorus, %	0.53	0.53	0.51	0.51	0.45	0.45

[a] *Pre-starter and starter feeds were formulated based on animals with 33–42 and 49–63 days of age, respectively, with 10.8 and 22.5 kg of body weight on average, respectively.*
[b] *90 kcal of metabolizable energy/kg of feed was the energy matrix attributed to the enzyme during diet formulation of nursery piglet feeds.*
[c] *Values were estimated considering the Brazilian Tables for Poultry and Swine (22).*
[d] *Standardized ileal digestible.*

All other simulations were developed considering a feed mill located in Concórdia (Santa Catarina, Brazil) since it represents a traditional pig and poultry producing region in Southern Brazil. Grain production was independently characterized in both Central-West (CW) and Southern (SO) regions in Brazil, as described by Andretta et al. (14). Crop farm locations were

TABLE 2 | Composition of growing pig feeds[a].

	Growing I			Growing II		
	Control	β-mannanase[b]		Control	β-mannanase	
		45 kcal	90 kcal		45 kcal	90 kcal
Ingredient (as-fed basis), %						
Corn	68.60	69.59	70.63	73.69	74.67	75.72
Soybean meal	23.43	23.31	23.19	19.73	19.61	19.49
Soybean oil	2.22	1.32	0.41	1.89	1.00	0.08
Meat and bone meal	3.35	3.34	3.33	2.39	2.38	2.37
L-lysine HCL	0.49	0.49	0.49	0.46	0.46	0.46
DL-methionine	0.19	0.19	0.19	0.15	0.15	0.15
L-threonine	0.19	0.19	0.19	0.17	0.17	0.16
L-tryptophane	0.06	0.06	0.06	0.05	0.05	0.05
L-valine	0.07	0.07	0.07	0.06	0.05	0.05
Salt	0.39	0.39	0.39	0.38	0.38	0.38
Limestone	0.51	0.52	0.52	0.54	0.54	0.55
Vitamin-mineral premix	0.50	0.50	0.50	0.50	0.50	0.50
Phytase	0.01	0.01	0.01	0.01	0.01	0.01
β-mannanase	-	0.03	0.03	-	0.03	0.03
Calculated chemical composition[c]						
Crude protein, %	18.31	18.33	18.35	16.51	16.53	16.56
SID[d] lysine, %	1.16	1.16	1.16	1.03	1.03	1.03
Metabolizable energy, kcal/kg	3,350	3,350	3,350	3,350	3,350	3,350
Digestible phosphorus, %	0.38	0.38	0.38	0.33	0.33	0.33

[a] *Growing I and growing II feeds were formulated based on animals 70–84 and 91–105 days of age, respectively, with 40 and 60 kg of body weight on average, respectively.*
[b] *45 and 90 kcal of metabolizable energy/kg of feed were the energy matrices attributed to the enzyme during diet formulation of growing pig feeds.*
[c] *Values were estimated considering the Brazilian Tables for Poultry and Swine (22).*
[d] *Standardized ileal digestible.*

chosen based on rankings of the largest corn- and soybean-producing municipalities within each region (15). Agricultural practices for grain production and the models used to calculate their emissions were adapted from Alvarenga (16), Alvarenga et al. (17), and Prudêncio da Silva et al. (18). The land transformation was estimated based on the data provided by Alvarenga (16), following the methodology described by Prudêncio da Silva et al. (18). Grain yield data were obtained from the Brazilian Institute of Geography and Statistics (15) for each municipality.

As pointed out by Prudêncio da Silva et al. (18), the environmental footprint of grain production depends on the Brazilian region being considered for crop cultivation. Thus, three geographic scenarios were simulated based on different crop cultivation locations: CW-CW, in which only grains from CW were used to produce feeds; CW-SO, in which soybean from CW and corn from SO were used to produce feeds; and SO-SO, in which only grains from SO were used to produce feeds. These scenarios differed in terms of road transportation distances, agricultural practices, and deforestation impact on recently opened agricultural frontiers (deforestation was assumed for the CW region but not included for the characterization of production in the SO region). Information from the Ecoinvent database (v. 3.0, Swiss Center for Life Cycle Inventories, Dübendorf, Switzerland) was used to characterize soybean oil production. A process with solvent was applied for obtaining the product, with no geographical scenarios considered for oil production.

The impact of phytase supplementation was simulated considering the information provided by Nielsen et al. (5). The scope of synthetic amino acid production was adapted from Mosnier et al. (19), distinguishing amino acids produced by chemical synthesis (DL-methionine) from those produced by fermentation (L-lysine, L-threonine, L-tryptophane, and L-valine). All other feed ingredients were based on available databases. The Ecoinvent database (v. 3.0, Swiss Center for Life Cycle Inventories, Dübendorf, Switzerland) was used to characterize the production of meat and bone meal, sodium chloride, and limestone. The environmental impacts of vitamin-mineral trace elements were assumed to be equal to those of limestone. On the other hand, the environmental impacts of soybean protein isolate and whey were based on the AgriFootPrint database (v. 5.0, Blonk Consultants, Gouda, The Netherlands).

Grain processing and storage conditions were adapted from previous reports (20, 21). Transportation of grains (from the farm to the feed factory), other ingredients (from the industry to the feed factory), and feeds (from the feed factory to the

TABLE 3 | Composition of finishing pig feeds[a].

	Finishing I			Finishing II		
	Control	β-mannanase[b]		Control	β-mannanase	
		45 kcal	90 kcal		45 kcal	90 kcal
Ingredient (as-fed basis), %						
Corn	78.98	79.97	78.66	84.68	85.67	84.61
Soybean meal	15.56	15.45	17.51	10.76	10.64	11.58
Soybean oil	1.60	0.71	-	1.33	0.43	-
Meat and bone meal	1.65	1.64	1.58	1.17	1.16	1.14
L-lysine HCL	0.43	0.43	0.37	0.39	0.39	0.36
DL-methionine	0.12	0.12	0.10	0.07	0.07	0.06
L-threonine	0.14	0.14	0.11	0.10	0.10	0.09
L-tryptophane	0.05	0.05	0.04	0.04	0.04	0.04
L-valine	0.04	0.04	0.00	0.02	0.02	0.00
Salt	0.36	0.36	0.36	0.35	0.35	0.35
Limestone	0.57	0.57	0.74	0.59	0.59	1.23
Vitamin-mineral premix	0.50	0.50	0.50	0.50	0.50	0.50
Phytase	0.01	0.01	0.01	0.01	0.01	0.01
β-mannanase	-	0.03	0.03	-	0.03	0.03
Calculated chemical composition[c]						
Crude protein, %	14.62	14.65	15.34	12.58	12.60	12.88
SID[d] lysine, %	0.90	0.90	0.90	0.75	0.75	0.75
Metabolizable energy, kcal/kg	3,350	3,350	3,350	3,350	3,350	3,350
Digestible phosphorus, %	0.28	0.28	0.28	0.25	0.25	0.25

[a] *Finishing I and finishing II feeds were formulated based on animals with 112–133 and 140–161 days of age, respectively, with 85 and 112.5 kg of body weight on average, respectively.*
[b] *45 and 90 kcal of metabolizable energy/kg of feed were the energy matrices attributed to the enzyme during diet formulation of finishing pig feeds.*
[c] *Values were estimated considering the Brazilian Tables for Poultry and Swine (22).*
[d] *Standardized ileal digestible.*

pig farm) were assumed to have been done by truck, with the exception of enzymes that also included marine transportation. The Google Earth software (Google Inc., Mountain View, CA) was used to estimate transportation distances. Information from the Agri-footprint database (v. 5, Blonk Consultants, Gouda, The Netherlands) was used to simulate the impact of transportation.

Feeding Practices

Ingredients commonly used in Brazil were used to formulate feeds. Soybean meal was the major protein source, combined with corn and refined soybean oil as the major energy suppliers. A total of 28 feeds (**Tables 1–4**) were formulated based on the nutritional requirements and feeding programs described in the Brazilian Tables for Poultry and Swine (22). For pigs, pre-starter, starter, growing I, growing II, finishing I, and finishing II feeds were formulated based on animals with 33–42, 49–63, 70–84, 91–105, 112–133, and 140–161 days of age, respectively, and 10.8, 22.5, 40, 60, 85, and 112.5 kg of body weight, respectively. For broilers, starter I, starter II, growing I, growing II, and finishing feeds were formulated based on animals with 1–7, 8–21, 22–33, 34–42, and 43–46 days of age, respectively, and 0.14, 0.59, 1.65, 2.78, and 3.48 kg of body weight, respectively. These feeds were formulated considering animals for slaughter only (excluding breeding animals) once they represent most of the feed produced in pig and poultry feeding programs, with complex and simple formulas being simulated for nursery pigs.

During diet formulation, the replacement of soybean oil was performed automatically by the formulation software (Formula 2000, Optimal Informatica, Campinas, Brazil). The least-cost formulation method was used considering real price averages practiced in a local industry over 12 months. The nutritional composition of the ingredients was obtained from the Brazilian Tables for Poultry and Swine (22). The metabolizable energy (ME) matrix of β-mannanase was chosen based on the most common values applied to the Brazilian industry (45 or 90 kcal of ME/kg of feed, depending on the species and rearing phase). Both matrices were simulated for growing-finishing pigs. While 45 kcal of ME/kg of feed was the energy matrix simulated for broilers, 90 kcal of ME/kg of feed was the energy matrix simulated for nursery pigs.

Modeling Environmental Impacts

Inputs and outputs were defined for each step of the life cycle and organized in a model using the SimaPro software (v. 9.1.1.1, PRE-Consultants, Amersfoort, The Netherlands). Environmental impacts related to capital assets (machinery, equipment, and buildings) were not considered in the model. The allocation of environmental burdens to by-products was based on economic criteria. The functional units considered were 1 kg of the enzyme

TABLE 4 | Composition of broiler feeds[a].

	Starter				Growing				Finishing	
	I		II		I		II			
	Control	βM[b]	Control	βM	Control	βM	Control	βM	Control	βM
Ingredient (as-fed basis), %										
Corn	43.59	44.54	45.38	46.32	50.52	51.44	59.20	60.12	63.80	64.73
Soybean meal	46.11	45.96	43.60	43.46	38.02	37.90	30.87	30.76	26.69	26.57
Soybean oil	5.50	4.66	6.60	5.76	7.13	6.30	6.24	5.40	6.13	5.30
L-lysine HCL	0.22	0.22	0.22	0.23	0.35	0.35	0.34	0.34	0.34	0.34
DL-methionine	0.41	0.41	0.39	0.39	0.38	0.38	0.31	0.30	0.26	0.26
L-threonine	0.07	0.07	0.06	0.06	0.09	0.09	0.06	0.06	0.05	0.04
L-valine	0.02	0.02	0.02	0.02	0.05	0.05	0.04	0.04	0.03	0.03
Salt	0.22	0.22	0.21	0.21	0.19	0.19	0.17	0.17	0.16	0.16
Limestone	1.07	1.07	0.96	0.97	0.91	0.92	0.76	0.76	0.69	0.69
Dicalcium phosphate	1.84	1.84	1.61	1.61	1.41	1.41	1.07	1.07	0.92	0.91
Sodium bicarbonate	0.45	0.45	0.45	0.45	0.44	0.44	0.44	0.44	0.44	0.44
Vitamin-mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
β-mannanase	-	0.03	-	0.03	-	0.03	-	0.03	-	0.03
Calculated chemical composition[c]										
Crude protein, %	24.56	24.57	23.56	23.57	21.55	21.57	18.95	18.97	17.39	17.41
SID[d] lysine, %	1.36	1.36	1.31	1.31	1.24	1.24	1.07	1.07	0.97	0.97
Metabolizable energy, kcal/kg	3,000	3,000	3,100	3,100	3,200	3,200	3,250	3,250	3,300	3,300
Digestible phosphorus, %	0.48	0.48	0.43	0.43	0.38	0.38	0.31	0.31	0.27	0.27

[a] *Starter I, starter II, growing I, growing II, and finishing feeds were formulated based on animals with 1–7, 8–21, 22–33, 34–42, and 43–46 days of age, respectively, and 0.14, 0.59, 1.65, 2.78, and 3.48 kg of body weight, respectively.*

[b] *β-mannanase supplementation, 45 kcal of metabolizable energy/kg of feed was the energy matrix attributed to the enzyme during diet formulation of broiler feeds.*

[c] *Values were estimated considering the Brazilian Tables for Poultry and Swine (22).*

[d] *Standardized ileal digestible.*

at the feed mill gate to account for the environmental impacts associated with β-mannanase supplementation and 1 kg of feed at the feed mill gate to evaluate the impact of feed production and the grain production scenarios.

Climate change, eutrophication, and acidification were the chosen environmental impact categories, the most common impact categories used to assess the environmental impacts of pig and poultry production (4). Results were obtained for each environmental impact category, stating the resources used in each production system and the aggregate emissions of each substance with the respective characterization factor. The CML-IA baseline method was used through the SimaPro software to calculate the environmental impacts (CO_2-eq, PO_4-eq, and SO_2-eq). Changes in potential environmental impacts associated with β-mannanase supplementation were estimated considering the total amount of feed used to raise a pig or a broiler (from hatch/weaning until slaughter, excluding feeds for breeders). For this simulation, feed intake was estimated using the Brazilian Tables for Poultry and Swine (22).

Simulating Another Formulation Strategy

Data obtained from a previous study (12) was also simulated to consider a different energy matrix released through β-mannanase supplementation. Even though formulas had the same ingredient base (corn and soybean meal), the formulation procedure differs from the one described in this study. Lv et al. (12) did not use the least-cost formulation method to formulate diets, with 150 kcal of digestible energy (DE)/kg of feed being released in β-mannanase supplemented diets.

Simulating the Environmental Impacts Associated With Energy Reduction

Under Brazilian pig and poultry production conditions, the main change in ingredients after the inclusion of β-mannanase during diet formulation is the reduction of soybean oil content. The association between the reduction of soybean oil content in feed formulas and the estimated mitigation of environmental impacts was evaluated using regression analysis. The significance ($P <$ 0.05) of each equation term was evaluated before interpretation. Since species was not significant, one regression was created for both pigs and broilers. Analyses were performed using the Minitab 20.2.0 software (23).

Another simulation was performed to estimate the minimum amount of energy matrix necessary to mitigate the environmental cost of producing and transporting the enzyme. In this case, due to changes in diet formulation with β-mannanase supplementation, the soybean oil impact was fully replaced by the impact of corn in the simulation. ME values for soybean oil and corn were those proposed in the Brazilian Tables for Poultry and Swine (22). Information from the Ecoinvent

TABLE 5 | Potential environmental impacts of control feeds[a] (1 kg at feed mill gate, formulated without β-mannanase) for nursery piglets in different grain production scenarios.

	Pre-Starter		Starter
	Complex	Simple	
SO-SO scenario[b]			
Climate change, g CO_2-eq	1,266	994	695
Eutrophication, g PO_4-eq	4.08	4.38	4.66
Acidification, g SO_2-eq	7.79	7.14	6.78
CW-SO scenario[c]			
Climate change, g CO_2-eq	1,336	1,138	914
Eutrophication, g PO_4-eq	4.06	4.34	4.59
Acidification, g SO_2-eq	8.13	7.84	7.85
CW-CW scenario[d]			
Climate change, g CO_2-eq	1,398	1,198	974
Eutrophication, g PO_4-eq	4.12	4.39	4.65
Acidification, g SO_2-eq	7.53	7.27	7.26

[a]*Pre-starter and starter feeds were formulated based on animals with 33–42 and 49–63 days of age, respectively, with 10.8 and 22.5 kg of body weight on average, respectively.*
[b]*SO-SO scenario: Soybean and corn produced in Southern Brazil.*
[c]*CW-SO scenario: Soybean produced in Central-West Brazil and corn produced in Southern Brazil.*
[d]*CW-CW scenario: Soybean and corn produced in Central-West Brazil.*

TABLE 6 | Potential environmental impacts of control feeds[a] (1 kg at feed mill gate, formulated without β-mannanase) for growing-finishing pigs in different grain production scenarios.

	Growing		Finishing	
	I	II	I	II
SO-SO scenario[b]				
Climate change, g CO_2-eq	633	614	596	577
Eutrophication, g PO_4-eq	4.33	4.25	4.15	4.03
Acidification, g SO_2-eq	7.81	8.18	8.55	8.95
CW-SO scenario[c]				
Climate change, g CO_2-eq	768	728	686	639
Eutrophication, g PO_4-eq	4.29	4.21	4.12	4.01
Acidification, g SO_2-eq	8.47	8.73	8.99	9.25
CW-CW scenario[d]				
Climate change, g CO_2-eq	844	809	773	732
Eutrophication, g PO_4-eq	4.36	4.28	4.20	4.10
Acidification, g SO_2-eq	7.74	7.95	8.15	8.36

[a]*Growing I, growing II, finishing I, and finishing II feeds were formulated based on animals 70–84, 91–105, 112–133, and 140–161 days of age, respectively, with 40, 60, 85, and 112.5 kg of body weight on average, respectively.*
[b]*SO-SO scenario: Soybean and corn produced in Southern Brazil.*
[c]*CW-SO scenario: Soybean produced in Central-West Brazil and corn produced in Southern Brazil.*
[d]*CW-CW scenario: Soybean and corn produced in Central-West Brazil.*

database (v. 3.0, Swiss Center for Life Cycle Inventories, Dübendorf, Switzerland) was used to characterize the soybean oil production. The environmental impact of oil reduction was estimated considering other references to include variability in the simulations (AgriFootPrint v. 5.0, Blonk Consultants, Gouda, The Netherlands), all based on Brazilian production scenarios.

RESULTS

The production of corn (functional unit: 1 kg at the feed mill gate) in the Southern region led to the emission of 491 g of CO_2-eq, 3.78 g of PO_4-eq, and 9.98 g of SO_2-eq. For the Central-West region, corn showed a higher impact concerning climate change (601 g of CO_2-eq; +22%) and eutrophication (3.88 g of PO_4-eq; +3%) but a lower impact concerning acidification (8.92 g of PO_4-eq;−11%) compared to the Southern region. The production of soybean meal (1 kg at the feed mill gate) in the Southern region was associated with the emission of 533 g of CO_2-eq, 5.82 g of PO_4-eq, and 2.62 g of SO_2-eq. In comparison with the Southern region, soybean meal from the Central-West region showed a higher impact concerning climate change (1,110 g of CO_2-eq; +108%) and acidification (5.43 g of SO_2-eq; +107%) but a lower impact concerning eutrophication (5.64 g of PO_4-eq;−3%). These differences among ingredient origins are highlighted in the impacts of producing complete feed formulas (functional unit: 1 kg at the feed mill gate) for pigs and broilers, which are presented in **Tables 5–7**.

The production of β-mannanase (1 kg at the feed mill gate) was associated with the emission of 1,800 g of CO_2-eq, 4.53 g of PO_4-eq, and 7.89 g of SO_2-eq. When feeds were reformulated considering the inclusion of the enzyme and its energy matrix, there were some modifications in the ingredient use, which lead to changes in the potential environmental impact associated with

TABLE 7 | Potential environmental impacts of control feeds[a] (1 kg at feed mill gate, formulated without β-mannanase) for broilers in different grain production scenarios.

	Starter		Growing		Finishing
	I	II	I	II	
SO-SO scenario[b]					
Climate change, g CO_2-eq	816	877	910	857	848
Eutrophication, g PO_4-eq	4.94	4.97	4.90	4.71	4.62
Acidification, g SO_2-eq	5.96	6.13	6.56	7.17	7.50
CW-SO scenario[c]					
Climate change, g CO_2-eq	1,082	1,128	1,130	1,035	1,002
Eutrophication, g PO_4-eq	4.86	4.89	4.83	4.66	4.58
Acidification, g SO_2-eq	7.26	7.35	7.63	8.04	8.25
CW-CW scenario[d]					
Climate change, g CO_2-eq	1,130	1,178	1,185	1,100	1,072
Eutrophication, g PO_4-eq	4.90	4.94	4.88	4.72	4.64
Acidification, g SO_2-eq	6.80	6.87	7.09	7.41	7.58

[a]*Starter I, starter II, growing I, growing II, and finishing feeds were formulated based on animals with 1–7, 8–21, 22–33, 34–42, and 43–46 days of age, respectively, and 0.14, 0.59, 1.65, 2.78, and 3.48 kg of body weight, respectively.*
[b]*SO-SO scenario: Soybean and corn produced in Southern Brazil.*
[c]*CW-SO scenario: Soybean produced in Central-West Brazil and corn produced in Southern Brazil.*
[d]*CW-CW scenario: Soybean and corn produced in Central-West Brazil.*

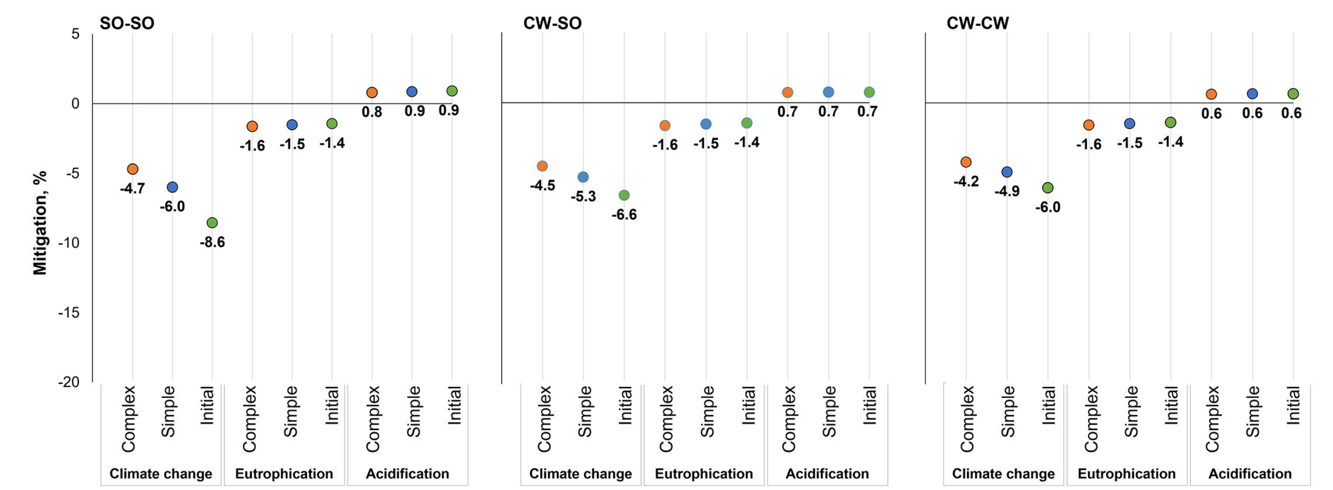

FIGURE 2 | Potential environmental impact mitigation associated with the production of piglet feeds, considering energy savings from an energy matrix of 90 kcal of ME/kg of feed released by β-mannanase supplementation in different grain production scenarios; SO-SO, Soybean and corn produced in Southern Brazil; CW-SO, Soybean produced in Central-West Brazil and corn produced in Southern Brazil; CW-CW, Soybean and corn produced in Central-West Brazil.

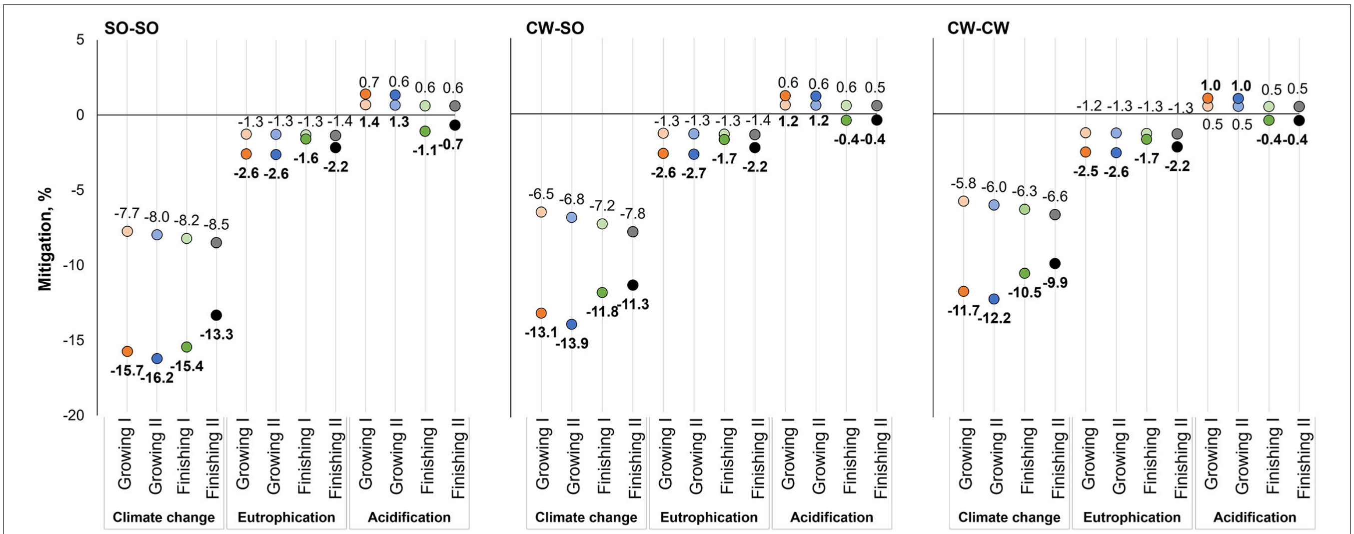

FIGURE 3 | Potential environmental impact mitigation associated with the production of growing-finishing pig feeds, considering energy savings from two energy matrices (45 kcal of ME/kg of feed, light circles; 90 kcal of ME/kg of feed, dark circles) released by β-mannanase supplementation in different grain production scenarios; SO-SO, Soybean and corn produced in Southern Brazil; CW-SO, Soybean produced in Central-West Brazil and corn produced in Southern Brazil; CW-CW, Soybean and corn produced in Central-West Brazil.

the same functional unit of feed production (**Figures 2–4**). The use of β-mannanase associated with an energy matrix of 90 kcal of ME/kg for pre-starter diets leads to the mitigation of 59 g of CO_2-eq per kg of produced feed, representing a greater percentage reduction in simple than complex diets. Using β-mannanase in growing-finishing pig feeds reduced the potential impact of climate change up to 16.2% when using an energy matrix of 90 kcal of ME/kg and up to 8.5% when considering 45 kcal of ME/kg. The eutrophication impact of producing the same feeds was reduced up to 2.7% when using an energy matrix of 90 kcal of ME/kg, while the reduction reached up to 1.4% when using an energy matrix of 45 kcal of ME/kg. The climate change impact of producing feeds for broilers was reduced up to 5.6%, while the eutrophication impact was reduced by up to 1.1%.

The effect of using β-mannanase on the acidification impact was not consistent among feeds. Although mitigation on the potential acidification impact was observed in feeds for finishing pigs, the use of β-mannanase (and the consequent modifications in other ingredients inclusion) increased the acidification impact associated with most other pig feeds (even when formulating considering an

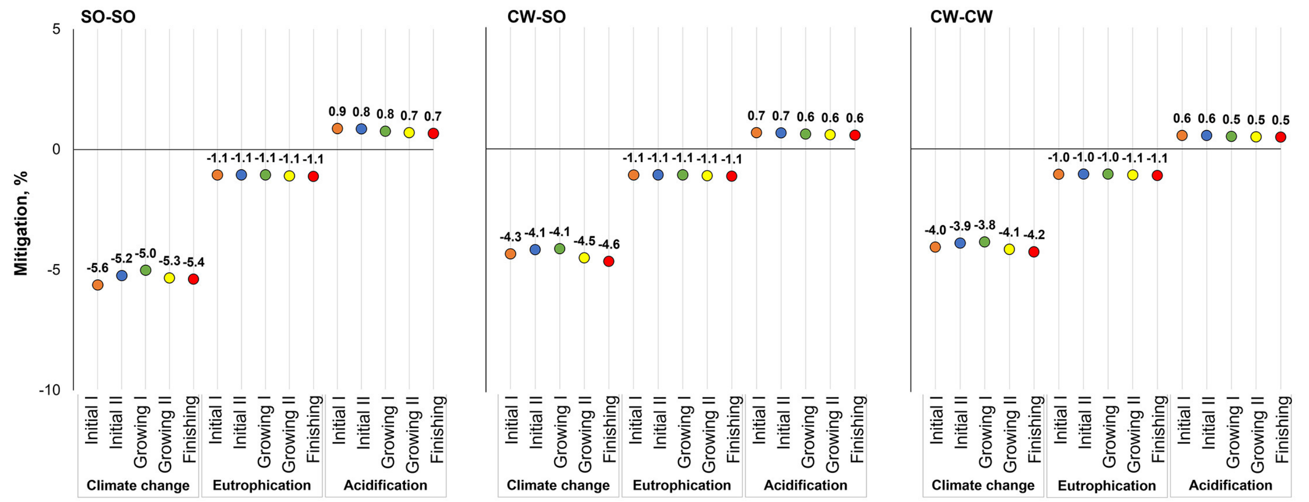

FIGURE 4 | Potential environmental impact mitigation associated with the production of broiler feeds, considering energy savings from an energy matrix of 45 kcal of ME/kg of feed released by *β*-mannanase supplementation in different grain production scenarios; SO-SO, Soybean and corn produced in Southern Brazil; CW-SO, Soybean produced in Central-West Brazil and corn produced in Southern Brazil; CW-CW, Soybean and corn produced in Central-West Brazil.

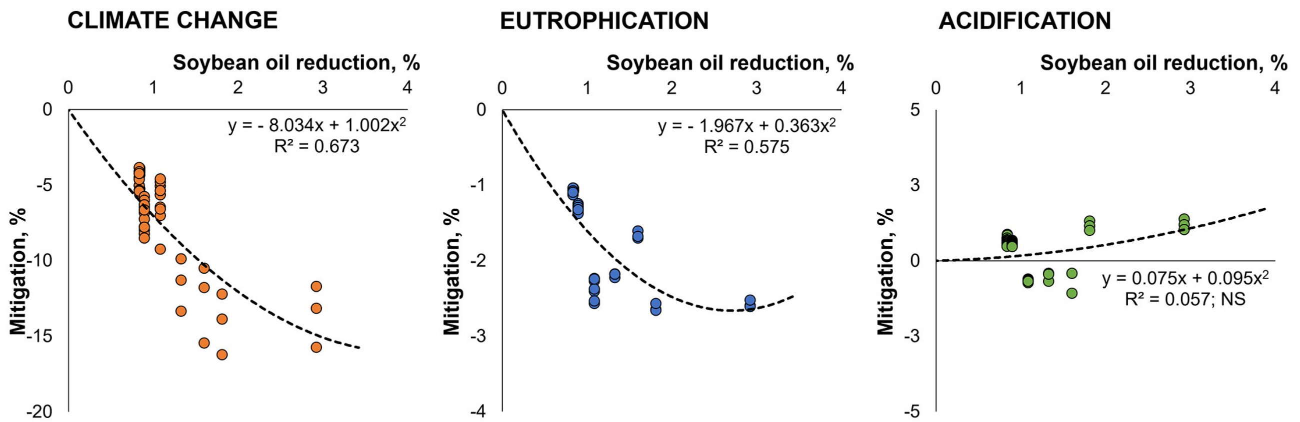

FIGURE 5 | Potential environmental impact mitigation associated with the production of pig and broiler feeds due to soybean oil reduction through energy savings released by *β*-mannanase supplementation. For this regression analysis, species was not significant ($P > 0.05$) in the model. In addition, for the acidification impact, all terms were not significant in the regression equation.

energy matrix of 90 kcal of ME/kg) and with all broiler feeds evaluated.

The use of *β*-mannanase allowed a reduction in the amount of soybean oil in the feed formulas, which is associated with a high environmental impact. Consequently, the potential impacts of climate change and eutrophication were mitigated. A quadratic regression explained the association between soybean oil reduction in feed formulas (as a consequence of *β*-mannanase supplementation) and the mitigation of both climate change and eutrophication impacts (**Figure 5**).

The mitigation effect of *β*-mannanase supplementation is comparable between species when the total amount of feed used to raise a pig or a broiler is considered in the simulation (i.e., the feeding program, considering the feed used from hatch/weaning until slaughter, excluding breeding phases; **Table 8**). *β*-mannanase supplementation produced greater changes on the potential impact of climate change (reduction of up to 4.7% in feeding programs for pigs and 5.2% in feeding programs for broilers) compared to the eutrophication (−1.6% in pigs and −1.1% in broiler). Changes in the acidification impact associated with *β*-mannanase supplementation are positive (i.e., increased environmental impact), however, lower than 1% in all studied scenarios. When simulating the impacts using another formulation strategy (**Table 9**), mitigation was higher: the potential impact of climate change was reduced up to 18%, while eutrophication and acidification were mitigated by 6 and 4%, respectively. However, it is hard to compare results since they originally used different ingredients and formulation methods.

TABLE 8 | Changes in the environmental impacts associated with β-mannanase supplementation when the total amount of feed used to raise a pig or a broiler (from hatch/weaning until slaughter, excluding breeding phases) is considered in the simulation[a].

	Pig	Broiler
SO-SO scenario[b]		
Climate change, kg CO_2-eq	−11.14 (−4.7%)	−0.271 (−5.2%)
Eutrophication, g PO_4-eq	−12.56 (−1.6%)	−0.308 (−1.1%)
Acidification, g SO_2-eq	+11.95 (+0.8%)	+0.297 (+0.7%)
CW-SO scenario[c]		
Climate change, kg CO_2-eq	−11.29 (−4.5%)	−0.276 (−4.3%)
Eutrophication, g PO_4-eq	−12.51 (−1.6%)	−0.307 (−1.1%)
Acidification, g SO_2-eq	+11.23 (+0.7%)	+0.277 (+0.6%)
CW-CW scenario[d]		
Climate change, kg CO_2-eq	−11.04 (−4.2%)	−0.270 (−4.0%)
Eutrophication, g PO_4-eq	−12.29 (−1.6%)	−0.301 (−1.1%)
Acidification, g SO_2-eq	+8.86 (+0.6%)	+0.219 (+0.5%)

[a]*Feed intake for each animal phase was estimated using the Brazilian Tables for Poultry and Swine (22). Values indicate the total amount mitigated/increased when β-mannanase is used in the formulations, followed by the percentage change compared to scenarios without β-mannanase supplementation.*
[b]*SO-SO scenario: Soybean and corn produced in Southern Brazil.*
[c]*CW-SO scenario: Soybean produced in Central-West Brazil and corn produced in Southern Brazil.*
[d]*CW-CW scenario: Soybean and corn produced in Central-West Brazil.*

TABLE 9 | Potential environmental impacts of feeds (1 kg at feed mill gate) formulated for growing pigs based on the 150 kcal digestible energy reduction per kg of feed, with β-mannanase supplemented diets compared to control diets.

	Treatments[a]		Mitigation, %
	Control	**β-mannanase**	
SO-SO scenario[b]			
Climate change, g CO_2-eq	587.19	479.41	−18
Eutrophication, g PO_4-eq	4.27	3.99	−6
Acidification, g SO_2-eq	7.88	7.56	−4
CW-SO scenario[c]			
Climate change, g CO_2-eq	717.01	596.31	−17
Eutrophication, g PO_4-eq	4.23	3.96	−6
Acidification, g SO_2-eq	8.42	8.13	−4
CW-CW scenario[d]			
Climate change, g CO_2-eq	790.11	666.82	−16
Eutrophication, g PO_4-eq	4.29	4.02	−6
Acidification, g SO_2-eq	7.72	7.45	−4

[a]*Control formula contains 3,400 kcal of digestible energy and formula supplemented with β-mannanase, considering an energy matrix of 150 kcal of digestible energy/kg of feed. Treatments proved to have similar performance and digestibility (12).*
[b]*SO-SO scenario: Soybean and corn produced in Southern Brazil.*
[c]*CW-SO scenario: Soybean produced in Central-West Brazil and corn produced in Southern Brazil.*
[d]*CW-CW scenario: Soybean and corn produced in Central-West Brazil.*

The simulation to estimate the minimum amount of energy matrix necessary to mitigate the environmental cost of producing and transporting the enzyme considered that soybean oil was replaced entirely by corn in the formulas to estimate the environmental cost of using β-mannanase in feeds for pigs and poultry, as the changes in soybean meal depend on the energy matrix used in the formulation (**Figure 6**). Considering that the inclusion of β-mannanase is 300 g per ton of feed and the functional unit of 1 kg of manufactured feed, the impact associated with the enzyme is 6 g of CO_2-eq, 0.0151 g of PO_4-eq, and 0.0262 g of SO_2-eq (indicated by an A within each panel). When the soybean oil impact is estimated considering Ecoinvent references, the production of soybean oil (1 kg at the feed mill gate) was associated with the emission of 6,008 g of CO_2-eq, 9.71 g of PO_4-eq, and 5.00 g of SO_2-eq. The mitigation of climate change occurred at an energy matrix of 5.4 kcal for pigs and 5.9 kcal for broilers (indicated by a B within each panel), while eutrophication was mitigated at 17 kcal for pigs and 18 kcal for broilers. However, the mitigation may occur even with a lower energy matrix (indicated by C and D within each panel) if other references were used to characterize the environmental impacts of soybean oil. It is worth mentioning that, in real-life conditions, it is recommended to use a percentage of soybean oil to stimulate the feed's palatability and improve mixing conditions.

DISCUSSION

With regards to the environmental impacts of using β-mannanase in feeds, our results are similar to those obtained by Nielsen et al. (5) when describing the phytase production (1,900 g of CO_2-eq, 2.20 g of PO_4-eq, and 4.80 g of SO_2-eq, 1 kg at the enzyme producer). On the other hand, for the environmental impacts of grain production, crop management practices and expansion rates varied among the Brazilian regions simulated in this study, and so did results. Alvarenga et al. (17) reported equivalent environmental impacts for broiler chicken diets produced in Brazil, but with a slighter lower climate change impact, especially for the CW-CW scenario. Cherubini et al. (25) also reported equivalent impacts in terms of carbon footprint when assessing diets for finishing pigs in Brazil. Both climate change and eutrophication impacts estimated in the current study were also comparable to those obtained by van der Werf et al. (26) and Mosnier et al. (19), who assessed finishing pig diets produced in France using Brazilian soybean. On the other hand, results concerning the use of β-mannanase on acidification were not consistent among feeds once the acidification impact was heavily associated with the feed formula being considered in the simulation. In other words, for some feeds, there was a slight increase in the acidification impact following β-mannanase supplementation; for other feeds, it was quite the opposite. For growing pig diets, the acidification impact was greater when considering the 90 kcal of ME/kg of feed matrix. However, for finishing pig diets, soybean oil was removed from the feed formula for this matrix, lowering its impact on acidification. The acidification impact varied with the corn/soybean proportion. Both climate change and eutrophication impacts are greater for soybean than corn. On the other hand, the acidification impact is greater for corn, especially the one from the Southern region. As this corn/soybean ratio varies from region to region, the same

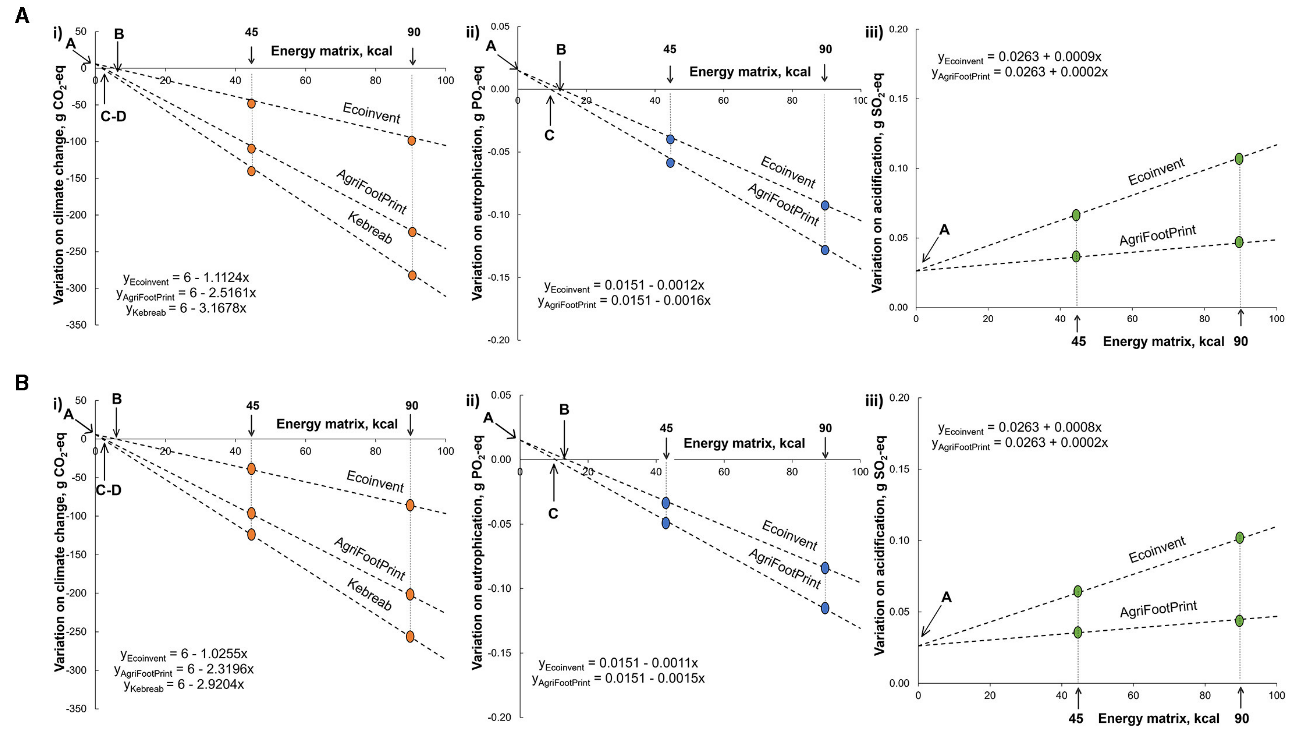

FIGURE 6 | Minimum energy matrix necessary to mitigate the environmental cost associated with β-mannanase supplementation in pig **(A)** and broiler **(B)** feeds. Within each panel, the letter A indicates the environmental cost of β-mannanase supplementation for 1 kg of feed, while letters B (Ecoinvent database v. 3.0, Swiss Center for Life Cycle Inventories, Dübendorf, Switzerland), C (AgriFootPrint v. 5.0, Blonk Consultants, Gouda, The Netherlands), and D [Kebreab et al. (24)] indicate the energy matrix able to mitigate this impact on climate change (i), eutrophication (ii), and acidification (iii) depending on the reference considered to account soybean oil production.

change (%) in the formula ends up increasing or mitigating the overall acidification impact.

For growing-finishing pig feeds, the energy matrix of β-mannanase was chosen based on the most common values applied to the Brazilian industry (45 or 90 kcal of ME/kg of feed). For feeds that do not use exogenous enzymes other than β-mannanase, the saving of 90 kcal of ME/kg of feed provided by β-mannanase is commonly considered. On the other hand, for feeds that include a mix of enzymes, a 45 kcal of ME/kg of feed matrix is more appropriate. Broiler feeds under Brazilian feeding programs commonly include multiple exogenous enzymes when formulated. Therefore, we only considered a 45 kcal of ME/kg of feed matrix in our simulations for broilers. Despite the differences among the several enzyme supplementation strategies available for nutritionists, all simulated values are much higher than the minimum matrix necessary to mitigate the environmental impact associated with the enzyme incorporation in the formula (i.e., 5.4 kcal of ME for pigs and 5.9 kcal of ME for broilers to mitigate the climate change impact, or 17 kcal of ME for pigs and 18 kcal of ME for broilers considering the eutrophication impact). The use of an energy matrix of 45 kcal of ME/kg of feed associated with β-mannanase reduced by 0.90 and 0.84 percent points the inclusion of soybean oil in feed formulas for pigs and broilers, respectively. Considering the recent price conditions in Brazil, the space created in the formula by the reduction of oil use is occupied by corn. However, the average inclusion of corn in the feeds with β-mannanase increased by 0.99 and 0.95 percent points for pigs and broilers, respectively, compared to control diets. Corn variation was higher than the reduction in oil use because soybean meal inclusion was also reduced (on average, a reduction of 0.12 percent points). From an environmental standpoint, these variations are favorable as soybean meal and soybean oil are associated with a higher environmental impact than corn. Formulating feeds for pigs and poultry through liquid energy is a reality in some countries. However, this is not the case in Brazil and therefore it was not included in our analyses.

Overall, the greater environmental impact observed for soybean oil compared to corn is mainly because of the greater amount of resources needed to obtain this ingredient, such as land and fertilizers. The impact of soybean oil considered in this study was based on the Ecoinvent database (v. 3.0, Swiss Center for Life Cycle Inventories, Dübendorf, Switzerland), which is lower than other references for the same product. Simulating the production of 1 kg of feed, the changes in feed formula (average reduction of soybean oil and meal, and increasing corn use) caused by β-mannanase can prevent the emission of 55 g of CO_2-eq and 0.11 g of PO_4-eq. If values from AgriFootPrint (v. 5.0, Blonk Consultants, Gouda, The Netherlands) were considered, the mitigation is raised to 112 g of CO_2-eq and 0.13 g of PO_4-eq. If the impact estimated by Kebreab et al. (24) for Brazilian soybean oil would be considered, the mitigation associated with β-mannanase could reach 140 g of CO_2-eq per kg of feed produced. These differences are mostly due to the grain production scenario considered in the database (Southern or Central-West origin).

Differences in the environmental impact of β-mannanase supplementation between pig and poultry feeding programs are mostly due to the ingredients included in diet formulations as each ingredient has it is own environmental impact. However, the pig and poultry sectors share some similarities not only from an organizational point of view but also from an environmental one. Pig and poultry production systems have been pointed out as large contributors to environmental impacts, such as climate change, eutrophication, and acidification (3, 27). Feeding both pigs and poultry requires tremendous amounts of feed resources, especially rich in protein and/or energy, with several studies indicating it as the most important source of environmental impact (2, 4). Novel feeding strategies are thus needed to tackle the challenges of these sectors. Our study has shown that β-mannanase supplementation can be considered as an eco-friendly feed strategy to reduce the environmental impacts of pig and poultry feeding programs. This was mostly because β-mannanase is a nutrient-sparing enzyme that breaks down β-mannans, leading to an increase in energy-use efficiency for both sectors.

CONCLUSION

β-mannanase supplementation reduced the amount of soybean oil in feed formulas, which is associated with high environmental impacts. Consequently, the potential impacts of climate change and eutrophication associated with producing feeds for pigs and broilers were substantially mitigated. These results suggest that β-mannanase supplementation is an eco-friendly feed strategy to reduce the environmental impacts of pig and poultry feeding programs. As feeding accounts for most of the environmental impacts associated with pig and poultry production, strategies such as β-mannanase supplementation that mitigate these impacts are desired. This feeding strategy improves the overall sustainability of pig and poultry production systems by increasing energy-use efficiency. It is worth mentioning that the β-mannanase supplementation described in this study is only one way to address the environmental impacts of feeding pigs and broilers. Several other approaches and techniques must be considered in an integrated way toward more sustainable animal systems.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

FH and IA assisted with data analysis, interpreted results, prepared tables and figures, and drafted the manuscript. MK assisted with data analysis, interpretation and discussion of results. GG, AR, JV, and M-PL-M were involved in the interpretation and discussion of results. All authors have contributed to this research and approved the final version of the manuscript.

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REFERENCES

1. Lovatto PA, Hauschild L, Hauptli L, Lehnen CR, Carvalho AD. Modelagem da ingestão, retenção e excreção de nitrogênio e fósforo pela suinocultura brasileira. *Revista Brasileira de Zootecnia.* (2005) 34:2348–54. doi: 10.1590/S1516-35982005000700022
2. McAuliffe GA, Chapman DV, Sage CL, A. thematic review of life cycle assessment (LCA) applied to pig production. *Environ Impact Assess Rev.* (2016) 56:12–22. doi: 10.1016/j.eiar.2015.08.008
3. Dourmad J-Y, Ryschawy J, Trousson T, Bonneau M, Gonzàlez J, Houwers H, et al. Evaluating environmental impacts of contrasting pig farming systems with life cycle assessment. *Animal.* (2014) 8:2027–37. doi: 10.1017/S1751731114002134
4. Andretta I, Kipper M, Remus A, Orso C, Franceschi C. Environmental impacts of feeding crops to pigs and poultry. In: Galanakis C, editor. *Environmental Impact of Agro-Food Industry and Food Consumption.* Cambridge: Academic Press (2021). p. 59–79. doi: 10.1016/B978-0-12-821363-6.00001-1
5. Nielsen PH, Oxenbøll KM, Wenzel H. Cradle-to-gate environmental assessment of enzyme products produced industrially in Denmark by Novozymes A/S. *Int J Life Cycle Assessment.* (2016) 12:432. doi: 10.1007/s11367-006-2651-4
6. Jegannathan KR, Nielsen PH. Environmental assessment of enzyme use in industrial production - a literature review. *J Cleaner Production, Elsevier.* (2013) 42:228–40. doi: 10.1016/j.jclepro.2012.11.005
7. Kim JS, Ingale SL, Lee SH, Kim HM, Kim JS, Lee JH, et al. Effects of energy levels of diet and β-mannanase supplementation on growth performance, apparent total tract digestibility and blood metabolites in growing pigs. *Anim Feed Sci Technol.* (2013) 186:64–70. doi: 10.1016/j.anifeedsci.2013.08.008
8. Shastak Y, Ader P, Feuerstein D, Ruehle R, Matuschek M. ß-Mannan and mannanase in poultry nutrition. *World's Poultry Science Journal.* (2015) 71:161–74. doi: 10.1017/S00439339150 00136
9. Ferreira HC, Hannas MI, Albino LFT, Rostagno HS, Neme R, Faria BD, Xavier MLJr, Rennó LN. Effect of the addition of β-mannanase on the performance, metabolizable energy, amino acid digestibility coefficients, and

immune functions of broilers fed different nutritional levels. *Poultry Sci.* (2016) 1848–57 doi: 10.3382/ps/pew076
10. Kipper M, Andretta I, Quadros VR, Schroeder B, Pires PGS, Franceschina CS, et al. Performance responses of broilers and pigs fed diets with β-mannanase. *Revista Brasileira de Zootecnia.* (2020) 49:e20180177. doi: 10.37496/rbz4920180177
11. Guinée JB. Handbook on life cycle assessment: operational guide to the ISO standards. Amsterdam, Springer Netherlands: Int J Life Cycle Assess (2002). p. 692. doi: 10.1007/BF02978897
12. Lv JN, Chen YQ, Guo XJ, Piao XS, Cao YH, Dong B. Effect of supplementation of β-mannanase in corn-soybean meal diets on performance and nutrient digestibility in growing pigs. *Asian-Australas J Anim Sci.* (2013) 26:579–87. doi: 10.5713/ajas.2012.12612
13. Gilpin GS, Andrae AS. Comparative attributional life cycle assessment of European cellulase enzyme production for use in second-generation lignocellulosic bioethanol production. *Int J Life Cycel Assess.* (2017) 22:1034–53. doi: 10.1007/s11367-016-1208-4
14. Andretta I, Hauschild L, Kipper M, Pires PGS, Pomar C. Environmental impacts of precision feeding programs applied in pig production. *Animal.* (2018) 12:1990–8. doi: 10.1017/S1751731117003159
15. Instituto Brasileiro de Geografia e Estatística. Sistema IBGE de recuperação automática – SIDRA: Produção Agrícola Municipal (2020). Available online at: https://sidra.ibge.gov.br/pesquisa/pam/tabelas (accessed April 12, 2021).
16. Alvarenga, RAF. Avaliação de métodos de AICV: um estudo de caso de quatro cenários de ração para frangos de corte. [dissertation/master's thesis]. Florianópolis (SC): Universidade Federal de Santa Catarina, Brazil (2010).
17. Alvarenga RAF, Silva Júnior VP, Soares SR. Comparison of the ecological footprint and a life cycle impact assessment method for a case study on Brazilian broiler feed production. *J Clean Prod.* (2012) 28:25–32. doi: 10.1016/j.jclepro.2011.06.023
18. Prudêncio da. Silva V, van der Werf HMG, Spies A, Soares SR. Variability in environmental impacts of Brazilian soybean according to crop production and transport scenarios. *J Environ Managem.* (2010) 91:1831–9. doi: 10.1016/j.jenvman.2010.04.001
19. Mosnier E, van der Werf HMG, Boissy J, Dourmad JY. Evaluation of the environmental implications of the incorporation of feed-use amino acids in the manufacturing of pig and broiler feeds using Life Cycle Assessment. *Animal.* (2011) 5:1972–83. doi: 10.1017/S1751731111001078
20. van Zeist WJ, Marinussen M, Broekema R, Groen E, Kool A, Dolman M, et al. LCI data for the calculation tool Feedprint for greenhouse gas emissions of feed production and utilization: Crushing industry. Gouda, the Netherlands: Blonk Consultants. (2012).
21. van Zeist WJ, Marinussen M, Broekema R, Groen E, Kool A, Dolman M, Blonk H. LCI data for the calculation tool Feedprint for greenhouse gas emissions of feed production and utilization: Dry milling industry. Gouda, the Netherlands: Blonk Consultants. (2012).
22. Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira RF, Lopes DC, et al. Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. Viçosa: Universidade Federal de Viçosa (2017). p.482.
23. Minitab 20.2.0 Statistical Software (2021). [Computer software]. State College, PA: Minitab, Inc. Available online at: www.minitab.com
24. Kebreab E, Liedke A, Caro D, Deimling S, Binder M, Finkbeiner M. Environmental impact of using specialty feed ingredients in swine and poultry production: A life cycle assessment. *J Anim Sci.* (2016) 94:2664–81. doi: 10.2527/jas.2015-9036
25. Cherubini E, Zanghelini GM, Tavares JMR, Belettini F, Soares SR. The finishing stage in swine production: influences of feed composition on carbon footprint. *Environ Dev Sustain.* (2015) 17:1313–28. doi: 10.1007/s10668-014-9607-9
26. van der Werf HMG, Petit J, Sanders J. The environmental impacts of the production of concentrated feed: the case of pig feed in Bretagne. *Agric Syst.* (2005) 83:153–77. doi: 10.1016/j.agsy.2004.03.005
27. Cappelaere L, Le Cour Grandmaison J, Martin N, Lambert W. Amino acid supplementation to reduce environmental impacts of broiler and pig production: a review. *Front Vet Sci.* (2021) 8:689259. doi: 10.3389/fvets.2021.689259

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be perceived as a potential conflict of interest.

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Environmental Impacts of Pig and Poultry Production: Insights From a Systematic Review

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Pig and poultry production systems have reached high-performance levels over the last few decades. However, there is still room for improvement when it comes to their environmental sustainability. This issue is even more relevant due to the growing demand for food demand since this surplus food production needs to be met at an affordable cost with minimum impact on the environment. This study presents a systematic review of peer-reviewed manuscripts that investigated the environmental impacts associated with pig and poultry production. For this purpose, independent reviews were performed and two databases were constructed, one for each production system. Previous studies published in peer-reviewed journals were considered for the databases if the method of life cycle assessment (LCA) was applied to pig (pork meat) or poultry (broiler meat or table eggs) production to estimate at least the potential effects of climate change, measured as CO_2-eq. Studies considering the cradle-to-farm gate were considered, as well as those evaluating processes up to the slaughterhouse or processor gate. The pig database comprised 55 studies, while 30 publications were selected for the poultry database. These studies confirmed feeding (which includes the crop cultivation phase, manufacturing processes, and transportation) as the main contributor to the environmental impact associated with pig and poultry production systems. Several studies evaluated feeding strategies, which were indicated as viable alternatives to mitigate the environmental footprint associated with both production chains. In this study, precision feeding techniques are highlighted given their applicability to modern pig and poultry farming. These novel feeding strategies are good examples of innovative strategies needed to break paradigms, improve resource-use efficiency, and effectively move the current productive scenario toward more sustainable livestock systems.

Keywords: sustainability, swine, broilers, environment, livestock, climate change, laying hens, precision feeding

INTRODUCTION

The increasing demand for food is an important challenge that society will face in the coming decades. The growing population will need more resources, leading to a relevant increase in food demand. The productive sector (including agriculture and livestock) needs to support the growing demands for food, however, without compromising the ability of the future generations to also meet their requirements. In other words, environmentally sustainable agri-food systems are mandatory requirements for a world with increasing urbanization and growing food demands.

In this context, the benefits of agri-food sectors for society need to be maximized (1), which can be achieved by improving the efficiency in which the resources are applied in the production chains. The current production methods will need to adapt to these new challenges (limited resources, increased production), with most surplus food production being supplied by innovative agri-food systems (2).

Pig and poultry production systems have reached high-performance levels over the last few decades. Together, these sectors provide a large amount of affordable and nutritious food, especially high-quality protein, contributing to food security worldwide. However, there is still room for improvement when it comes to their environmental sustainability. Feeding pigs and poultry requires tremendous amounts of feed resources, with several studies indicating it as an important source of environmental impact (3). In addition, pigs and broilers excrete annually large amounts of nitrogen and phosphorus to the environment, which conditions the production sustainability of these chains (4).

Conventionally, the impacts of pig and poultry production have been assessed by methodologies that used an "animal basis" approach (e.g., studies focusing on reducing nutrient excretion). These are very important studies; however, few mitigation strategies have focused on the efficiency of resource use, which is critical in a global context. Considering the relevance of the topic, it is important to investigate feeding practices that mitigate the environmental impacts associated with the entire production system. Thus, we carried out a systematic review to summarize, analyze, and compare studies that used life cycle assessment (LCA) to evaluate the environmental impacts associated with pig and poultry production systems.

MATERIALS AND METHODS

This systematic review was based on structured and elaborated research performed using online search methods. The search strategy was planned and carried out to identify as many studies as possible on the subject. Papers were rigorously selected and those focusing on feeding practices were further evaluated.

Independent searches were performed for pig and poultry production systems. The strategy "PICo" was applied to build the research question by identifying "Population" (database 1: "pig"; database 2: "poultry"), "Interest" ("life cycle assessment"), and "Context" ("climate change") for both searches. Alternative terms for population and interest were listed using synonymous words in English to compose the final search strategy. Context was applied later (through full-text reads) to avoid missing any study in which the response was not mentioned among the main terms (title, abstract, and keywords). The final search terms were:

Database 1:

(*pig OR pigs OR swine*) *AND* ("*life cycle assessment*" *OR* "*life cycle*" *OR* "*carbon emission*" *OR* "*carbon footprint*" *OR* "*greenhouse gas**" *OR* "*global warming*" *OR LCA*)

Database 2:

(*poultry OR broiler* OR chicken* OR hen*) *AND* ("*life cycle assessment*" *OR* "*life cycle*" *OR* "*carbon emission*" *OR* "*carbon footprint*" *OR* "*greenhouse gas**" *OR* "*global warming*" *OR LCA*).

The search was conducted in March 2020, considering only original peer-reviewed studies published in scientific journals available in PubMed, Scopus, and Web of Science. A snowball approach using forward (e.g., databases) and backward research methods (e.g., direct journal search, reference lists, studies listed in previously published reviews) was performed to increase the chance of including as many relevant studies as possible. No limitations on the geographic origin or year of publication were applied in both searches.

Each database was exported to the reference software (EndNote X9, Philadelphia, PA) used to organize references and manage part of the study selection. Duplicate references were identified and excluded. Studies were critically evaluated regarding their relevance and quality by examining titles and abstracts, followed by a complete review of the LCA study. Two reviewers performed a critical evaluation of the study eligibility. A study was not considered in the final database (removed) after mutual agreement, with a third reviewer reassessing studies that differed in terms of eligibility.

The selection criteria were stated as (i) original papers published in peer-reviewed journals, (ii) environmental impact evaluated using the LCA methodology; (iii) evaluation of pig (pork meat) or poultry (broiler meat or table eggs) production systems; (iv) scopes including cradle-to-farm, to the slaughterhouse, or to processor gate; (v) estimation of at least the potential impact of climate change, in CO_2-eq. The quality of selected studies was further evaluated and information relevant to describe the proposed theoretical model was transferred to the pig and poultry spreadsheets. Finally, cross-study comparisons were performed considering the subject, scope, and main results observed.

RESULTS

Studies Focusing on Pig Production

The research process until obtaining the final pig database is described in **Figure 1A**. Articles obtained by online searches (4,237 references) were critically evaluated and successive exclusions were performed. The main exclusions (more related to methodological aspects of the original studies) were performed when assessing the full-text, when 36 references were eliminated (criterium i and ii: 15 publications; criterium iii: 2 publications; criterium iv: 13 publications; and criterium v: 6 publications). The final list of 55 selected studies is described in **Table 1**.

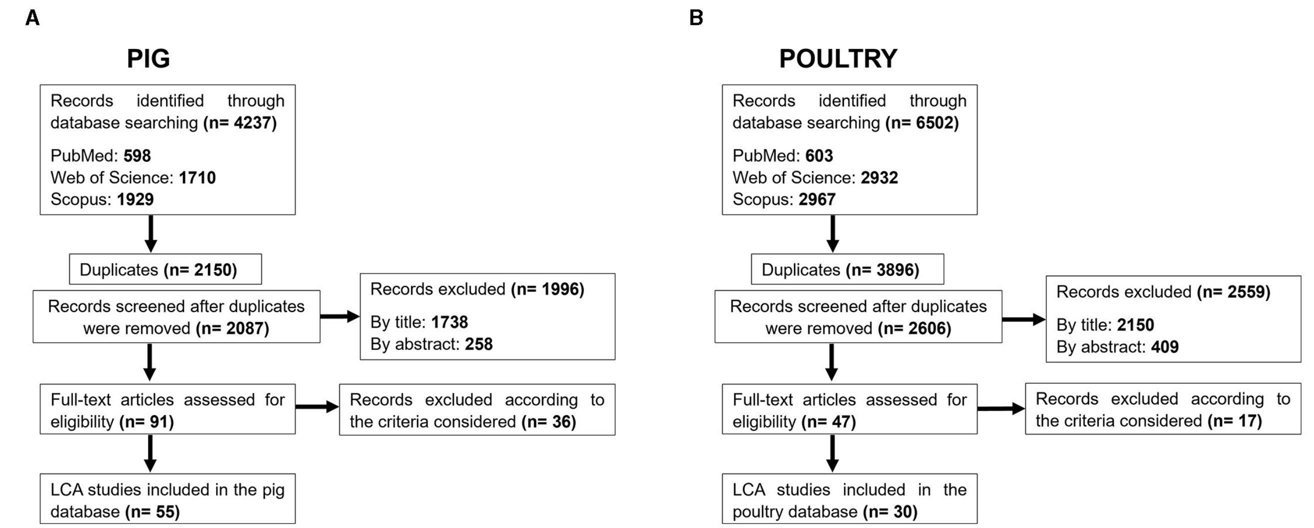

FIGURE 1 | Study selection diagram for pig **(A)** or poultry **(B)** databases.

The first LCA study identified in the pig database was published in 2005. Considering the entire database, 26 journals reported publications, with 16 papers being published in Journal of Cleaner Production and 5 papers in Animal. Production scenarios located in Brazil (which was considered in eight studies), Spain (considered in six studies), France (considered in five studies), and China (considered in four studies) were assessed in the selected papers, as illustrated in **Figure 2A**. The frequency of studied countries is highly related to the location of the main research groups. However, it is important to highlight that the order of most studied countries is not in complete agreement with the pork production ranking (led by China). Another important aspect related to the geographical characteristics of the papers is that five studies were developed by researchers from countries different than the one (or at least one of the regions) considered in the simulations, with Brazil or South America being studied in three of them.

A scope described as cradle-to-farm gate was used in the majority of the studies, which means that all phases comprised from the crop cultivation (and its inputs/outputs) up to the animal rearing phase were considered in these projects. The impacts associated with slaughtering and processing were considered in nine publications only.

Climate change was the focus of our study. However, the LCA studies also reported other impact categories (**Figure 3A**). From those variables, the most prevalent were eutrophication and acidification, followed by the use of energy and land.

The main subjects under evaluation in the studies focusing on pig production are presented in **Table 2**. The characterization of pig production in the region or country was the main objective in 18 studies. Another important objective in the studies was the comparison of production systems (including organic or alternative housing systems), which was the main subject in nine papers.

Changes in feeding practices (diet composition or feeding programs) were studied in 25% of the papers. The relative participation of feed production (which includes each ingredient's life cycle, fabrication, and transport) varied from 31 to 76% of the overall greenhouse gas (GHG) emissions in the pig database (**Figure 4A**). Despite the importance of feeding to the total pig production impact, the diet composition used in the inventory was described by the minority of the papers. Only 38% of the papers described the ingredient formulas, while only 29% of the studies showed any description for dietary nutritional composition, limited sometimes to crude protein. In addition, the environmental impacts related to the production of individual ingredients were presented in only 9% of the papers. The proportion of total impact associated with feed was highlighted in most of the studies. However, the impact of feed production (considering as a functional unit; e.g., 1 ton of feed) was presented in only 15% of the publications. These data would be of great value for further investigations on feeding practices that may mitigate the potential environmental impact of pig production. In addition, more information on feeding practices would allow a better comparison among studies, as great variability exists between the final results (impact of pig production) presented by the studies even for the same functional unit.

As previously stated, crop production is a major contributor to the overall impacts of the pig production chain. The globalization of feed ingredient markets is relevant to LCA studies because it disconnects commodity production from its use/consumption. In a context in which most of the ingredients used for feed production are internationally traded, it is important to highlight that the impacts associated with a certain product are virtually shared with several countries involved in the international trade. The most frequent example of this intercontinental sharing was the use of soybean imported from South America, mainly from Brazil, in European countries. Considering the pig database, 49%

TABLE 1 | Summary of the LCA studies on pig production in terms of location, functional unit, and climate change potential.

Code	Study	Country	Functional unit	Climate change potential[a], CO_2-eq
1	Basset-Mens and van der Werf (5)	France	1 kg of live weight	2.30–3.97 kg
2	Eriksson et al. (6)	Sweden	1 kg of live weight gain	1.36–1.51 kg
3	Basset-Mens et al. (7)	France	1 kg of live weight	2.30 kg
4	Basset-Mens et al. (8)	France	1 t of pig	0.88–1.39 t
5	Liang et al. (9)	Japan	1 kg of carcass weight	5.02 kg
6	Halberg et al. (10)	Denmark	1 kg of live weight	2.80–3.30 kg
7	Halberg et al. (10)	United States	1 t live weight pig	2.47–3.33 kg
8	Aramyan et al. (11)	Europe, several countries	1 kg of slaughter weight	2.55–2.97 kg
9	Bonesmo et al. (12)	Norway	1 kg of carcass weight	2.65 kg
10	Devers et al. (13)	United Kingdom	1 kg of cut pork	2.55–4.5 kg
11	Dolman et al. (14)	Netherlands	100 kg of live weight	473–637 kg
12	Stone et al. (15)	United States	1 pig (118kg)	398.20 kg
13	De Moraes et al. (16)	World, several countries	1 kg of live weight pig	5.36–5.57 kg
14	Luo et al. (17)	China	1 farm (1,956 units of 500 kg each)	5,611–5,714 t
15	Ogino et al. (18)	Japan	1 kg of meat after dressing	7.12–7.12 kg
16	Reckmann et al. (19)	Germany	1 market pig	346–370 kg
17	Dourmad et al. (20)	Europe, several countries	1 kg of slaughter weight	3.20–3.25 kg
18	Jacobsen et al. (21)	Belgium	1 kg of live weight pig	2.25–3.47 kg
19	Sasu-Boakye et al. (22)	Sweden	1 kg of deboned pork	5.70 kg
20	Cherubini et al. (23)	Brazil	1 kg carcass weight	2.10–2.20 kg
21	Cherubini et al. (24)	Brazil	1 t of swine carcass	3.11–3.55 t
22	González-García et al. (25)	Portugal	30 kg of weight gain (finishing phase)	67.15–76.02 kg
23	Mackenzie et al. (26)	Canada	1 kg of meat (carcass weight)	3.34 kg
24	Reckmann and Krieter (27)	Germany	1 kg of carcass weight	2.81 kg
25	van Zanten et al. (28)	Netherlands	1 kg of slaughter weight	3.09–3.36 kg
26	Wang et al. (29)	China	1 kg of live weight pig	2.50 kg
27	Groen et al. (30)	Netherlands	1,000 pigs	9.08E+04 kg
28	Kebreab et al. (31)	Europe, North, and South America	1 kg of live weight	2.61 kg
29	Lamnatou et al. (32)	Spain	1 t of live weight pig	1.98–2.46 t
30	Mackenzie et al. (33)	Canada	1 kg of meat (live or carcass weight)	3.2–5.5 kg
31	Monteiro et al. (34)	Brazil and France	1 market pig (105 kg)	336–460 kg
32	Noya et al. (35)	Spain	1 kg of carcass weight	1.95–2.55 kg
33	Pirlo et al. (36)	Italy	1 kg of weight gain (fattening phase)	2.27–3.00 kg
34	Sagastume Gutiérrez et al. (37)	Cuba	1 kg of live-weight pig	6.70 kg
35	Wang et al. (38)	China	1 kg of carcass pork	8.70 kg
36	Ali et al. (39)	Brazil	1 kg of cut pork	10.3 kg
37	Bava et al. (40)	Italy	1 kg of live weight gain	3.3 kg
38	Li et al. (41)	China	1 pig (120kg)	1,019 kg
39	Monteiro et al. (42)	Brazil	1 market pig	2.29–3.19 kg
40	Noya et al. (43)	Spain	1 kg of live weight pig	1.13–1.96 kg
41	Noya et al. (44)	Spain	1 kg of live weight pig	2.69–5.81kg
42	Six et al. (45)	Belgium	1 market pig	248.53 kg
43	Andretta et al. (46)	Brazil	1 kg of weight gain (fattening phase)	2.57–2.67 kg
44	Rudolph et al. (47)	Europe, several countries	1 kg of cut pork	4.96 kg
45	Arrieta and González (48)	Argentina	100 kg live weight pig	342 kg
46	Monteiro et al. (49)	Brazil	100 g of pork	0.46 kg
47	Monteiro et al. (50)	Europe, several countries	1 t live weight pig	1.78–2.36 t
48	Ottosen et al. (51)	Denmark	1 t live weight pig	1.47–2.71 t
49	Reyes et al. (52)	Cuba	1 t of live weight pig	0.89–0.94 t
50	Anestis et al. (53)	Greece	1 kg of weight gain (nursery phase)	1.76–2.45 kg

(Continued)

TABLE 1 | Continued

Code	Study	Country	Functional unit	Climate change potential[a], CO_2-eq
51	Cadero et al. (54)	France	1 kg of live weight pig	5.07–9.35 kg
52	Garcia-Gudino et al. (55)	Spain	1 kg of live weight pig	4.18 kg
53	Horrillo and Gaspar (56)	Spain	1 kg of live weight pig	6.87–9.65 kg
54	Monteiro et al. (57)	Brazil	1 kg of live weight pig	3.85–4.15 kg
55	Pexas et al. (58)	Denmark	1 kg of live weight gain	2.16–2.48 kg

[a]*Original results were preserved, however, some conversions were needed for the purpose of having the same weight unit as the functional unit.*

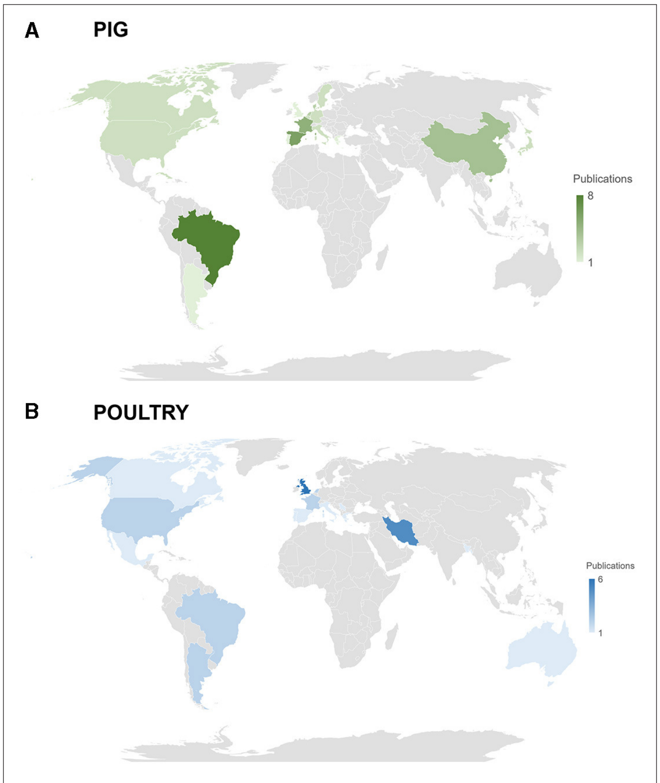

FIGURE 2 | Location of the LCA studies focusing on pig **(A)** or poultry **(B)** production. Studies that simulated two or more countries, or even an entire continent, are not displayed in the figure.

of the studies mentioned the use of Brazilian soybean. For that reason, several papers also mentioned the inclusion of overseas transport during the inventory characterization.

Studies Focusing on Poultry Production

The research process until obtaining the final poultry database is described in **Figure 1B**. Articles obtained by online searches (6,502 references) were critically evaluated, which resulted in several exclusions. Seventeen references were excluded when assessing the full-text (criterium i and ii: 2 publications; criterium iii: 1 publication; criterium iv: 7 publications; and criterium v: 7 publications). The final list of 30 selected studies is described in **Table 3**.

The first study identified in the poultry database was published in 2006. Considering the entire database, 13 journals reported publications, with 10 papers being published in Journal of Cleaner Production and 5 papers in Poultry Science. Broiler production was evaluated in 18 studies, eggs were the main product evaluated in 10 studies, and both products were assessed in two papers. Production scenarios located in the United Kingdom and Iran (which were considered in 6 studies each); followed by Argentina, Brazil, France, Netherlands, and the USA, which were considered in two studies each; as illustrated in **Figure 2B**.

Likewise to the pig database, the scope described as cradle-to-farm gate was used in most studies focusing on poultry production. Impacts associated with slaughtering and processing were considered in 10 publications. Besides climate change, studies presented other impact categories (**Figure 3B**), such as acidification, eutrophication, and the use of energy and land. The characterization of the meat or egg production in the region or country was the main objective in 15 studies (**Table 4**).

Three papers described the environmental impacts of replacing ingredients in feed formulas, while one paper described the impacts of dietary supplementation with protease. Feeding was highlighted as the major source of environmental impact in most studies, accounting for 28–82% of the overall impact of climate change (**Figure 4B**). Despite the importance of feeding to the total impact, the diet composition used in the inventory was not described in most studies. Only 13% of the papers described the ingredient formulas, while only 10% of the studies showed any description for dietary nutritional composition. In addition, the environmental impacts related to the production of individual ingredients were presented in only 13% of the papers, with the impact of feed production (considering as a functional unit; e.g., 1 ton of feed) being presented in only 20% of the publications. The use of Brazilian soybean was reported by 30% of the studies, highlighting the importance of international trade also for the environmental impact of poultry production.

DISCUSSION

The availability of peer-reviewed publications using LCA to assess the environmental impacts of pig and poultry production systems has increased over the years (**Figure 5**). The first studies of each database were published in close years for both pig (2005) and poultry (2006) production chains. However, the

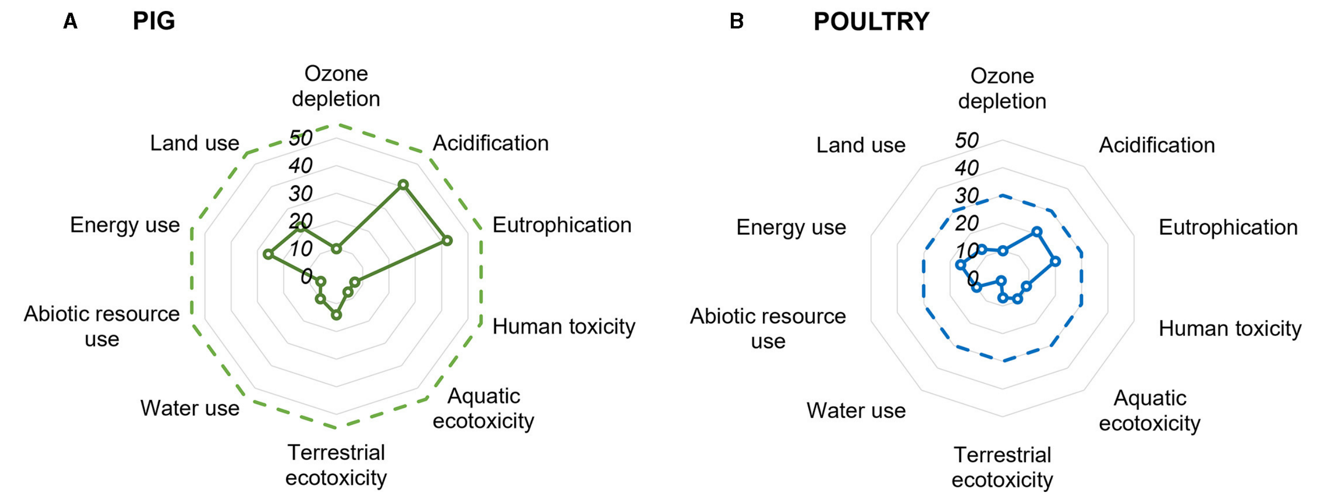

FIGURE 3 | Environmental impact categories evaluated in the LCA studies focusing on pig **(A)** or poultry **(B)** production systems, with dashed lines indicating the total number of publications included in each database.

availability of studies focusing on pig production evolved greatly in the following years, mainly after 2014. In most research areas, the number of studies on poultry production is great than the number of publications available in a comparable topic in pigs. However, the opposite was found in this systematic review, probably due to the higher risk and concern with the environmental impacts of pig production compared to poultry systems.

The interest in using LCA to investigate the sustainability of a given production originates from its capability to quantify and evaluate the resources consumed and the emissions released at each phase needed for its production (8). Concerns about food safety and climate change have greatly increased in recent years. In response, the livestock industry must then reduce the utilization of resources by increasing its efficiency while reducing its environmental impact.

The impacts estimated for both production systems varied greatly across studies, mainly due to the heterogeneity of functional units and the amplitude of the considered life-cycle scopes. However, other attributes may also be listed as sources of variability when comparing publications. In particular, this heterogeneity may be related to the production systems under analysis (10, 62, 63), as well as to regional characteristics (31, 69). The conditions considered for housing (58), farm size (38, 79), level of intensification (20), and manure management (23) are also reported as important factors determining the final impact associated to the product. When focusing on animal aspects, some welfare (68) and genetic traits (50, 51, 73), as well as sanitary aspects (54) were reported.

Feed production was highlighted in several papers due to its relevant contribution to the total environmental impact. This phase was simulated including each ingredient's life cycle, fabrication, and transportation to the feed mill or to the farm in most studies. The reported contribution of the feed production phase relative to the overall GHG emissions varied from 31 to 76% in the pig database. In the poultry database, it accounted for 28–82% of the total climate change impact. Regardless of the exact environmental impact attributed to the feeding phase, almost all studies identified feeding as the production factor having the greatest environmental impact. These findings support the hypothesis that eco-friendly feeding practices can mitigate the environmental impacts of pig and poultry production.

Importance of Rearing System Scenarios

Even though a comparison between organic and conventional systems will not be deeply reviewed, it is important to highlight that several studies indicated the production system as one of the important aspects determining the relative contribution of feeding to the overall environmental impact (due to the feed ingredient composition, number of feeding phases, among others). Conventional production systems were considered in most simulations (i.e., conventional feed ingredients). However, some studies evaluated the environmental impacts of adopting alternative production systems (e.g., organic, free-range, certified labels). According to Leinonen et al. (62, 63), the global warming impact necessary to obtain a given functional unit of feed (e.g., 1 ton) can be low in organic farms in comparison to conventional farms. However, a higher feed amount is generally necessary for organic farms than in conventional production systems to obtain the same functional unit. Several reasons are indicated in the papers, as the impairment in feed conversion ratio, an increase in feed consumption, or even waste of feed or products. Thus, when the total cycle is analyzed, a greater global warming potential impact may be associated with feeding animals in organic than in conventional systems (10, 62, 63).

The environmental impacts of a given rearing system are highly correlated with animal performance, especially feed efficiency (27, 87). Thus, technologies that improve animal performance usually have great potential to mitigate

TABLE 2 | Summary of the LCA studies on pig production in terms of main subject under analysis and scope boundary.

Code	Main study subject	Scope final boundary
1	Production systems	At farm gate
2	Feed choice	At farm gate
3	Implications of uncertainty and variability	At farm gate
4	Production systems	At farm gate
5	Production in Japan	At farm gate
6	Production systems (organic)	At farm gate
7	Production systems	At farm gate
8	Production system in Europe	At farm gate
9	Production in Norway	At farm gate
10	Production in Western Cape and Flanders	Delivered to the distribution center
11	Production systems	At farm gate
12	Production in the United States	At farm gate
13	Immunological castration	At farm gate
14	Manure management	At farm gate
15	Low-protein diet supplemented with amino acids	At slaughterhouse gate
16	Production in Germany	At farm gate
17	Production systems	At slaughterhouse gate
18	Production in Flanders	At farm gate
19	Protein sources for feed production	At pork cutting gate
20	Manure management	At farm gate
21	Feed composition for finishing pigs	At slaughterhouse gate
22	Production in Portugal	At farm gate
23	Production in Canada	At the slaughterhouse gate
24	Farm performance	At farm gate
25	Replacing soybean meal with rapeseed meal	At slaughterhouse gate
26	Production in North China	At farm gate
27	Sensitivity analysis	At farm gate
28	Specialty feed ingredients	At farm gate
29	Production in Spain	At farm gate
30	Utilizing co-products as feed	At farm gate
31	Protein source, feeding programs (including precision feeding), amino acids inclusion	At farm gate
32	Production in Catalonia	At farm gate
33	Production in Italy (heavy pig)	At farm gate
34	Manure management	At farm gate
35	Husbandry on different scale	At slaughterhouse gate
36	Using co-products in the diets of finishing pigs	At slaughterhouse gate
37	Production system in Italy (heavy pig)	At farm gate
38	Crop-swine integrated system	At farm gate
39	Reduced dietary protein levels	At farm gate
40	Production in Catalonia	At farm gate
41	Production in Galicia	At farm gate
42	Supply chain management	At farm gate
43	Precision feeding	At farm gate
44	Production systems (organic)	At pork cutting gate
45	Production in Argentina	At farm gate

(Continued)

TABLE 2 | Continued

Code	Main study subject	Scope final boundary
46	Individual data of performance and excretion	At retail gate
47	European local breeds	At farm gate
48	Altering genetic components of individual traits	At farm gate
49	Production system in Cuba	At farm gate
50	Dietary modification for fattening pigs	At farm gate
51	Feeding practices, animal health, and farm infrastructure	At farm gate
52	Production in Spain	At farm gate
53	Agroecosystems	At farm gate
54	Source of performance and excretion data	At farm gate
55	Housing conditions and manure management	At farm gate

life cycle environmental impacts. In this particular aspect, some technologies were assessed in the reviewed studies. Immunological castration and feed additives are some of these factors (16, 31, 53, 75), but probably many more aspects still need to be evaluated in future research.

Another important factor evaluated in some studies was the impact of innovative practices during the cultivation or processing of feed ingredients. The use of maize genetically modified (59), different processing methods for soybean (48), or crop-animal integrated systems (41) were evaluated, and impacts in the final product (i.e., functional unit) were reported.

Importance of Feeding Scenarios

The use of alternative feed ingredients is an important strategy in livestock systems. Some studies presented environmental advantages when using co-products in the assessed feeds (28, 33, 39). Other papers indicated that these advantages may be related to the calculation method, with favorable results being reported only when the impacts were not co-allocated between the main and the co-products (35, 88). The environmental cost to obtain co-products cannot be ignored in the LCA analysis, and this should be probably further evaluated in future research. Another limitation to be considered when comparing studies are the different ingredient choices given the difficulties in data acquisition, especially for local or limited ingredients as well as the great variability among processes used to obtain co-products (64, 88).

The distance between feedstuff production location and their place of use is an important argument in favor of using ingredient choice (or replacement) as a strategy to mitigate environmental impacts. Feed ingredients are products with cross-border flows, which are a consequence of globalization. Reducing the distance from producers to consumers means fewer transportation needs, and consequently fewer costs and emissions. Using this argument, several studies were developed proposing the use of locally grown ingredients instead of products cultivated in

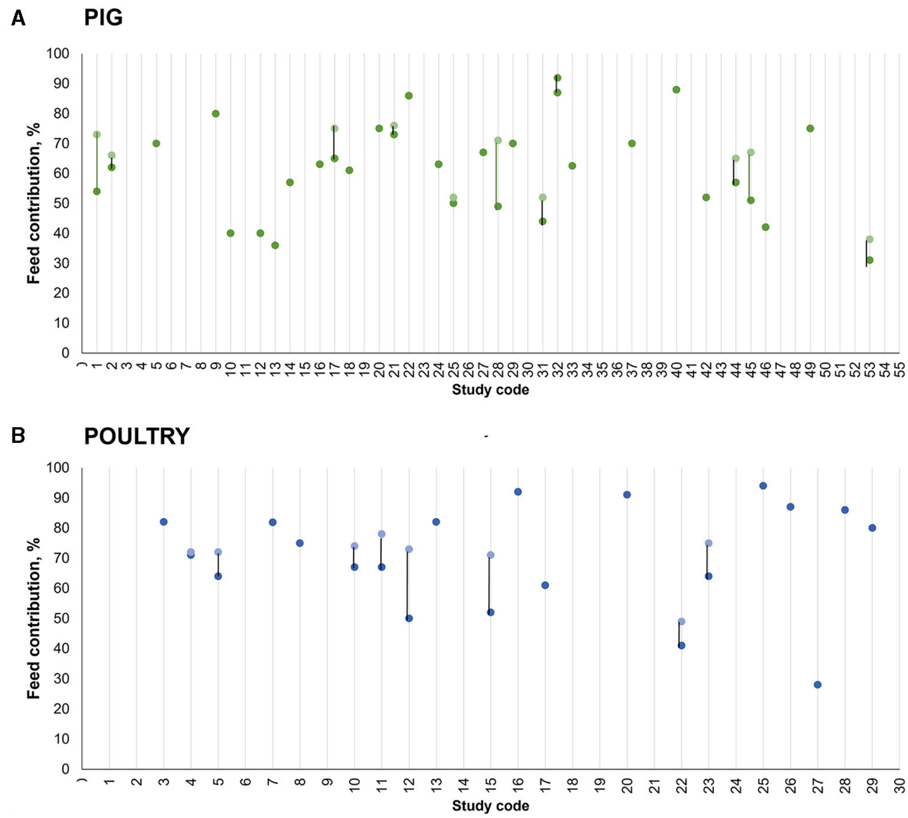

FIGURE 4 | Feed contribution to the potential impact of climate change in LCA studies focusing on pig **(A)** or poultry **(B)** production. Study codes are the same as those presented in **Tables 1**, **2** for pigs and poultry, respectively. Blank lines were used for studies where the exact information was not presented in the original publication (text or tables, as the exact value could not be obtained when information was presented in figures).

different countries or even continents (22, 43, 64). This is particularly important for local protein-ingredients that replace imported soybean and soybean meal (64). However, the use of local ingredients must not impair feed conversion (24, 66). Otherwise, the advantages may be lost caused by increased demand for feed to reach the same final weight.

Feed composition in terms of ingredients is also a way to reduce the excretion of nutrients and, consequently, manure composition. For that reason, the choice of ingredients needs to be made always with caution, focusing on the origin, but also on the nutritional quality of the product. Nitrogen excretion in manure is highly correlated with diet formulation. If an increase in nitrogen losses in the manure is related to a given ingredient choice, it is expected that this modification will lead to higher GHG emissions and probably other major consequences too (65). In this context, strategies that mitigate nutrient excretion, such as enzyme supplementation (75), synthetic amino acid partially replacing protein crops, or the use of low-protein diets (6, 42, 57), can potentially mitigate the environmental impacts of both pig and poultry production. The modification of the feed formulation method (89) and the adoption of precision feeding techniques (46, 90) are also very important and innovative tools. Due to its relevance for future animal production, precision feeding will be further discussed in the next section, with a focus on pig production.

Precision Feeding as an Eco-Friendly Strategy to Mitigate the Environmental Impacts of Pig Production

Feeding is a major source of environmental impacts, as previously discussed. When correctly applied, precision feeding is an efficient tool to decrease production and environmental costs (91). Pigs and poultry are usually fed according to group requirements, disregarding individual particularities. This means that all animals receive the same feed for an extended period, with part of the population receiving nutrients above their requirements (92). The animals that receive nutrients above their needs excrete this excess. An increased protein intake decreases protein efficiency utilization, resulting in larger nitrogen excretions (93). In many pig commercial systems, the nitrogen retention in conventional phase-feeding programs will rarely exceed 35%, being that the efficiency of nitrogen utilization used in many LCA studies (94). However, nitrogen efficiency varies depending on age, sanitary status, and crude protein levels (95, 96).

Precision feeding consists in providing the right amount of feed with the right balance composition to each animal at

TABLE 3 | Summary of the LCA studies on poultry production in terms of location, focus, functional unit and climate change potential.

Code	Study	Country	Focus	Functional unit	Climate change potential[a], CO_2-eq
1	Bennett et al. (59)	Argentina	Meat	1 kg (body weight) of broiler	NA
2	Mollenhorst et al. (60)	Netherlands	Egg	1 kg of eggs	3.9–4.6 kg
3	Pelletier (61)	United States	Meat	1 t (live weight) of broiler	1.40 t
4	Leinonen et al. (62)	United Kingdom	Meat	1 t of expected carcass	4.41–5.66 t
5	Leinonen et al. (63)	United Kingdom	Egg	1 t of marketable eggs	2.92–3.45 t
6	Leinonen et al. (64)	United Kingdom	Meat/Egg	1 t of expected carcass weight	3.54–4.39 t
7	Pelletier et al. (65)	United States	Egg	1 t of marketable eggs	2.95–3.46 t
8	Thévenot et al. (66)	Reunion Island (France)	Meat	1 t of produced eggs	4.20–6.10 t
9	González-García et al. (67)	Portugal	Meat	1 t of produced eggs	4.32–6.45 t
10	Leinonen et al. (68)	United Kingdom	Meat/Egg	1 t of liquid eggs	4.95–7.48 t
11	Prudêncio da Silva et al. (69)	Brazil, France	Meat	1 t of whole chickens packed	2.49 t
12	Taylor et al. (70)	United Kingdom	Egg	1 kg (live weight) of broiler	1.62 kg
13	Ghasempour and Ahmadi (71)	Iran	Egg	1 kg of chicken meat packed	2.46 kg
14	Kalhor et al. (72)	Iran	Meat	1 t of expected carcass weight	4.22–4.42 t
15	Kebreab et al. (31)	Europe, North, and South America	Meat	1 t of marketable eggs	2.83–2.92 t
16	Leinonen et al. (73)	United Kingdom	Meat	1 t (live weight) of broiler	1.45–2.70 t
17	Cesari et al. (74)	Italy	Meat	1 t of packaged chicken	1.95–4.02 t
18	Giannenas et al. (75)	Greece	Meat	1 dozen eggs	1.9–2.5 kg
19	Mainali et al. (76)	Bangladesh	Egg	1 kg of expected carcass	4.07 kg
20	Payandeh et al. (77)	Iran	Meat	1 t (live weight) of broiler	1.39–3.25 t
21	Pelletier (78)	Canada	Egg	1 t of packed meat	2.93–5.36 t
22	Pishgar-Komleh et al. (79)	Iran	Meat	1 t (live weight) of broiler	1.12–1.34 t
23	Wiedemann et al. (80)	Australia	Meat	1 kg (live weight) of broiler	3.03–3.84 kg
24	Abín et al. (81)	Spain	Egg	1 kg of carcass	5.52 kg
25	Skunca et al. (82)	Serbia	Meat	1 t of expected carcass weight	2.76 t
26	Arrieta and González (48)	Argentina	Meat	1 kg (live weight) of broiler	1.63–4.21 kg
27	Duarte da Silva Lima et al. (83)	Brazil	Meat	10,000 eggs	1.74 t
28	Ramedani et al. (84)	Iran	Meat	1 t (live weight) of broiler	5.00–5.78 t
29	van Hal et al. (85)	Netherlands	Egg	1 t of produced eggs	1.37–2.44 t
30	Estrada-González et al. (86)	Mexico	Egg	1,000 broilers	17.36–20.25 t

[a]*Original results were preserved, however, some conversions were needed for the purpose of having the same weight unit as the functional unit.*

the right time. Thus, precision feeding can be defined as the technology that provides each animal the nutrients tailored to meet in real-time the animal requirements (91). Nitrogen and phosphorus excretions can be decreased by 40% and consequently reduce production costs by 10% when using an individual precision feeding program (93, 97).

In this context, precision feeding can improve the sustainability of pig production systems. Automatic feeding stations allow pigs to be fed individually with a diet whose composition is appropriate to their growth potential (91). This strategy is an important pattern shift in animal nutrition because at this point nutritional requirements are no longer consider static, but as dynamic processes that develop differently for each individual.

The use of precision feeding instead of conventional group feeding systems already demonstrated several benefits. The increased nutrient-use efficiency and the consequent reduction in the excretion of polluting substances to the environment, improving the overall sustainability of the production system, are the main advantages presented by this feeding system (91). In addition, studies have shown that it is possible to considerably reduce soybean meal and dicalcium phosphate in diet formulations compared to conventional programs. In validation studies (93, 97), individual feeding allowed a reduction in lysine intake by up to 26%, and nitrogen and phosphorus excretion by 30 and 14%, respectively, without affecting the productive pig performance.

Environmental Impacts of Applying Precision Feeding Techniques

Before applying precision feeding techniques, it is necessary to study the environmental impacts of adopting these techniques. An LCA study performed by Andretta et al. (46) intends to estimate the environmental impact of precision feeding techniques applied to pig production. Once again, in Brazilian scenarios, feeding was the largest source of environmental impact. In addition, the study showed that

TABLE 4 | Summary of the LCA studies on poultry production in terms of main subject under analysis and scope boundary.

Code	Main study subject	Focus	Scope final boundary
1	Conventional and genetically modified maize	Meat	At processing plant door
2	Production systems	Egg	At farm gate
3	Production system in the United States	Meat	At farm gate
4	Production system in the United Kingdom	Meat	At farm gate
5	Production system in the United Kingdom	Egg	At farm gate
6	Alternative protein crops	Meat/Egg	At farm gate
7	Production system in the United States	Egg	At farm gate
8	Accounting for farm diversity	Meat	At farm gate
9	Production system in Portugal	Meat	At shell egg processor facilities
10	Welfare-enhancing system changes	Meat/Egg	At breaker facilities
11	Large and small-scale production in Brazil and France	Meat	At processor door
12	Production systems (free-range)	Egg	At farm gate
13	Production system in Iran	Egg	At processor door
14	Production system in Iran	Meat	At farm gate
15	Specialty feed ingredients	Meat	At farm gate
16	Genetic changes	Meat	At farm gate
17	Production system in Italy	Meat	At processor gate
18	Protease and replacement of soybean meal	Meat	At farm gate
19	Litter management	Egg	At farm gate
20	Mitigating environmental impacts by data envelopment analysis	Meat	At farm gate
21	Production system in Canada and housing systems	Egg	At processor door
22	Production system in Iran	Meat	At farm gate
23	Production system in Australia	Meat	At farm gate
24	Production system in Spain	Egg	At slaughterhouse gate
25	Chicken meat chain	Meat	At farm gate
26	Production system in Argentina	Meat	At farm gate
27	Production system in Brazil	Meat	At farm gate
28	Comparing ostrich and chicken production	Meat	At farm gate
29	Feed-food competition	Egg	At processor door
30	Production system in Mexico	Egg	At farm gate

replacing conventional group feeding with daily group feeding (nutrient supply adjusted daily to meet the group requirements) could decrease the potential impact of eutrophication by 4% and acidification by 3%. The mitigation was even greater (up to 6% for the potential impact of climate change and 5% for eutrophication and acidification) when the program was applied to each animal individually (pigs received diets daily tailored to their requirements).

The study also highlighted a reduction over time in the potential impact of climate change associated with pig feed production related to reducing the expected dietary nutrient levels. In the simulated population, reducing the dietary standardized ileal digestible lysine level by one percentage point led to a reduction of up to 194.7 kg of CO_2-eq per ton of feed, depending on the simulated scenario. Certainly, the main advantage of this method was the improved nutrient use efficiency. In other words, the same amount of product was produced using fewer resources. Monteiro et al. (34) performed a similar study considering Brazilian and French scenarios with simulated data (the previous study used data collected *in vivo*). In their study, a precision feeding system that fed pigs individually was able to reduce the impact of climate change by 7%.

Future Challenges

Animals are exposed to several conditions during their lives and these factors may impact directly their nutrient requirements (98). For example, sanitary challenges affect the way amino acids are used by the animal because the nutrients that would be used for protein deposition are directed to cope with the immune system (98). Sanitary challenges also impact the growth performance of pigs and broilers (99), reducing feed efficiency and consequently increasing the environmental impact associated with this production (27). Cadero et al. (54) reported a significant effect of impaired health status on the carbon footprint of pig production. This is only one of several topics that need to be more evaluated in the future, especially in a scenario with reduced use of antibiotics in animal production.

Several studies on the environmental impact of animal production have been published, but only a few have worked using precision feeding programs or considering sanitary challenges. More studies must be carried out to better understand their environmental impact on modern pig and poultry production. Despite all the variability found in livestock, precision systems can foster some eco-friendly solutions by the possibility of managing animals as an individual, having their diets tailored based on real-time data.

Important Aspects to Be Considered When Applying LCA to Animal Science

LCA is a well-known and established method to evaluate environmental impacts, particularly for complex production chains as those in the livestock sector. However, LCA has its limitation like any other scientific method. Some of these limitations have been described by Finkbeiner et al. (100). Some of these gaps may apply in the studies described in this systematic review.

One important limitation observed in the studies was the assessment of water use. Many studies did not include this impact category or they did not consider water consumption (water not returned to the system), which is very relevant for agriculture (100, 101).

The great variability in functional units is certainly another important limitation to be highlighted. The unit choice is a challenging task because it impacts directly on the results and is also related to the objective and scope (100). However, the

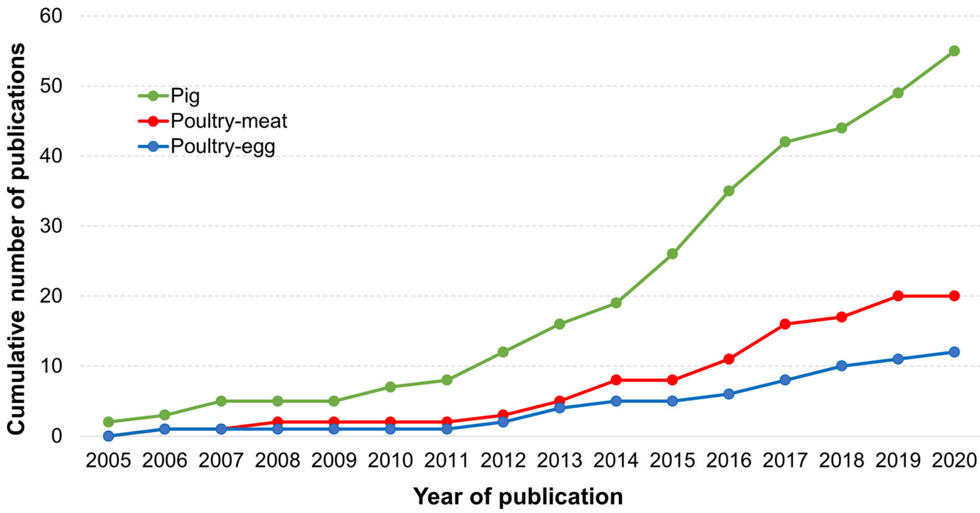

FIGURE 5 | Cumulative number of LCA studies focusing on pig or poultry production.

variability among studies is a great limitation when comparing results since transformations are sometimes not possible or precise (e.g., results expressed for 1 ton of live pig are difficult to compare to those expressed for 1 ton of carcass because there are more processes included and sometimes the carcass yield is not fully known).

In addition, impacts on human health are probably insufficiently covered in LCA studies dealing with pig and poultry production. Soil contamination, noise, and odors are some of these impacts that are not commonly addressed in LCA studies. Additionally, the LCA method fails to consider other aspects, such as biodiversity, welfare, and social aspects (100). The positive impact of specific activities may be also disregarded.

Finally, the choice of a single scenario to represent the reality of an entire production chain is another important limitation of some reviewed LCA studies. The issue related to data gathering was previously highlighted (102). A single model (e.g., data collected in a single scenario) are not able to describe the pig and poultry production systems worldwide, and neither probably across regions. Even in integrated systems that are characterized by a higher level of uniformity, it is possible to observe a different performance in each producer (for the same genetic type, with the same feed, and similar management practices). Thus, variability is something that needs to be considered in future LCA studies.

CONCLUSION

This systematic review confirmed feeding as the largest source of environmental impact associated with pig and poultry production systems. This supports the hypothesis that novel feeding techniques may mitigate the environmental footprint associated with both production chains. Precision feeding is highlighted as a way to optimize nutrient-use efficiency and, for that reason, as a promising tool toward more sustainable animal production systems. It is still a challenging task to properly consider and compare the variability among LCA studies. Despite these issues, LCA is a comprehensive way to assess sustainability from a global perspective and its application on pig and poultry production systems is very encouraged in future research.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon request.

AUTHOR CONTRIBUTIONS

AR, CF, CO, MK, and IA searched articles for the systematic review and interpreted results. IA interpreted results, prepared figures, and tables, and wrote the first draft of the manuscript. M-PL-M and CP were involved in the interpretation and discussion of results. FH and AM contributed to manuscript revision, read, and drafted the final version of the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

1. Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural sustainability and intensive production practices. *Nature.* (2002) 418:671–7. doi: 10.1038/nature01014
2. Simmons J. Making safe, affordable and abundant food a global reality. *Range Beef Cow Symposium.* Lincoln (2011).
3. McAuliffe GA, Chapman DV, Sage CL. A thematic review of life cycle assessment (LCA) applied to pig production. *Environ Impact Assess Rev.* (2016) 56:12–22. doi: 10.1016/j.eiar.2015.08.008
4. Lovatto PA, Hauschild L, Hauptli L, Lehnen CR, Carvalho Ad'A. Modelagem da ingestão, retenção e excreção de nitrogênio e fósforo pela suinocultura Brasileira. *J Rev Bras Zootecn.* (2005) 34:2348–54. doi: 10.1590/S1516-35982005000700022
5. Basset-Mens C, van der Werf HMG. Scenario-based environmental assessment of farming systems: the case of pig production in France. *Agric Ecosyst Environ.* (2005) 105:127–44. doi: 10.1016/j.agee.2004.05.007
6. Eriksson IS, Elmquist H, Stern S, Nybrant T. Environmental systems analysis of pig production: the impact of feed choice. *Int J Life Cycle Assess.* (2005) 10:143–54. doi: 10.1065/lca2004.06.160
7. Basset-Mens C, van der Werf HMG, Durand P, Leterme P. Implications of uncertainty and variability in the Life Cycle Assessment of pig production systems. *Int J Life Cycle Assess.* (2006) 11:298–304. doi: 10.1065/lca2005.08.219
8. Basset-Mens C, van der Werf HMG, Robin P, Morvan T, Hassouna M, Paillat JM, et al. Methods and data for the environmental inventory of contrasting pig production systems. *J Clean Prod.* (2007) 15:1395–405. doi: 10.1016/j.jclepro.2006.03.009
9. Liang R, Taniguchi K, Kawashima H, Kikuchi E, Soma T. Estimation of global warming emissions associated with a pig production system by Life Cycle Assessment. *J Life Cycle Assess.* (2007) 3:178–83. doi: 10.3370/lca.3.178
10. Halberg N, Hermansen JE, Kristensen IS, Eriksen J, Tvedegaard N, Petersen BM. Impact of organic pig production systems on CO_2 emission, C sequestration and nitrate pollution. *Agron Sustain Dev.* (2010) 30:721–31. doi: 10.1051/agro/2010006
11. Aramyan L, Hoste R, van den Broek W, Groot J, Soethoudt H, Nguyen TL, et al. Towards sustainable food production: a scenario study of the European pork sector. *J Chain Netw Sci.* (2011) 11:177–89. doi: 10.3920/JCNS2011.Qpork8
12. Bonesmo H, Little SM, Harstad OM, Beauchemin KA, Skjelvåg AO, Sjelmo O. Estimating farm-scale greenhouse gas emission intensity of pig production in Norway. *Acta Agric Scand Sect A Anim Sci.* (2012) 62:318–25. doi: 10.1080/09064702.2013.770913
13. Devers L, Kleynhans TE, Mathijs E. Comparative life cycle assessment of Flemish and Western Cape pork production. *Agrekon.* (2012) 51:105–28. doi: 10.1080/03031853.2012.741208
14. Dolman MA, Vrolijk HCJ, de Boer IJM. Exploring variation in economic, environmental and societal performance among Dutch fattening pig farms. *Livest Sci.* (2012) 149:143–54. doi: 10.1016/j.livsci.2012.07.008
15. Stone JJ, Dollarhide CR, Benning JL, Gregg Carlson C, Clay DE. The life cycle impacts of feed for modern grow-finish Northern Great Plains US swine production. *Agric Syst.* (2012) 106:1–10. doi: 10.1016/j.agsy.2011.11.002
16. De Moraes PJU, Allison J, Robinson JA, Baldo GL, Boeri F, Borla P. Life cycle assessment (LCA) and environmental product declaration (EPD) of an immunological product for boar taint control in male pigs. *J Environ Assess Policy Manag.* (2013) 15:1–26. doi: 10.1142/S1464333213500014
17. Luo Y, Stichnothe H, Schuchardt F, Li G, Huaitalla RM, Xu W. Life cycle assessment of manure management and nutrient recycling from a Chinese pig farm. *Waste Manag Res.* (2013) 32:4–12. doi: 10.1177/0734242X13512715
18. Ogino A, Osada T, Takada R, Takagi T, Tsujimoto S, Tonoue T, et al. Life cycle assessment of Japanese pig farming using low-protein diet supplemented with amino acids. *Soil Sci Plant Nutr.* (2013) 59:107–18. doi: 10.1080/00380768.2012.730476
19. Reckmann K, Traulsen I, Krieter J. Life Cycle Assessment of pork production: a data inventory for the case of Germany. *Livest Sci.* (2013) 157:586–96. doi: 10.1016/j.livsci.2013.09.001
20. Dourmad JY, Ryschawy J, Trousson T, Bonneau M, Gonzàlez J, Houwers HWJ, et al. Evaluating environmental impacts of contrasting pig farming systems with life cycle assessment. *Animal.* (2014) 8:2027–37. doi: 10.1017/S1751731114002134
21. Jacobsen R, Vandermeulen V, Van Huylenbroeck G, Gellynck X. Carbon footprint of pigmeat in Flanders. *Int J Agric Sustain.* (2014) 12:54–70. doi: 10.1080/14735903.2013.798896
22. Sasu-Boakye Y, Cederberg C, Wirsenius S. Localising livestock protein feed production and the impact on land use and greenhouse gas emissions. *Animal.* (2014) 8:1339–48. doi: 10.1017/S1751731114001293
23. Cherubini E, Zanghelini GM, Alvarenga RAF, Franco D, Soares SR. Life cycle assessment of swine production in Brazil: a comparison of four manure management systems. *J Clean Prod.* (2015) 87:68–77. doi: 10.1016/j.jclepro.2014.10.035
24. Cherubini E, Zanghelini GM, Tavares JMR, Belettini F, Soares SR. The finishing stage in swine production: influences of feed composition on carbon footprint. *Environ Dev Sustain.* (2015) 17:1313–28. doi: 10.1007/s10668-014-9607-9
25. González-García S, Belo S, Dias AC, Rodrigues JV, Costa RRD, Ferreira A, Andrade LPD, Arroja L. Life cycle assessment of pigmeat production: Portuguese case study and proposal of improvement options. *J Clean Prod.* (2015) 100:126–39. doi: 10.1016/j.jclepro.2015.03.048
26. Mackenzie SG, Leinonen I, Ferguson N, Kyriazakis I. Accounting for uncertainty in the quantification of the environmental impacts of Canadian pig farming systems1. *J Anim Sci.* (2015) 93:3130–43. doi: 10.2527/jas.2014-8403
27. Reckmann K, Krieter J. Environmental impacts of the pork supply chain with regard to farm performance. *J Agric Sci.* (2015) 153:411–21. doi: 10.1017/S0021859614000501
28. van Zanten HH, Bikker P, Mollenhorst H, Meerburg BG, de Boer IJM. Environmental impact of replacing soybean meal with rapeseed meal in diets of finishing pigs. *Animal.* (2015) 9:1866–74. doi: 10.1017/S1751731115001469
29. Wang X, Dadouma A, Chen Y, Sui P, Gao W, Jia L. Sustainability evaluation of the large-scale pig farming system in North China: an emergy analysis based on life cycle assessment. *J Clean Prod.* (2015) 102:144–64. doi: 10.1016/j.jclepro.2015.04.071
30. Groen EA, van Zanten HHE, Heijungs R, Bokkers EAM, de Boer IJM. Sensitivity analysis of greenhouse gas emissions from a pork production chain. *J Clean Prod.* (2016) 129:202–11. doi: 10.1016/j.jclepro.2016.04.081
31. Kebreab E, Liedke A, Caro D, Deimling S, Binder M, Finkbeiner M. Environmental impact of using specialty feed ingredients in swine and poultry production: a life cycle assessment1. *J Anim Sci.* (2016) 94:2664–81. doi: 10.2527/jas.2015-9036
32. Lamnatou C, Ezcurra-Ciaurriz X, Chemisana D, Plà-Aragonés LM. Environmental assessment of a pork-production system in North-East of Spain focusing on life-cycle swine nutrition. *J Clean Prod.* (2016) 137:105–15. doi: 10.1016/j.jclepro.2016.07.051
33. Mackenzie SG, Leinonen I, Ferguson N, Kyriazakis I. Can the environmental impact of pig systems be reduced by utilising co-products as feed? *J Clean Prod.* (2016) 115:172–81. doi: 10.1016/j.jclepro.2015.12.074
34. Monteiro A, Garcia-Launay F, Brossard L, Wilfart A, Dourmad JY. Effect of feeding strategy on environmental impacts of pig fattening in different contexts of production: evaluation through life cycle assessment. *J Anim Sci.* (2016) 94:4832–47. doi: 10.2527/jas.2016-0529
35. Noya I, Aldea X, Gasol CM, Gonzalez-Garcia S, Amores MJ, Colon J, et al. Carbon and water footprint of pork supply chain in Catalonia: From feed to final products. *J Environ Manage.* (2016) 171:133–43. doi: 10.1016/j.jenvman.2016.01.039
36. Pirlo G, Care S, Casa GD, Marchetti R, Ponzoni G, Faeti V, et al. Environmental impact of heavy pig production in a sample of Italian farms. A cradle-to-farm gate analysis. *Sci Total Environ.* (2016) 565:576–85. doi: 10.1016/j.scitotenv.2016.04.174
37. Sagastume Gutiérrez A, Cabello Eras JJ, Billen P, Vandecasteele C. Environmental assessment of pig production in Cienfuegos, Cuba: alternatives for manure management. *J Clean Prod.* (2016) 112:2518–28. doi: 10.1016/j.jclepro.2015.09.082
38. Wang X, Wu X, Yan P, Gao W, Chen Y, Sui P. Integrated analysis on economic and environmental consequences of livestock

husbandry on different scale in China. *J Clean Prod.* (2016) 119:1–12. doi: 10.1016/j.jclepro.2016.01.084

39. Ali BM, van Zanten HHE, Berentsen P, Bastiaansen JWM, Bikker P, Lansink AO. Environmental and economic impacts of using co-products in the diets of finishing pigs in Brazil. *J Clean Prod.* (2017) 162:247–59. doi: 10.1016/j.jclepro.2017.06.041
40. Bava L, Zucali M, Sandrucci A, Tamburini A. Environmental impact of the typical heavy pig production in Italy. *J Clean Prod.* (2017) 140:685–91. doi: 10.1016/j.jclepro.2015.11.029
41. Li Z, Sui P, Wang X, Yang X, Long P, Cui J, et al. Comparison of net GHG emissions between separated system and crop-swine integrated system in the North China Plain. *J Clean Prod.* (2017) 149:653–64. doi: 10.1016/j.jclepro.2017.02.113
42. Monteiro ANTR, Bertol TM, de Oliveira PAV, Dourmad JY, Coldebella A, Kessler AM. The impact of feeding growing-finishing pigs with reduced dietary protein levels on performance, carcass traits, meat quality and environmental impacts. *Livestock Sci.* (2017) 198:162–9. doi: 10.1016/j.livsci.2017.02.014
43. Noya I, Aldea X, Gonzalez-Garcia S, Gasol CM, Moreira MT, Amores MJ, et al. Environmental assessment of the entire pork value chain in Catalonia - a strategy to work towards Circular Economy. *Sci Total Environ.* (2017) 589:122–9. doi: 10.1016/j.scitotenv.2017.02.186
44. Noya I, Villanueva-Rey P, Gonzalez-Garcia S, Fernandez MD, Rodriguez MR, Moreira MT. Life Cycle Assessment of pig production: a case study in Galicia. *J Clean Prod.* (2017) 142:4327–38. doi: 10.1016/j.jclepro.2016.11.160
45. Six L, De Wilde B, Vermeiren F, Van Hemelryck S, Vercaeren M, Zamagni A, et al. Using the product environmental footprint for supply chain management: lessons learned from a case study on pork. *Int J Life Cycle Assess.* (2017) 22:1354–72. doi: 10.1007/s11367-016-1249-8
46. Andretta I, Hauschild L, Kipper M, Pires P, Pomar C. Environmental impacts of precision feeding programs applied in pig production. *Animal.* (2018) 12:1990––8. doi: 10.1017/S1751731117003159
47. Rudolph G, Hörtenhuber S, Bochicchio D, Butler G, Brandhofer R, Dippel S, et al. Effect of three husbandry systems on environmental impact of organic pigs. *Sustainability.* (2018) 10:3796. doi: 10.3390/su10103796
48. Arrieta EM, González AD. Energy and carbon footprints of chicken and pork from intensive production systems in Argentina. *Sci Total Environ.* (2019) 673:20–8. doi: 10.1016/j.scitotenv.2019.04.002
49. Monteiro A, Dourmad JY, Fachinello MR, Diaz-Huepa LM, Sitanaka NY, Partyka AVS, et al. Effect of observed individual data of performance and excretion on life cycle assessment of piglets. *Sci Agric.* (2019) 76:102–11. doi: 10.1590/1678-992x-2017-0094
50. Monteiro ANTR, Wilfart A, Utzeri VJ, Batorek Lukač N, TomaŽin U, Costa LN, et al. Environmental impacts of pig production systems using European local breeds: the contribution of carbon sequestration and emissions from grazing. *J Clean Prod.* (2019) 237:117843. doi: 10.1016/j.jclepro.2019.117843
51. Ottosen M, Mackenzie SG, Wallace M, Kyriazakis I. A method to estimate the environmental impacts from genetic change in pig production systems. *Int J Life Cycle Assess.* (2019) 25:523–37. doi: 10.1007/s11367-019-01686-8
52. Reyes YA, Barrera EL, Valle AS, Gil MP, Garcia OH, Dewulf J. Life Cycle Assessment for the Cuban pig production: case study in Sancti Spiritus. *J Clean Prod.* (2019) 219:99–109. doi: 10.1016/j.jclepro.2019.02.047
53. Anestis V, Papanastasiou DK, Bartzanas T, Giannenas I, Skoufos I, Kittas C. Effect of a dietary modification for fattening pigs on the environmental performance of commercial pig production in Greece. *Sustain Prod Consum.* (2020) 22:162–76. doi: 10.1016/j.spc.2020.03.002
54. Cadero A, Aubry A, Dourmad JY, Salaun Y, Garcia-Launay F. Effects of interactions between feeding practices, animal health and farm infrastructure on technical, economic and environmental performances of a pig-fattening unit. *Animal.* (2020) 14:s348–s359. doi: 10.1017/S1751731120000300
55. Garcia-Gudino J, Monteiro A, Espagnol S, Blanco-Penedo I, Garcia-Launay F. Life Cycle Assessment of Iberian traditional pig production system in Spain. *Sustainability.* (2020) 12:627. doi: 10.3390/su12020627
56. Horrillo A, Gaspar P. Organic farming as a strategy to reduce carbon footprint in dehesa agroecosystems: a case study comparing different livestock products. *Animals.* (2020) 10:162. doi: 10.3390/ani10010162
57. Monteiro ANTR, Dourmad JY, Moreira CA, Rossi RM, Ferreira LFM, Costa AER, et al. The source of performance and excretion data affects the environmental impact of pig rearing estimated by life cycle assessment. *Can J Anim Sci.* (2020) 100:184–92. doi: 10.1139/cjas-2019-0022
58. Pexas G, Mackenzie SG, Wallace M, Kyriazakis I. Environmental impacts of housing conditions and manure management in European pig production systems through a life cycle perspective: a case study in Denmark. *J Clean Prod.* (2020) 253:120005. doi: 10.1016/j.jclepro.2020.120005
59. Bennett RM, Phipps RH, Strange AM. The use of life cycle assessment to compare the environmental impact of production and feeding of conventional and genetically modified maize for broiler production in Argentina. *J Anim Feed Sci.* (2006) 15:71–82. doi: 10.22358/jafs/66843/2006
60. Mollenhorst H, Berentsen PBM, De Boer IJM. On-farm quantification of sustainability indicators: an application to egg production systems. *Br Poult Sci.* (2006) 47:405–17. doi: 10.1080/00071660600829282
61. Pelletier N. Environmental performance in the US broiler poultry sector: life cycle energy use and greenhouse gas, ozone depleting, acidifying and eutrophying emissions. *Agric Syst.* (2008) 98:67–73. doi: 10.1016/j.agsy.2008.03.007
62. Leinonen I, Williams AG, Wiseman J, Guy J, Kyriazakis I. Predicting the environmental impacts of chicken systems in the United Kingdom through a life cycle assessment: broiler production systems. *Poult Sci.* (2012) 91:8–25. doi: 10.3382/ps.2011-01634
63. Leinonen I, Williams AG, Wiseman J, Guy J, Kyriazakis I. Predicting the environmental impacts of chicken systems in the United Kingdom through a life cycle assessment: egg production systems. *Poult Sci.* (2012) 91:26–40. doi: 10.3382/ps.2011-01635
64. Leinonen I, Williams AG, Waller AH, Kyriazakis I. Comparing the environmental impacts of alternative protein crops in poultry diets: The consequences of uncertainty. *Agric Syst.* (2013) 121:33–42. doi: 10.1016/j.agsy.2013.06.008
65. Pelletier N, Ibarburu M, Xin H. A carbon footprint analysis of egg production and processing supply chains in the Midwestern United States. *J Clean Prod.* (2013) 54:108–14. doi: 10.1016/j.jclepro.2013.04.041
66. Thévenot A, Aubin J, Tillard E, Vayssières J. Accounting for farm diversity in Life Cycle Assessment studies - the case of poultry production in a tropical island. *J Clean Prod.* (2013) 57:280–92. doi: 10.1016/j.jclepro.2013.05.027
67. González-García S, Gomez-Fernández Z, Dias AC, Feijoo G, Moreira MT, Arroja L. Life Cycle Assessment of broiler chicken production: a Portuguese case study. *J Clean Prod.* (2014) 74:125–34. doi: 10.1016/j.jclepro.2014.03.067
68. Leinonen I, Williams AG, Kyriazakis I. The effects of welfare-enhancing system changes on the environmental impacts of broiler and egg production. *Poult Sci.* (2014) 93:256–66. doi: 10.3382/ps.2013-03252
69. Prudêncio da Silva V, van der Werf HMG, Soares SR, Corson MS. Environmental impacts of French and Brazilian broiler chicken production scenarios: An LCA approach. *J Environ Manag.* (2014) 133:222–31. doi: 10.1016/j.jenvman.2013.12.011
70. Taylor RC, Omed H, Edwards-Jones G. The greenhouse emissions footprint of free-range eggs. *Poult Sci.* (2014) 93:231–7. doi: 10.3382/ps.2013-03489
71. Ghasempour A, Ahmadi E. Assessment of environment impacts of egg production chain using life cycle assessment. *J Environ Manage.* (2016) 183:980–7. doi: 10.1016/j.jenvman.2016.09.054
72. Kalhor T, Rajabipour A, Akram A, Sharifi M. Environmental impact assessment of chicken meat production using life cycle assessment. *Inf Process Agric.* (2016) 3:262–71. doi: 10.1016/j.inpa.2016.10.002
73. Leinonen I, Williams AG, Kyriazakis I. Potential environmental benefits of prospective genetic changes in broiler traits. *Poult Sci.* (2016) 95:228–36. doi: 10.3382/ps/pev323
74. Cesari V, Zucali M, Sandrucci A, Tamburini A, Bava L, Toschi I. Environmental impact assessment of an Italian vertically integrated broiler system through a Life Cycle approach. *J Clean Prod.* (2017) 143:904–11. doi: 10.1016/j.jclepro.2016.12.030
75. Giannenas I, Bonos E, Anestis V, Filioussis G, Papanastasiou DK, Bartzanas T, et al. Effects of protease addition and replacement of soybean meal by corn gluten meal on the growth of broilers and on the environmental performances of a broiler production system in Greece. *PLoS One.* (2017) 12:e0169511. doi: 10.1371/journal.pone.0169511

76. Mainali B, Emran SB, Silveira S. Greenhouse gas mitigation using poultry litter management techniques in Bangladesh. *Energy.* (2017) 127:155–66. doi: 10.1016/j.energy.2017.03.103
77. Payandeh Z, Kheiralipour K, Karimi M, Khoshnevisan B. Joint data envelopment analysis and life cycle assessment for environmental impact reduction in broiler production systems. *Energy.* (2017) 127:768–74. doi: 10.1016/j.energy.2017.03.112
78. Pelletier N. Life cycle assessment of Canadian egg products, with differentiation by hen housing system type. *J Clean Prod.* (2017) 152:167–80. doi: 10.1016/j.jclepro.2017.03.050
79. Pishgar-Komleh SH, Akram A, Keyhani A, van Zelm R. Life cycle energy use, costs, and greenhouse gas emission of broiler farms in different production systems in Iran-a case study of Alborz province. *Environ Sci Pollut Res.* (2017) 24:16041–9. doi: 10.1007/s11356-017-9255-3
80. Wiedemann SG, McGahan EJ, Murphy CM. Resource use and environmental impacts from Australian chicken meat production. *J Clean Prod.* (2017) 140:675–84. doi: 10.1016/j.jclepro.2016.06.086
81. Abín R, Laca A, Laca A, Díaz M. Environmental assessment of intensive egg production: a Spanish case study. *J Clean Prod.* (2018) 179:160–8. doi: 10.1016/j.jclepro.2018.01.067
82. Skunca D, Tomasevic I, Nastasijevic I, Tomovic V, Djekic I. Life cycle assessment of the chicken meat chain. *J Clean Prod.* (2018) 184:440–50. doi: 10.1016/j.jclepro.2018.02.274
83. Duarte da Silva Lima N, de Alencar Nääs I, Garcia RG, Jorge de Moura D. Environmental impact of Brazilian broiler production process: evaluation using life cycle assessment. *J Clean Prod.* (2019) 237:117752. doi: 10.1016/j.jclepro.2019.117752
84. Ramedani Z, Alimohammadian L, Kheialipour K, Delpisheh P, Abbasi Z. Comparing energy state and environmental impacts in ostrich and chicken production systems. *Environ Sci Pollut Res.* (2019) 26:28284–93. doi: 10.1007/s11356-019-05972-8
85. van Hal O, Weijenberg AAA, de Boer IJM, van Zanten HHE. Accounting for feed-food competition in environmental impact assessment: Towards a resource efficient food-system. *J Clean Prod.* (2019) 240:118241. doi: 10.1016/j.jclepro.2019.118241
86. Estrada-González I, Taboada-González P, Guerrero-García-Rojas H, Marquez-Benavides L. Decreasing the environmental impact in an egg-producing farm through the application of LCA and lean tools. *Appl Sci.* (2020) 10:1352. doi: 10.3390/app10041352
87. Pelletier N, Lammers P, Stender D, Pirog R. Life cycle assessment of high- and low-profitability commodity and deep-bedded niche swine production systems in the Upper Midwestern United States. *Agric Syst.* (2010) 103:599–608. doi: 10.1016/j.agsy.2010.07.001
88. Stone J, Aurand K, Clay S, Thaler R. Life cycle assessment of tylosin and chlortetracycline antimicrobial use at swine production facilities. In: *Rocky Mountain - 62nd Annual Meeting. Geological Society of America Abstracts with Programs*. Denver, CO (2010) 42:17.
89. Jean dit Bailleul P, Rivest J, Dubeau F, Pomar C. Reducing nitrogen excretion in pigs by modifying the traditional least-cost formulation algorithm. *Livest Prod Sci.* (2001) 72:199–211. doi: 10.1016/S0301-6226(01)00224-X
90. Monteiro ANTR, Garcia-Launay F, Brossard L, Wilfart A, Dourmad JY. Effect of precision feeding on environmental impact of fattening pig production. In: *European Conference on Precision Livestock Farming (ECPLF).* Nantes (2017). p. 565–73.
91. Pomar C, van Milgen J, Remus A. Precision livestock feeding, principle and practice. In: Hendriks WH, Verstegen MWA, Babinszky L, editors. *Poultry an Pig Nutrition Challenges of the 21st century.* Wageningen: Wageningen Academic Publishers (2019). p. 397–418. doi: 10.3920/978-90-8686-884-1_18
92. Hauschild L, Pomar C, Lovatto PA. Systematic comparison of the empirical and factorial methods used to estimate the nutrient requirements of growing pigs. *Animal.* (2010) 4:714–23. doi: 10.1017/S1751731109991546
93. Andretta I, Pomar C, Rivest J, Pomar J, Radünz J. Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. *Animal.* (2016) 10:1137–47. doi: 10.1017/S1751731115003067
94. Dourmad J, Guingand N, Latimier P, Seve B. Nitrogen and phosphorus consumption, utilisation and losses in pig production: France. *Livest Sci.* (1999) 58:199–211. doi: 10.1016/S0301-6226(99)00009-3
95. Rakhshandeh A, Htoo JK, Karrow N, Miller SP, de Lange CF. Impact of immune system stimulation on the ileal nutrient digestibility and utilisation of methionine plus cysteine intake for whole-body protein deposition in growing pigs. *Br J Nutr.* (2013) 111:101–10. doi: 10.1017/S0007114513001955
96. Remus A, Hauschild L, Pomar C. Simulated amino acid requirements of growing pigs differ between current factorial methods. *Animal.* (2020) 14:725–30. doi: 10.1017/S1751731119002660
97. Andretta I, Pomar C, Rivest J, Pomar J, Lovatto P, Radünz Neto J. The impact of feeding growing-finishing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition. *J Anim Sci.* (2014) 92:3925–36. doi: 10.2527/jas.2014-7643
98. Le Floc'h N, Gondret F, Matte JJ, Quesnel H. Towards amino acid recommendations for specific physiological and patho-physiological states in pigs. *Proc Nutr Soc.* (2012) 71:425–32. doi: 10.1017/S0029665112000560
99. Pastorelli H, van Milgen J, Lovatto P, Montagne L. Meta-analysis of feed intake and growth responses of growing pigs after a sanitary challenge. *Animal.* (2012) 6:952–61. doi: 10.1017/S1751731111 00228X
100. Finkbeiner M, Ackermann R, Bach V, Berger M, Brankatschk G, Chang Y-J, et al. Challenges in life cycle assessment: an overview of current gaps and research needs. In: W Klöpffer, editor, *Background and Future Prospects in Life Cycle Assessment.* Dordrecht: Springer (2014). p. 207–58.
101. Shiklomanov I, Rodda JC. *World Water Resources at the Beginning of the 21st Century*. International Hydrology Series. Cambridge: Cambridge University Press (2003).
102. Hospido A, Davis J, Berlin J, Sonesson U. A review of methodological issues affecting LCA of novel food products. *Int J Life Cycle Assess.* (2010) 15:44–52. doi: 10.1007/s11367-009-0130-4

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Feeding Strategies to Reduce Nutrient Losses and Improve the Sustainability of Growing Pigs

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The efficiency of pig production using nutrients has increased over the years. Still, better efficiency of nutrient utilization can be achieved by feeding pigs with diets adjusted to their estimated requirements. An increase in nutrient efficiency of utilization represents economic gains while maximizing environmental performance. The objective of this paper is to review the impact of different methods of diet formulation that provide farm animals with the amount of nutrients to satisfy their needs while minimizing nutrient excretion and greenhouse gas emissions. Diet formulation is one tool that can help to maximize nitrogen and energy utilization by decreasing crude protein content in diets. The use of local feedstuff and non-human-edible products (e.g., canola meal) associated with synthetic amino acid inclusion in the diet are valuable techniques to reduce carbon footprint. Precision feeding and nutrition is another powerful tool that allows not only daily tailoring of diets for maximal nutrient efficiency of utilization but also to reduce costs and improve nitrogen efficiency of utilization. In this review, we simulated through mathematical models the nitrogen and energy efficiency of utilization resulting from crude protein reduction in the diet. An 8% crude protein reduction in the diet can increase nitrogen efficiency of utilization by 54% while costing 11% less than a control diet without synthetic amino acids. The same reduction in crude protein represented a major improvement in available energy due to the decrease of energetic losses linked to protein deamination. Urinary and hindgut fermentation energy losses were 24% lower for pigs fed with low-protein diets when compared to control diets. In terms of modern feeding techniques and strategies, precision feeding and nutrition can decrease nitrogen excretion by 30% when compared to group phase feeding. The benefits of feeding pigs with low-protein diets and precision feeding techniques are additive and might result in a 61% nitrogen efficiency of utilization. There is room for improvement in the way nutrient requirements are estimated in pigs. Improving the understanding of the variation of nutrient utilization among pigs can contribute to further environmental gains.

Keywords: low protein diets, sustainable pig production, precision feeding, precision nutrition, nutrient utilization, nutrient efficiency of utilization

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INTRODUCTION

Farm animals are raised to produce commodities such as meat, dairy products, and fiber. Energy, amino acids (AA), minerals, vitamins, and water are used by animals for body maintenance, growth, reproduction, and lactation. Body maintenance and the synthesis of body tissues (i.e., lean, fat, etc.) are dependent upon an adequate supply of dietary nutrients (1). The energy and nutrient losses associated with the conversion of dietary energy and nutrients into animal products increase production costs and may also contribute to an environmental load of animal farms by the excessive application of nitrogen (N), phosphorus, or trace minerals from manure or by the carbon and methane losses. The conversion rate of dietary nutrients into animal products is generally low. Dietary crude protein (i.e., nitrogen), which is one of the most limiting and expensive nutrients in monogastric feeds, is converted to body protein by pigs with efficiencies that vary between 15 (2) and 33% (3). Similar figures are found for beef cattle and broilers, in which the efficiency ranges from 10 to 20% and from 30 to 40%, respectively (2). Nonetheless, given the global human population growth and the increasing demand for vegetable protein for human and livestock production, the method we are using to evaluate production efficiency needs to be redefined (4–6). For the efficiencies of conversion of human-edible livestock feeds into human-edible animal products, it may be more appropriate to evaluate these efficiencies in the actual context of limited global land resources and food security rather than just the efficiency of conversion of livestock feeds into units of animal products (4, 7, 8). For instance the use of digestible indispensable amino acids score to quantify differences in protein quality together with the concept of human-edible protein conversion efficiency allows to quantify the net protein contribution of a system (9, 10). Pig and chicken net protein contribution are around 0.64 and 0.76, respectively, while dairy cows will reach a 3.6 score (11). A score >1 indicates that the animal chain has a positive impact on providing human nutrients. Although these calculations are highly impacted by the feedstuff used in pig and poultry diet, the numbers are pointing for a competition for food between humans and non-ruminants. The challenge to animal scientists and the livestock sector is to improve the efficiency of use of feed resources by matching available nutrients to the animal requirements while reducing the livestock dependence on human-edible feeds (6, 7, 12, 13).

The efficiency by which farm animals convert the dietary nutrient provisions into animal products depends on many factors. These factors can be associated with the animal (i.e., its metabolism, age, and species), the feeding method (i.e., feed composition, feeding phases), and the environment (i.e., housing system). Within the animal, there are various causes of nutrient inefficiency. Thus, part of the ingested nutrients are used for basal metabolic processes involving degradation (catabolism) and synthesis (anabolism) or are lost in the digestive tract through desquamation and endogenous secretions (14). These nutrient losses are generally referred to as maintenance losses. Nutrients are also lost during the synthesis of animal products (e.g., body lean). In growing animals, the losses associated with the utilization of the first-limiting AA for body protein deposition can largely be attributed to its inevitable catabolism (14, 15). These inevitable AA losses should be differentiated from other metabolic losses related to the preferential AA catabolism, which results from the catabolism of AA given in excess, from the excretion of chemically unavailable absorbed AA (e.g., heat-damaged proteins) (16, 17), and to a minor extent from integumental AA losses and from the use of AA for the synthesis of non-protein body compounds (14). In growing pigs fed with cereal-based diets, the sum of the undigested N and the losses associated with digestion, maintenance functions, and body protein deposition may represent 33% of the total ingested N, and similar values are obtained for dietary P (3). These sources of nutrient inefficiency are difficult to reduce because they are inherent to the animal metabolism and occur during digestion and metabolic processes (18).

Other sources of nutrient losses are related to the composition of the feeds and the methods we use to provide these feeds to the animals. Because these losses are related to the way we are feeding and raising the animals, there is great potential for improvement. Indeed, the feeds are responsible for the largest part (70%) of the environmental impact caused by pig production (19, 20). This is because in practical conditions most of the pigs within the herd receive more nutrients than they need (21–23), and all excess nutrients are excreted and contribute to the overall nutrient inefficiency. To reduce the supply of excess nutrients and thus reduce their excretion, it is essential to: (a) precisely estimate the amount of dietary nutrients that will be available for the animals' metabolism; (b) estimate the amount of nutrients required by each animal throughout the growing period; (c) formulate balanced diets that limit excess nutrients; and (d) concomitantly adjust the dietary supply of nutrients to match the animals' estimated requirements (24). The estimation of available nutrients in the available feed ingredients and the determination of nutrient requirements have been previously addressed (25, 26). Additionally, the environmental impact of livestock production must also include the direct and indirect contribution of farm animals and manure disposal to greenhouse gas (GHG) emissions, which in some cases, like in pig and poultry production, may contribute to around 9.5% of the global livestock GHG emissions (27, 28). The objective of this paper is to review the impact of the different methods of diet formulation to provide growing pigs with the amount of nutrients that satisfy their needs and concomitantly minimize their excretion and GHG emissions.

FORMULATING BALANCED DIETS TO REDUCE NUTRIENT LOSSES AND EXCRETION

Formulating a compound feed for farm animals refers to the determination of a blend of feed ingredients and additives that will have the concentration of nutrients that will allow the achievement of the production goals at an optimized feed cost (29). A compound feed is said to be complete when it provides all the nutrients required by animals. Many farm animals are fed today with complete diets.

One of the essential requisites for diet formulation is to precisely know the nutrients in feed ingredients that will be available to the animals after digestion and the amount of nutrients that are required by the animal to live and produce. Linear programming is the most widely used method for diet formulation and involves determining the level of incorporation of the available feed ingredients that, by respecting a series of linear constraints, will minimize (or maximize) an objective function, typically the cost of the blend. Other methods, such as goal programming, are proposed as an extension of linear programming to include several optimization criteria (30). Nonetheless, the main characteristics of these methods are the result of the linear nature of the objective function and constraints (31), which requires the verification of important assumptions such as the additivity (the value of the objective function is the sum of the contributions of each ingredient, and, similarly, the nutritional contribution of a blend of ingredients is the sum of the nutrient contribution of each ingredient), proportionality (the change in the contribution of an ingredient in a blend changes the nutritional value and cost of the blend in proportion to the change) and divisibility (the incorporation of an ingredient in a mixture is divisible indefinitely, and there are no ingredient or nutrient interactions).

For any nutrient, feed ingredient provisions and animal requirements can be expressed in different units or within different nutritional systems. The system and units used to appraise the potential nutrient contribution of feed ingredients and those required by animals have to verify these assumptions of the formulation method. For example, the apparent ileal digestibility of AA does not satisfy the additivity assumption, because animal responses to increasing levels of an AA are not necessarily linear (32). The use of net energy and standardized ileal digestible AA systems circumvent these limitations (32–34).

Furthermore, AA requirements are often expressed based on the concept of the ideal protein. The ideal protein concept was proposed more than 50 years ago and refers to a protein in which all dietary essential AA and the pool of dietary non-essential AA are co-limiting so that AA supply exactly matches the AA requirement (35, 36). Lysine has traditionally been used as the reference AA because it is the first limiting AA when pigs and poultry are fed with corn-soybean meal based feeds. The utilization of the ideal protein concept greatly simplifies practical animal nutrition and feed formulation, because the nutritionist only has to evaluate the requirement of lysine and extend the requirements of the other AA using the ideal protein profile.

Nonetheless, the scope of the conventional diet formulation methods is to satisfy the nutritional constraints while minimizing the cost of the blended feed and the supply of excess nutrients when adding environmental constraints. Other than the limitations inherent to the linearity of the objective function and constraints, and the assumptions identified above, linear programming is limited by the objective function, which is normally proposed to minimize the cost of the feed (i.e., the blend). In other words, what counts is to provide the necessary nutrients independently of their origin. Thus, two diets are assumed to be equivalent if they satisfy all the nutritional constraints of the formulation method independently of the nutrient excesses they provide. Unfortunately, reducing the environmental footprint by adding environmental objectives in the diet formulation method is often considered a complex and costly task that adversely affects production competitiveness. Introducing environmental objectives in the diet formulation algorithms can be accomplished by modifying the traditional least-cost formulation algorithm (37–39), using goal and other programming techniques (30, 40–42) and others. However, whatever formulation method is chosen, the environmental criteria to be minimized must be those that will have the greatest impact on the environmental footprint of production. The use of life cycle assessment to globally quantify this environmental footprint is a promising avenue (43) but has the downside that it attributes to the livestock feed the environmental footprint associated with the production of ingredients, fertilizers, etc. The resulting solution may be optimal for society in general, but it will not necessarily be optimal for the production sector or the producer himself. The practical use of this approach will require the adoption of national and international policies allowing the sharing of the environmental costs between consumers and the various stakeholders in the sector (4). Only the environmental footprint associated with animal feeding is considered in this study.

Mitigate the Carbon Footprint by Feed Formulation

With the increasing demand from society to reduce the global environmental carbon footprint of animal production systems with a focus on improving the sustainability of the production of feed ingredients, the utilization of these ingredients by livestock and the disposal of manure is warranted. Thus, other than formulating the feeds to reduce nutrient losses and excretion, more strategies are required to mitigate global production carbon footprint. Thus, (1) formulating feeds using local ingredients, (2) using by-products from the food and bio-energy industry, (3) formulating low-protein diets by increasing the use of crystalline AA, and (4) using more efficient crops with reduced fertilizer (e.g., precision farming) have been proposed (44). Between all these strategies, the use of more efficient crops can help to decrease the carbon footprint. However, when considering changes in land use, low-protein diets with crystalline AA seems to be the most efficient strategy to mitigate the carbon footprint (44). Crystalline AA are synthetically made but present with the same configuration as naturally occurring AA. The use of feed-grade AA allows replacing bound protein by synthetic and crystalline AA (45, 46). Amino acids can be produced by the different methods such as: extraction from protein hydrolysates, chemical synthesis, and microbial processes; each method presenting different economic and environmental advantages (45, 47). Crystalline AA are the product of bacterial fermentation which is purified by crystallization (45). In production contexts like in Europe, where feed ingredients are frequently imported from distant countries like Brazil and Argentina, reducing the utilization of soybean meal by using feed-grade AA significantly decreases land use, carbon footprint, and GHG emissions (43, 48, 49). Reducing soybean meal utilization can be attained by formulating low-protein diets by incorporating crystalline AA, by using precision feeding, or both. Nonetheless, these feeding alternatives are environmentally viable only if they do not compromise growth performance (50, 51).

Simulated Impact of Low-Protein Diets in Nutrient Efficiency, Nutrient Excretion, and Carbon Footprint

Energy, AA, minerals, vitamins, and water are essential nutrients needed by animals to live (maintenance), grow, and produce (reproduction, lactation, etc.). When formulating a diet, it is necessary to consider that animals must be provided with all these essential nutrients in adequate amounts and in forms that are palatable, digestible, and metabolically available in order to optimize growth, reproduction and production (1). It is also assumed that for many nutrients, and particularly for AA, their excess will not compromise performance. In fact, the excreted N originates from the undigested, unbalanced, and chemically unavailable dietary protein fractions, from the protein given in excess to the animals, and from the inevitable protein catabolism (14). With the increasing availability of crystalline AA such as L-lysine, DL-methionine (or its analogs), L-threonine, L-tryptophane, and L-valine, it is now possible to formulate low-protein diets with a well-balanced AA content. When providing pigs and other monogastric animals with the required amount of essential AA, including the pool of non-essential AA does not affect animals' growth (1, 52–54).

The impact of low-protein diets in nutrient efficiency, nutrient excretion, and carbon footprint was evaluated by simulation feeding growing pigs with five feeds formulated to lower dietary crude protein (CP) content with the inclusion of different crystalline AA based on studies addressing the use of low-protein diets (44, 55). The feeds were formulated to meet the requirements of 25–50-kg body weight growing pigs (23, 56, 57) using the nutritional matrix of the NRC (1) for feed ingredients, the standardized ileal digestibility values of EvaPig® software (v. 1.4.0.1; INRA, Saint-Gilles, France), and recognized ideal protein AA profile (54, 58). The feed ingredients used to formulate the basal diet (Diet 1) contained corn, wheat, soybean meal, canola meal, vegetable oil, mineral sources (micro-mineral premix, calcium carbonate, dicalcium phosphate hydrated), and phytase. These feed ingredients were chosen from local sources when possible, while costs were those of January 2021 expressed in US dollars using the conversion rate of January 15, 2021.

Pig performance was simulated (59) based on previous study results (18, 58, 60), assuming that during a 28-d feeding phase starting at 25 kg BW, pigs will have an average daily feed intake (ADFI) of 2 kg, average daily weight gain (ADG) of 0.95 kg, and an average protein deposition (PD) of 152 g/d. Daily lysine requirements (g/d) were calculated by adding maintenance and growth requirements as generally suggested in the literature (53, 56, 57). Fecal energy losses were estimated by the difference between the gross and digestible dietary energy in diets. Urinary energy losses were calculated as suggested by van Milgen et al. (53), assuming that they originated from the deamination of two nitrogenous component fractions, one obligatory and another variable. The obligatory energy loss fraction is associated with maintenance, while the variable urinary energy excretion fraction is proportional to the excess protein supply. The difference between digestible and metabolizable energy represents the methane loss from fiber fermentation. Heat losses were obtained by determining the difference between the metabolizable and net dietary energy. These values were multiplied by ADFI to estimate average energy losses (MJ/day). Nitrogen and other nutrient excretion values were obtained by subtracting the estimated retention from the respective nutrient intake values.

In relation to growing pig AA requirements, corn is poor in lysine (1), which is generally the first limiting AA in the diets of many growing animals, including pigs. Because of this limited lysine content in corn, a higher amount of soybean meal has to be included in conventional corn-soybean meal diets to meet the lysine requirement of pigs, which results in high CP levels (55). The basal diet (Diet 1) formulated to satisfy the AA requirements of pigs without the addition of crystalline AA was mainly composed of soybean and canola meal, whose inclusion accounted for 38% of the diet and resulted in 22.3% CP diet (**Figure 1A**, **Table 1**). Supplementing this basal diet with L-lysine until the second essential AA becomes limiting (i.e., threonine; Diet 2) reduced dietary CP by 7% (**Figure 1B**). This decrease in dietary CP resulted from a decrease in soybean and canola meals and an increase in corn and wheat. In relation to the basal diet, a reduction of 10% in dietary CP can be obtained by supplementing the basal diet with L-lysine and L-threonine until the third AA becomes limiting (Diet 3). At this point, tryptophane and methionine became limiting, and by supplementing with these four feed-grade AA, a 17% CP reduction (19% CP content) can be obtained (Diet 4). Valine becomes the next limiting AA. Supplementing the basal diet with L-lysine, L-tryptophan, L-threonine, MHA-methionine, and L-valine resulted in a 26% reduction in the CP in Diet 5 (16% CP content). It is important to stress that the order of the limiting AA and the potential CP reduction in the diet depends on the nutritional matrix used, the ideal AA profile chosen, the economical scenario, and the estimated AA requirements of the animal.

The use of five feed-grade crystalline AA allowed a decrease in soybean and canola meals by 50% in relation to the basal diet (Diet 1). These feed ingredients accounted for 38% of this reference diet. Such reductions in protein-providing feed ingredients in livestock diets not only significantly reduces N excretion, but also contributes to reductions in land use and carbon footprint (48, 49). Nitrogen excretion was reduced in the present study by 8% per percent unit reduction in dietary CP, which is in agreement with Wang et al. (55), who reported reductions of N excretion of 8–10% for each percent unit reduction in dietary CP. In the present simulation study, the efficiency of N retention increased from 40 to 54% when pigs were fed with diets 1–5, respectively. Concomitantly, reducing dietary CP also reduced feed cost (**Figure 2**). Although feed cost continuously changes over time and across production contexts, Diet 5 was 11% cheaper than the control diet, resulting from the reduction of soybean and canola meal inclusion in the AA-supplemented diets.

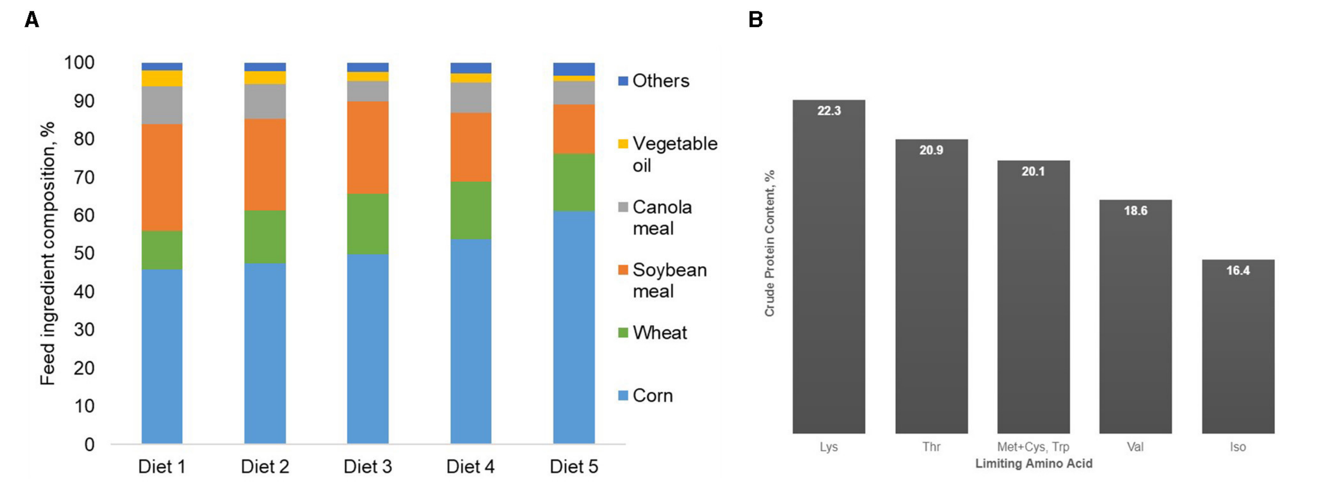

FIGURE 1 | Feed ingredient composition of diets formulated for grower pigs (25–50 kg BW) in standard Canadian pig diets when soybean and canola meals are gradually replaced by corn and feed-grade amino acids **(A)**. Crude protein content and limiting AA of diets formulated for grower pigs (25–50 kg BW) in standard Canadian conditions when soybean and canola meals are gradually replaced by corn and feed-grade amino acids **(B)**.

Dietary gross energy is not totally available for meeting the requirements of animals, since some energy is lost in feces, in urine, as fermentation gases (methane, hydrogen) and as heat (i.e., heat increment). The energy losses that are found in the feces come from the organic matter of the diet that has not been digested by the animal (61). Fecal energy losses may represent 14% of the gross energy intake, while urinary and fermentation losses may represent 8% in non-supplemented diets. Feeding pigs with low-protein diets will reduce fecal losses by 11% given the higher energy digestibility of energy in cereals than in soybean and canola meals. Furthermore, low-protein diets will significantly decrease protein deamination, which was therefore the energy loss component that presented the greatest difference in energetic cost. Urinary and hindgut fermentation energy losses were 24% greater for pigs fed with the basal diet (22% CP) than with low-CP diet (16% CP), likely because 31.1 kJ of energy is needed to deaminate and excrete each g of excess N in the urine (1). Heat increment decreased by 13% between diets 5 and 1. Such a change in heat increment is mainly due to the change in the proportion of starch and protein content in the diet. Given that glucose is used more efficiently than protein as an ATP source (62), reducing excess protein also decreases heat increment. Furthermore, high dietary CP content stimulates body protein turnover, a process which increases energy expenditure (63).

PRECISION FEEDING AS A TOOL TO IMPROVE NUTRIENT EFFICIENCY OF UTILIZATION

Reducing the excretion of excess nutrients and restricting the use of non-renewable resources are essential components in the development of sustainable livestock production systems. The amount of nutrients that are excreted depends mainly on how much nutrients are ingested, how metabolically available they are, and how their supply by the diet is balanced with the animals' requirements. In growing animals, the optimal concentration of nutrients in the diet progressively decreases over time (1). Therefore, an efficient way to reduce the excretion of excess nutrients is to concomitantly adjust their supply to the animals' requirements (64, 65). The economic and environmental benefits of this concomitant nutrient adjustment increase with the number of feeding phases (64, 66, 67). However, increasing the number of feeding phases complicates feed management and sometimes increases facility costs. The development of feeding systems that allow blend feeding and the automatic distribution of two feeds that, when combined in variable ratios, can meet the requirements of pigs throughout their growing period (64, 68) makes the phase-feeding technique promising again because nutrient excretion can be significantly reduced without increasing feeding costs (69). Nonetheless, there are two important sources of variation to be controlled in farm animals, which are the between-animal variation and the overtime variation on nutrient requirements (70, 71). Conventional farm animals are fed with the same feed during long periods (1, 72). Therefore, only the overtime variation can be controlled by increasing the number of feeding phases. Furthermore, given that for most nutrients underfed animals will exhibit reduced performance, whereas the overfed ones exhibit near-optimal performance, nutrients are provided to satisfy the requirements of the most demanding animals in the herd to ensure optimal production performance (i.e., growth) (21, 22, 73). In this situation, almost all animals receive more nutrients than they need. Furthermore, to account for the lack of information to precisely estimate the optimal level of nutrients to be provided to the group, the composition of feed ingredients, and other uncontrolled and unknown factors (e.g., environment, health), nutritionists include safety margins when formulating diets for maximum population responses.

TABLE 1 | Estimated nutrient composition and simulated results of the diets formulated for grower pigs (25–50 kg body weight) in standard Canadian diets when soybean and canola meals are gradually replaced by corn and feed-grade amino acids.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Estimated energy and nutrient composition					
Dry matter	87.74	87.63	87.46	87.45	87.26
Ash	4.96	4.82	4.64	4.66	4.39
Crude protein	22.34	20.88	20.11	18.64	16.48
Crude fat	6.63	5.96	5.07	5.07	4.29
NDF	9.8	9.81	9.42	9.78	9.58
ADF	4.7	4.52	4.1	4.27	3.9
Starch	35.8	38.96	41.43	43.4	47.77
Gross Energy, MJ/kg diet	17.23	16.99	16.74	16.65	16.36
Digestible Energy, MJ/kg diet	14.87	14.69	14.57	14.44	14.25
Metabolizable Energy, MJ/kg diet	14.15	14.01	13.91	13.82	13.7
Net Energy, MJ/kg diet	10.39	10.38	10.35	10.38	10.43
Total Lys, %/kg diet	1.19	1.17	1.16	1.15	1.13
SID* Lys, %/kg diet	1.02	1.02	1.02	1.02	1.02
Simulated animal responses[a]					
Fecal energy losses, MJ/d	4.72	4.6	4.34	4.42	4.22
Urinary energy losses, MJ/d	1.44	1.36	1.32	1.24	1.10
Heat increment[c], MJ/d	7.52	7.26	7.12	6.88	6.54
Nitrogen intake, g/d	69.70	65.15	62.74	58.16	51.29
Nitrogen retained as protein[b], g/d	24	24	24	24	24
Nitrogen for maintenance[b, c], g/d	3.4	3.4	3.4	3.4	3.4
Nitrogen excreted, g/d	42.26	37.71	35.30	30.72	23.85
Nitrogen retention, %	39.37	42.12	43.73	47.18	53.50

*Standardized ileal digestible Lysine (Lys).
[a]Assuming an ADFI of 1.95 kg/d, an ADG of 0.95 kg/d, and that 16% of ADG is deposited as protein.
[b]Hauschild et al. (56).
[c]Van Milgen et al. (53).

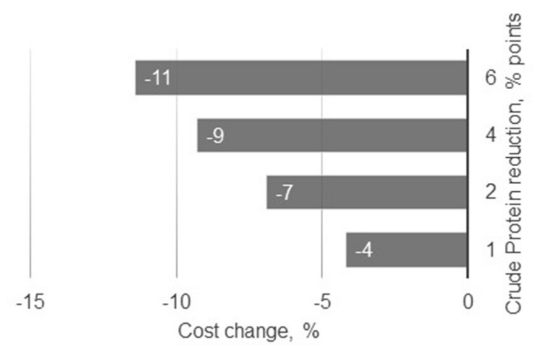

FIGURE 2 | Changes in estimated feeding costs as result of changes in crude protein content in the diet.

Precision feeding or precision animal nutrition is the practice of feeding animals with diets tailored daily (71). Precision feeding and nutrition is part of the precision livestock farming approach and involves the use of feeding techniques that allow the proper amount of feed with the suitable composition to be supplied in a timely manner to individual animals or groups of animals (24, 74). The automatic collection of data by the use of interconnected smart sensors and devices and the use of big data analysis techniques combined with conventional mathematical and data-driven models using deep learning algorithms and control devices (i.e., automatic feeders) are required for precision feeding applications (71). The application of precision feeding at the individual level is only possible where measurements, data processing, and control actions are taken at the individual animal level (71).

The use of real-time feed-intake and body-weight information allows estimating the required amount of nutrients that a group of pigs (22) or each pig in the herd (56) needs to grow at its potential. For example, a real-time modeling-control approach was used by Pomar et al. (64) to control the time-dependent variation of group-housed pigs offered feed *ad libitum*. In this system only two feeds are used throughout the grow-finishing period: feed A, which has high nutritional density, and feed B, which has a low nutritional density (24, 74). The daily tailored diet is obtained by mixing the right proportion of these two feeds to each individual (individual precision feeding) or for a group of animals [daily-phase group-feeding system; (75)]. Comparing a conventional three-phase feeding system to a daily-phase group-feeding system, these authors concluded that CP intake could be reduced by 7% while N excretion is reduced by 12%.

Controlling the time-dependent and between-animal variation can further help to reduce nutrient intake and excretion. The modeling approach proposed by Hauschild et al. (56) was used to estimate real-time nutrient requirements of individual pigs. The performance of growing pigs fed according to a conventional three-phase feeding system, similar to the one used by Pomar et al. (64), or using precision feeding were compared by Andretta et al. (75, 76) and Remus et al. (77), who observed that feeding pigs with diets in which the concentration of standardized ileal digestibility (SID) lysine is adjusted daily to the estimated requirements of each animal resulted in a 27% reduction in total lysine supply without detrimental effects on growth. This additional 20% reduction in SID lysine intake in relation to group-fed pigs was obtained by feeding the animals individually and thus simultaneously controlling the time-dependent and between-animal variation. Although feed cost reduction depends to a great extent on feed prices, it is expected that feed cost can be reduced by 1–3% when only controlling the time-dependent variation, while an 8–10% reduction can be obtained when controlling both sources of variation. Nitrogen excretion was reduced by nearly 30% when pigs were fed with daily tailored diets. The efficiency with which dietary protein was used for body protein retention was improved by 12.5% (75) and 13.4 % (76). Moreover, crude and SID lysine were improved in these trials by 30 and 23%, respectively. These differences between the CP and lysine efficiencies resulted from the fact that the experimental diets were not formulated to minimize CP content and the lysine to CP contents were different between feeds A and B.

Formulating Low-Protein Diets for Precision Feeding

The benefits of feeding pigs with low-protein diets and precision feeding techniques are additive. Therefore, formulating diets for precision-fed pigs with crystalline feed-grade AA can dramatically reduce the carbon footprint of growing-finishing pig production. Thus, if the diets in the trial of Andretta et al. (76) would have been formulated as proposed for Diet 5 in the previous simulation exercise, we could theoretically expect reductions in N excretion up to 43% instead of the observed 26%, with an N efficiency moving from 54 to 61%. It is important to see from these trials that young animals are much more efficient than older ones and that feeding pigs under requirements dramatically improves N and other nutrients efficiencies. Indeed, feeding pigs at 90% of the estimated SID Lys requirements would decrease protein retention by about 5%, while N excretion can be reduced by nearly 20% in relation to pigs fed to requirements. This reduction is, however, very sensitive to the formulation method. In fact, the feeds formulated for young animals are more concentrated in all nutrients, including AA. Therefore, the use of feed-grade crystalline AA will have a greater effect on the reduction of total protein than feeds formulated for heavier animals. When the diets are formulated for precision feeding, again, the more concentrated feed responds more to the incorporation of AA than the less concentrated feed. On the other hand, the less concentrated feed (i.e., feed B) is normally formulated for the least demanding animals when they are the least demanding. Therefore, at the end of the growing period, the required levels of AA and other nutrients are low and they are less affected by the incorporation of crystalline AA. These less concentrated feeds do not require the incorporation of any protein-providing ingredient, given that even the AA concentration of cereals exceeds the required level for this feed.

The Limitations of Actual Methods to Formulate Low-Protein Diets in the Context of Conventional and Precision Feeding Systems

The formulation of low-protein diets can have a great impact on livestock sustainability, but it is in using low-protein diets in precision feeding settings where the impact can be greater, given the additivity of both feeding techniques. Feeds and feeding remain the most important production factors to reduce the carbon footprint given that they account for around 70% of the environmental impact of pig and poultry production (20). Nonetheless, despite the tangible benefits of using low-protein diets and feeding pigs with efficient precision feeding systems, there are limitations to the actual principles we are using to formulate low-protein diets (23, 55) and for precision feeding systems (71).

Precisely adjusting the supply of nutrients to the needs of animals is the key issue to optimize the efficiency of use of feed nutrients and minimize their excretion and the environmental footprint of animal production systems. In the practice, the digestible AA content in the complete diet is obtained, assuming that the digestibility values of the feed ingredients are additive and independent of the animal, feed intake, and ingredient composition (32, 78, 79). However, these principles are weak, as low levels of feed intake may increase the estimated values of apparent ideal digestibility and SID of CP and AA in diets (78) and the inclusion of dietary insoluble fiber decreases the digestibility of most dietary components, including AA (80, 81). These phenomena may lead to the lack of additivity and the under- or overestimation of the available AA in the complete diet (78). Our ability to precisely estimate the available nutrients in feed ingredients and the final diet remains an important limitation to formulating low-protein diets or providing pigs with the amount of nutrients animals need for production.

On the other hand, the determination of the amount of nutrients that the animals need to produce may also be challenging. For specific nutrients (e.g., essential AA), and when all other nutrients are provided at adequate levels, nutrient requirements can be defined as the amount of nutrients needed for specified production purposes, which in farm animals are production outputs such as growth rate, protein deposition, and milk yield (82, 83). Depending on the production purpose and the nutrient, this required nutrient amount can be considered as the minimum amount that will prevent signs of deficiency and allow the animal to perform its necessary functions in a normal manner. Nutrient requirements are modulated by factors that are related to the animal (e.g., genetic potential, age, weight, and sex), the feed (e.g., anti-nutritional factors), and the environment (e.g., temperature and space allowance) (84) and they are estimated for a given animal at a given point in time as the sum of the requirements for maintenance and production (26). When applied to pig populations, however, the requirements for a nutrient should rather be defined as the amount needed for specified production purposes such as optimal growth rate, protein deposition and feed efficiency (22). That is, the concept of nutrient requirements when applied to populations should be considered in the context of nutrients provided to heterogeneous populations over long periods (73, 85, 86). Individual animals' response to dietary nutrient supply may differ in magnitude and pattern from the response of a population (73), and population nutrient requirements should be seen as the optimal balance between the proportion of pigs that are going to be overfed and underfed, acknowledging that this proportion will change over time (25).

The empirical and factorial methods are two methods used in practice to estimate the nutrient requirements of growing animals (29). In the empirical method, nutrient requirements are estimated by feeding groups of pigs with increasing levels of the nutrient under evaluation and measuring one or several sets of performance parameters (e.g., growth rate). In this empirical method, the nutrient level at which the optimal population response is observed is identified as the population requirement for this nutrient and this growing interval. In the factorial method, however, daily requirements are estimated as the sum of the requirements for maintenance and production (82). These requirements are estimated for each nutrient or its precursor and take into account the efficiency with which each nutrient is used for each metabolic function (53, 87). Because pigs within a population differ in terms of BW and growth potential, each pig has its requirement, and this requirement evolves over time according to each pig's own pattern of feed intake and growth. When the factorial method is used to estimate the nutrient requirements of a population of animals, it is common practice to use the average pig to represent the population. However, care has to be taken with this assumption, since using the average pig to feed the population implies that half of the population will be overfed while the other half will be underfed (21, 22, 26), thus leading to undesired population performance. Nonetheless, some factorial methods may have been calibrated to estimate the requirements of the population using average population values (23). Furthermore, unlike the empirical method, the factorial method estimates nutritional requirements using information from one individual at one specific point in time. Thus, changes that occur during the growing interval under study are not evaluated. Ultimately, both methods of estimating nutrient requirements are based on experimental results from trials studying the relationship between nutrient intakes and animal responses. In the empirical method, this relationship is used to estimate the optimal response to varying nutrient levels of a population of animals showing some degree of heterogeneity. In contrast, the factorial method estimates the required amount of nutrients for one animal at a given point in time. Thus, when the factorial method is used to estimate population nutrient requirements, the chosen individual should be the right representative of the population and not necessarily the average animal (22, 23, 73).

Mechanistic mathematical models that implement the factorial approach are used to represent the complexity of animal responses and the numerous factors modulating them. These models have been developed to simulate the growth of a single animal (1, 53) or a population (86, 88). These models must, however, be calibrated a priori using data collected from bygone reference populations. Furthermore, these models are challenged by the difficulty of identifying the right reference population for its calibration, the inadequacy of most of these models to represent population heterogeneity, and the fact that animals from actual populations may follow different feed intake and growth patterns than the ones observed in the reference population. Therefore, model users have to be very careful to identify any differences that may exist between the reference and the target populations as well as any changes in the evolution of this target population during growth (26).

From a nutritional perspective, animal variation is much larger than the variation in feed intake and protein deposition potential as represented in actual factorial methods and models (1, 53). The actual principles used in the factorial methods to estimate nutrient requirements or to formulate low-protein diets are based on the assumptions that for many nutrients, in particular for AA, (1) digestibility is constant and is only a feed attribute [e.g., 74% for lysine in corn; NRC (1)], (2) observed (i.e., SID) AA utilization efficiency is constant for production [e.g., 72% for lysine deposition in body protein; (15)] across animals and ages (some variation is considered in the NRC 2012 model), (3) body protein amino acid composition is constant across animals and ages [e.g., 7% for lysine; (89)] and AA are needed and retained according to an ideal protein profile (1, 53, 54). However, these assumptions do not always hold true. Indeed, as indicated earlier in this document, feed ingredient AA digestibility is affected by the composition of the diet [e.g., fiber content (80, 90)], feed processing (91) and animal factors such as feed intake (78), and body weight (91). Factors affecting nutrient digestibility should be taken into account to formulate low-protein diets. In addition to this, the efficiency with which animals use the available nutrients may not be constant. For instance, the efficiency of use of the absorbed AA for protein deposition is affected by many factors in pigs, and production conditions may be one of the most important ones. Thus, in growing pigs fed below lysine requirements, the estimated SID lysine efficiency ranged from 73 to 94% (58) and from 83 to 100% (92). Similar figures were observed for threonine, where the estimated efficiency ranged from 54 to 84% (58). Amino acid supply also affects the AA composition of body proteins, and different body proteins are affected differently by the AA supply. Indeed, the splanchnic tissues are less affected than carcass muscles by AA supply, and different muscles respond differently to dietary AA supply (58, 77, 93–95). Some proteins (collagen, albumin, C-reactive protein) are also more affected than others (58). The use of constant digestibility values, AA efficiency and AA composition of body protein and animal products in the estimation of AA provisions and requirements can lead to biased estimations that can limit animal performance when trying to minimize excess nutrients supply.

The concept of the ideal protein refers to a protein with an AA profile that exactly meets the animal's requirement, and in this context all the AA acids are equally limiting (54, 77, 96). There are important implications to this concept. First, the animal response is driven by the first limiting AA, independently of the others.

Second, the animal response is proportional to the available limiting AA until another AA becomes limiting or the maximal response is reached. Third, excess AA does not limit maximal response. And finally, there is no interaction between AAs. In an optimal setting, all the animals will respond similarly to a given supply of AA. However, the ideal protein concept explains a small portion of the observed variation in the animals' responses. That is, for any given level of AA supply, there is a large variation in animal responses, often larger than the variation across AA supply levels (58, 97–99). Remus et al. (77) also noted that, for growing pigs, optimal performances were obtained at different threonine/lysine ratios when pigs were fed in conventional or precision feeding systems. In both feeding systems, however, the between-animal variation was high, thus confirming that the ideal protein profile explains a relatively small proportion of the observed animal response variation. It is possible that the between-animal variation in terms of AA digestibility, the efficiency of use of available dietary AA, and AA body protein composition may be responsible for part of the unexplained animals' response variation in AA supply.

Furthermore, the utilization of the ideal protein concept is limited when a quadratic response is observed (100) or when deficiencies or excess AA affect other AA responses (AA interactions). For example, valine supplementation decreased ADG when using a diet marginal in tryptophan, whereas it increased ADG when using a tryptophan-sufficient diet (101). Valine deficiency or branched-chain AA imbalance in the diet reduced feed intake and growth performance in another trial (102). Amino acids are much more than building blocks for production. They are also essential substrates for the synthesis of many molecules (e.g., glutathione, carnitine, carnosine, etc.) crucial to the animal metabolism and they have a crucial role in neurological regulations, gene expression, and small intestine growth (103, 104). Some AA are essential to the immune system (i.e., sulfur AA) to maintain the integrity of the gut barrier (i.e., threonine), and their supply should be reviewed in pigs under poor sanitary conditions (55, 105). Functional AA are those involved in the regulation of key pathways associated with the improvement of health, growth, reproduction lactation and reproduction (106). These AA have been linked to possible metabolic disease prevention and treatment, and might have great influence on intestinal health (106, 107). Pigs in poor sanitary conditions have different AA and energy requirements than those in better conditions (108, 109). Health challenges result in shifts of AA that could be used for protein deposition being used for maintenance functions related to the immune system (108, 110, 111). As consequence non-ruminants decrease growth performance (110, 111), and this loss in efficiency using feed for growth results in increased environmental impact (112). Cadéro et al. (113) simulated 96 scenarios using a LCA model that takes into account the variability among pigs aiming to simulate the impact o health status and feeding practices on economic and environmental traits. They concluded that impaired health has a major impact on the carbon foot print, and improving practices that increase the health status also help to improve economic results. The authors point out that feeding pigs with diets that closely meet their requirements (e.g., individual precision feeding) help to improve the economic results of health impaired populations. Additionally, daily feeding groups or individually feeding pigs improved the economic and environmental performance independent of the health conditions of the herd. It is possible that the changes in functional AA concentration might help pigs overcome the sanitary challenge, especially in precision feeding systems.

FUTURE PERSPECTIVES

Formulating feeds with low-protein diets and feeding pigs individually or in groups with daily tailored diets can have a major impact on N excretion and overall livestock sustainability. Indeed, the ingested nutrients that are not retained by the animal or in animal products are excreted and contribute to increasing the production cost and to reducing the sustainability of the farm. Reducing the supply of AA as happens in low- and very-low-protein diets for conventional and precision feeding production systems requires integration in the estimation of AA requirements not only of their role in production (i.e., meat, milk, etc.) but also other essential metabolic functions. It also requires ensuring that other functional nutrients (e.g., fermentable carbohydrates, probiotics, etc.) are supplied to maximize the integrity of the intestinal morphology and microbiota, immune system, etc. We need to better understand AA digestion and metabolic use to quantify the animal needs and their response to AA supply in interaction with the animal microbiota and production environments.

The formulation of very-low-protein diets and the implementation of precision feeding techniques rely on the utilization of sound nutritional concepts and comprehensive biological models developed to precisely estimate individual real-time nutrient requirements and animal responses. Combining knowledge- and data-driven models will further enhance our ability to use real-time farm data, opening up new opportunities that will enhance farm profitability, nutrient efficiency, and the sustainability of the overall animal production system. With the development of advanced computer and communication technologies and high-speed data-collection sensors, it is possible today to obtain numerous measurements at the animal, feed, building, and other farm levels. Besides the availability of these new technologies and data gathering, knowledge remains the most limiting factor to precisely providing each animal or a group of animals with the amount of nutrients it needs to produce at the desired level. Understanding the metabolic processes responsible for the observed variation between individual animals in their ability to use dietary nutrients is challenging for nutritionists and modelers, but is required to further improve the efficiency of livestock production systems.

AUTHOR CONTRIBUTIONS

AR formulated the diets, ran the simulations, prepared tables and figures, and drafted part of the manuscript. CP reviewed the simulations and calculations provided in this document and wrote the first draft. CP, AR, and IA reviewed and edited the document. All authors read and approved the final version of the manuscript.

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REFERENCES

1. NRC. *Nutrient Requirements of Swine*. Washington, DC: National Academy Press (2012).
2. Flachowsky G, Kamphues J. Carbon footprints for food of animal origin: what are the most preferable criteria to measure animal yields? *Animals.* (2012) 2:108–26. doi: 10.3390/ani2020108
3. Dourmad JY, Seve B, Latimier P, Boisen S, Fernandez J, Van Der Peet-Schwering C, et al. Nitrogen consumption, utilisation and losses in pig production in France, The Netherlands and Denmark. *Livestock Production Science.* (1999) 58:261–4. doi: 10.1016/S0301-6226(99)00015-9
4. Gerber P, Key N, Portet F, Steinfeld H. Policy options in addressing livestock's contribution to climate change. *Animal.* (2010) 4:393–406. doi: 10.1017/S1751731110000133
5. Niemann H, Kuhla B, Flachowsky G. Perspectives for feed-efficient animal production. *J Anim Sci.* (2011) 89:4344–63. doi: 10.2527/jas.2011-4235
6. Wilkinson JM. Re-defining efficiency of feed use by livestock. *Animal.* (2011) 5:1014–22. doi: 10.1017/S175173111100005X
7. CAST. *Animal Agriculture and Global Food Supply*. Ames, IA: Council for Agricultural Science and Technology (CAST) (1999).
8. Gill M, Smith P, Wilkinson JM. Mitigating climate change: the role of domestic livestock. *Animal.* (2010) 4:323–33. doi: 10.1017/S1751731109004662
9. Ertl P, Knaus W, Zollitsch W. An approach to including protein quality when assessing the net contribution of livestock to human food supply. *Animal.* (2016) 10:1883–9. doi: 10.1017/S1751731116000902
10. Baber JR, Sawyer JE, Wickersham TA. Estimation of human-edible protein conversion efficiency, net protein contribution, and enteric methane production from beef production in the United States. *Transl Anim Sci.* (2018) 2:439–50. doi: 10.1093/tas/txy086
11. Ertl P, Steinwidder A, Schönauer M, Krimberger K, Knaus W, Zollitsch W. Net food production of different livestock: a national analysis for Austria including relative occupation of different land categories [Netto-Lebensmittelproduktion der Nutztierhaltung: Eine nationale Analyse für Österreich inklusive relativer Flächenbeanspruchung]. *J Land Manag Food Environ.* (2016) 67:91–103. doi: 10.1515/boku-2016-0009
12. Wilkinson JM, Lee MRF. Review: use of human-edible animal feeds by ruminant livestock. *Animal.* (2018) 12:1735–43. doi: 10.1017/S175173111700218X
13. Rauw WM, Rydhmer L, Kyriazakis I, Overland M, Gilbert H, Dekkers JC, et al. Prospects for sustainability of pig production in relation to climate change and novel feed resources. *J Sci Food Agric.* (2020) 100:3575–86. doi: 10.1002/jsfa.10338
14. Moughan PJ. An overview of energy and protein utilisation during growth in simple-stomached animals. *Anim Prod Sci.* (2018) 58:646–54. doi: 10.1071/AN15791
15. Mohn S, Gillis AM, Moughan PJ, De Lange CF. Influence of dietary lysine and energy intakes on body protein deposition and lysine utilization in the growing pig. *J Anim Sci.* (2000) 78:1510–9. doi: 10.2527/2000.786 1510x
16. Van Barneveld RJ, Batterham ES, Norton BW. The effect of heat on amino acids for growing pigs 3. The availability of lysine from heat-treated field peas (Pisum sativum cultivar Dundale) determined using the slope-ratio assay. *Br J Nutr.* (1994) 72:257–75. doi: 10.1079/BJN19940028
17. Almeida FN, Htoo JK, Thomson J, Stein HH. Effects of balancing crystalline amino acids in diets containing heat-damaged soybean meal or distillers dried grains with solubles fed to weanling pigs. *Animal.* (2014) 8:1594–602. doi: 10.1017/S175173111400144X
18. Remus A, Del Castillo JRE, Pomar C. Improving the estimation of amino acid requirements to maximize nitrogen retention in precision feeding for growing-finishing pigs. *Animal.* (2020) 14:2032–41. doi: 10.1017/S1751731120000798
19. Mcauliffe GA, Chapman DV, Sage CL. A thematic review of life cycle assessment (LCA) applied to pig production. *Environ Impact Assess Rev.* (2016) 56:12–22. doi: 10.1016/j.eiar.2015.08.008
20. Andretta I, Remus A, Franceschi CH, Orso C, Kipper M. Chapter 3 - environmental impacts of feeding crops to poultry and pigs. In: Galanakis CM, editor. *Environmental Impact of Agro-Food Industry and Food Consumption*. London: Academic Press (2021). p. 59–79. doi: 10.1016/B978-0-12-821363-6.00001-1
21. Brossard L, Dourmad JY, Rivest J, Van Milgen J. Modelling the variation in performance of a population of growing pig as affected by lysine supply and feeding strategy. *Animal.* (2009) 3:1114–23. doi: 10.1017/S1751731109004546
22. Hauschild L, Pomar C, Lovatto PA. Systematic comparison of the empirical and factorial methods used to estimate the nutrient requirements of growing pigs. *Animal.* (2010) 4:714–23. doi: 10.1017/S1751731109991546
23. Remus A, Hauschild L, Pomar C. Simulated amino acid requirements of growing pigs differ between current factorial methods. *Animal.* (2020) 14:725–30. doi: 10.1017/S1751731119002660
24. Pomar C, Hauschild L, Zhang GH, Pomar J, Lovatto PA. Applying precision feeding techniques in growing-finishing pig operations. *Rev Brasil Zoot.* (2009) 38:226–37. doi: 10.1590/S1516-35982009001300023
25. Pomar C, Pomar J, Rivest J, Cloutier L, Letourneau-Montminy MP, Andretta I, et al. Estimating real-time individual amino acid requirements in growing-finishing pigs : towards a new definition of nutrient requirements? In: Sakomura NK, Gous R, Kyriazakis I, Hauschild L, editors. *Nutritional Modelling for Pigs and Poultry*. Wallingford: CAB International (2015). p. 157–74. doi: 10.1079/9781780644110.0157
26. Pomar C, Andretta I, Hauschild L. Meeting individual nutrient requirements to improve nutrient efficiency and the sustainability of growing pig production systems. In: Wiseman J, editor. *Achieving Sustainable Production of Pig Meat*. Sawston; Cambridge: Burleigh Dodds Science Publishing (2017). p. 287–98. doi: 10.19103/AS.2017.0013.31
27. Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, et al. *Tackling Climate Change Through Livestock – A Global Assessment of Emissions and Mitigation Opportunities*. Roma: Food and Agriculture Organization of the United Nations (FAO) (2013).
28. Tullo E, Finzi A, Guarino M. Review: Environmental impact of livestock farming and Precision Livestock Farming as a mitigation strategy. *Sci Total Environ.* (2019) 650:2751–60. doi: 10.1016/j.scitotenv.2018.10.018
29. Patience JF, Thacker PA, De Lange CFM. *Swine Nutrition Guide*. Saskatoon, SK: University of Saskatchewan; Prairie Swine Center (1995).
30. Heidari MD, Gandasasmita S, Li E, Pelletier N. Proposing a framework for sustainable feed formulation for laying hens: a systematic review of recent developments and future directions. *J Clean Prod.* (2021) 288:125585. doi: 10.1016/j.jclepro.2020.125585
31. Wilton JW, Morris CA, Jenson EA, Leigh AO, Pfeiffer WC. A linear programming model for beef cattle production. *Can J Anim Sci.* (1974) 54:693–707. doi: 10.4141/cjas74-084
32. Stein HH, Seve B, Fuller MF, Moughan PJ, De Lange CFM. Invited review: amino acid bioavailability and digestibility in pig

feed ingredients: terminology and application. *J Anim Sci.* (2007) 85:172–80. doi: 10.2527/jas.2005-742
33. Jondreville C, Dourmad JY. Le phosphore dans la nutrition des porcs. *INRA Prod Anim.* (2005) 18:183–92. doi: 10.20870/productions-animales.2005.18.3.3523
34. Sanjayan N, Heo JM, Nyachoti CM. Nutrient digestibility and growth performance of pigs fed diets with different levels of canola meal from *Brassica napus* black and *Brassica juncea* yellow. *J Anim Sci.* (2014) 92:3895–905. doi: 10.2527/jas.2013-7215
35. Mitchell HH. *Comparative Nutrition of Man and Domestic Animals*. New York, NY: Academic Press (1962).
36. Fuller MF, Mcwilliam R, Wang TC, Giles LR. The optimum dietary amino acid pattern for growing pigs. *Br J Nutr.* (1989) 62:255–67. doi: 10.1079/BJN19890028
37. Jean Dit Bailleul P, Rivest J, Dubeau F, Pomar C. Reducing nitrogen excretion in pigs by modifying the traditional least-cost formulation algorithm. *Livestock Prod Sci.* (2001) 72:199–211. doi: 10.1016/S0301-6226(01)00224-X
38. Castrodeza C, Lara P, Pena T. Multicriteria fractional model for feed formulation: economic, nutritional and environmental criteria. *Agric Syst.* (2005) 86:76–96. doi: 10.1016/j.agsy.2004.08.004
39. Pomar C, Dubeau F, Létourneau Montminy MP, Boucher C, Julien P-O. Reducing phosphorus concentration in pig diets by adding an environmental objective to the traditional feed formulation algorithm. *Livest Sci.* (2007) 111:16–27. doi: 10.1016/j.livsci.2006.11.011
40. Lara P, Stancu-Minasian I. Fractional programming: a tool for the assessment of sustainability. *Agric Syst.* (1999) 62:131–41. doi: 10.1016/S0308-521X(99)00062-1
41. Tozer PR, Stokes JR. A multi-objective programming approach to feed ration balancing and nutrient management. *Agric Syst.* (2001) 67:201–15. doi: 10.1016/S0308-521X(00)00056-1
42. Niemi JK. A dynamic programming model for optimising feeding and slaughter decisions regarding fattening pigs. *Agric Food Sci.* (2006) 15:6–121. doi: 10.23986/afsci.5855
43. Garcia-Launay F, Dusart L, Espagnol S, Laisse-Redoux S, Gaudré D, Méda B, et al. Multiobjective formulation is an effective method to reduce environmental impacts of livestock feeds. *Br J Nutr.* (2018) 120:1298–309. doi: 10.1017/S0007114518002672
44. Meul M, Ginneberge C, Van Middelaar CE, De Boer IJM, Fremaut D, Haesaert G. Carbon footprint of five pig diets using three land use change accounting methods. *Livest Sci.* (2012) 149:215–23. doi: 10.1016/j.livsci.2012.07.012
45. Selle PH, De Paula Dorigam JC, Lemme A, Chrystal PV, Liu SY. Synthetic and crystalline amino acids: alternatives to soybean meal in chicken-meat production. *Animals.* (2020) 10:729. doi: 10.3390/ani10040729
46. Cappelaere L, Le Cour Grandmaison J, Martin N, Lambert W. Amino acid supplementation to reduce environmental impacts of broiler and pig production: a review. *Front Vet Sci.* (2021) 8:689259. doi: 10.3389/fvets.2021.689259
47. Ikeda M. Amino acid production processes. Adv Biochem Eng Biotechnol. (2003) 79:1–35. doi: 10.1007/3-540-45989-8_1
48. Van Der Werf HMG, Petit J, Sanders J. The environmental impacts of the production of concentrated feed: the case of pig feed in Bretagne. *Agric Syst.* (2005) 83:153–77. doi: 10.1016/j.agsy.2004.03.005
49. Garcia-Launay F, Van Der Werf HMG, Nguyen TTH, Le Tutour L, Dourmad JY. Evaluation of the environmental implications of the incorporation of feed-use amino acids in pig production using Life Cycle Assessment. *Livest Sci.* (2014) 161:158–75. doi: 10.1016/j.livsci.2013.11.027
50. Monteiro ANTR, Bertol TM, De Oliveira PV., Dourmad JY, Coldebella A, Kessler AM. The impact of feeding growing-finishing pigs with reduced dietary protein levels on performance, carcass traits, meat quality and environmental impacts. *Livestock Sci.* (2017) 198:162–9. doi: 10.1016/j.livsci.2017.02.014
51. Monteiro ANTR, Dourmad J-Y, Pozza PC. Life cycle assessment as a tool to evaluate the impact of reducing crude protein in pig diets %J Ciência Rural. *Ciência Rural.* (2017) 47:1–8. doi: 10.1590/0103-8478cr20161029
52. Le Bellego L, Noblet J. Performance and utilization of dietary energy and amino acids in piglets fed low protein diets. *Livestock Prod Sci.* (2002) 76:45–58. doi: 10.1016/S0301-6226(02)00008-8
53. Van Milgen J, Valancogne A, Dubois S, Dourmad J-Y, Seve B, Noblet J. InraPorc: A model and decision support tool for the nutrition of growing pigs. *Anim Feed Sci Technol.* (2008) 143:387–405. doi: 10.1016/j.anifeedsci.2007.05.020
54. Van Milgen J, Dourmad J-Y. Concept and application of ideal protein for pigs. *J Anim Sci Biotechnol.* (2015) 6:15. doi: 10.1186/s40104-015-0016-1
55. Wang Y, Zhou J, Wang G, Cai S, Zeng X, Qiao S. Advances in low-protein diets for swine. *J Anim Sci Biotechnol.* (2018) 9:60. doi: 10.1186/s40104-018-0276-7
56. Hauschild L, Lovatto PA, Pomar J, Pomar C. Development of sustainable precision farming systems for swine: estimating real-time individual amino acid requirements in growing-finishing pigs. *J Anim Sci.* (2012) 90:2255–63. doi: 10.2527/jas.2011-4252
57. Hauschild L, Kristensen AR, Andretta I, Remus A, Santos LS, Pomar C. Toward better estimates of the real-time individual amino acid requirements of growing-finishing pigs showing deviations from their typical feeding patterns. *Animal.* (2020) 14:s371–81. doi: 10.1017/S1751731120001226
58. Remus A, Hauschild L, Corrent E, Letourneau-Montminy MP, Pomar C. Pigs receiving daily tailored diets using precision-feeding techniques have different threonine requirements than pigs fed in conventional phase-feeding systems. *J Anim Sci Biotechnol.* (2019) 10:16. doi: 10.1186/s40104-019-0328-7
59. Remus, A., Pomar, C., and Andretta, I. (2021). *Estratégias para maximar a retenção de proteína e energia*. Available online at: https://www.3tres3.com.br.; https://www.3tres3.com.br/guia333/empresas/laboratorio-de-ensino-zootecnico-ufrgs/posts/730 (accessed September 28, 2021).
60. Remus A, Hauschild L, Methot S, Pomar C. Precision livestock farming: real-time estimation of daily protein deposition in growing-finishing pigs. *Animal.* (2020) 14:s360–70. doi: 10.1017/S1751731120001469
61. Noblet J. Net energy evaluation of feeds and determination of net energy requirements for pigs. *Rev Brasil Zoot.* (2007) 36:277–84. doi: 10.1590/S1516-35982007001000025
62. Van Milgen J, Noblet J, Dubois S. Energetic efficiency of starch, protein and lipid utilization in growing pigs. *J Nutr.* (2001) 131:1309–18. doi: 10.1093/jn/131.4.1309
63. Reeds PJ, Fuller MF, Cadenhead A, Lobley GE, Mcdonald JD. Effects of changes in the intakes of protein and non-protein energy on whole-body protein turnover in growing pigs. *Br J Nutr.* (1981) 45:539–46. doi: 10.1079/BJN19810132
64. Pomar C, Pomar J, Dubeau F, Joannopoulos E, Dussault J-P. The impact of daily multiphase feeding on animal performance, body composition, nitrogen and phosphorus excretions, and feed costs in growing–finishing pigs. *Animal.* (2014) 8:704–13. doi: 10.1017/S1751731114000408
65. Moore KL, Mullan BP, Kim JC. An evaluation of the alternative feeding strategies, blend feeding, three-phase feeding or a single diet, in pigs from 30 to 100 kg liveweight. *Anim Feed Sci Technol.* (2016) 216:273–80. doi: 10.1016/j.anifeedsci.2016.04.001
66. Letourneau Montminy MP, Boucher C, Pomar C, Dubeau F, Dussault JP. The impact of different formulation methods and feeding programs on feeding cost and nitrogen and phosphorus excretion in growing-finishing pigs. In: *International Workshop on Green Pork Production: "Porcherie verte", A Research Initiative on Environment-Friendly Pig Production* (Paris) (2005).
67. Brossard L, Vautier B, Van Milgen J, Salaun Y, Quiniou N. Comparison of *in vivo* and *in silico* growth performance and variability in pigs when applying a feeding strategy designed by simulation to control the variability of slaughter weight. *Anim Prod Sci.* (2014) 54:1939–45. doi: 10.1071/AN14521
68. Feddes JJR, Ouellette CA, Leonard JJ. A system for providing protein for pigs in intermediately sized grower/finisher barns. *Canad Agric Eng.* (2000) 42:209–13.
69. Joannopoulos E, Dubeau F, Haddou M, Dussault J-P, Pomar C. Nouvelle méthode d'alimentation hybride: un mixte de l'alimentation traditionnelle par phases et par mélange. In: *49th Journées Recherche Porcine*. Paris (2017). p. 93–8.
70. Black JL, Banhazi TM. Economic and social advantages from Precision Livestock Farming in the pig industry. In: *Precision Livestock Farming 2013 - Papers Presented at the 6th European Conference on Precision Livestock Farming, ECPLF 2013.* Leuven (2013). p. 199–208.
71. Pomar C, Van Milgen J, Remus A. Precision animal nutrition, principle and practice. In: Hendriks W, Verstegen M, Babinszky

L, editors. *Poultry and Pig Nutrition: Challenges of the 21st Century*. Wageningen: Wageningen Academic Publishers (2019). p. 397–418. doi: 10.3920/978-90-8686-884-1_18

72. Rostagno HS, Albino LFT, Hannas MI, Donzele JL, Sakomura NK, Perazzo FG, et al. (2017). *Brazilian Tables for Poultry and Swine: Composition of Feedstuffs and Nutritional Requirements*. Viçosa: UFV.
73. Pomar C, Kyriazakis I, Emmans GC, Knap PW. Modeling stochasticity: dealing with populations rather than individual pigs. *J Anim Sci.* (2003) 81:E178–86. doi: 10.2527/2003.8114_suppl_2E178x
74. Pomar C, Remus A. Precision pig feeding: a breakthrough toward sustainability. *Anim Front.* (2019) 9:52–9. doi: 10.1093/af/vfz006
75. Andretta I, Pomar C, Rivest J, Pomar J, Lovatto PA, Radunz Neto J. The impact of feeding growing-finishing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition. *J Anim Sci.* (2014) 92:3925–36. doi: 10.2527/jas.2014-7643
76. Andretta I, Pomar C, Rivest J, Pomar J, Radunz J. Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. *Animal.* (2016) 10:1137–47. doi: 10.1017/S1751731115003067
77. Remus A, Hauschild L, Létourneau-Montminy MP, Corrent E, Pomar C. The ideal protein profile for late-finishing pigs in precision feeding systems: threonine. *Anim Feed Sci Technol.* (2020) 265:114500. doi: 10.1016/j.anifeedsci.2020.114500
78. Liu JB, Yan HL, Liao YP, Xie ZJ, Yin YL. Effects of feed intake level on the additivity of apparent and standardized ileal digestibility of amino acids in diets for growing pigs. *Anim Feed Sci Technol.* (2020) 266:114525. doi: 10.1016/j.anifeedsci.2020.114525
79. Oliveira MSF, Abelilla JJ, Jaworski NW, Htoo JK, Stein HH. Crystalline amino acids do not influence calculated values for standardized ileal digestibility of amino acids in feed ingredients included in diets for pigs. *J Anim Sci.* (2020) 98. doi: 10.1093/jas/skaa333
80. Acosta JA, Stein HH, Patience JF. Impact of increasing the levels of insoluble fiber and on the method of diet formulation measures of energy and nutrient digestibility in growing pigs. *J Anim Sci.* (2020) 98. doi: 10.1093/jas/skaa130
81. Chen T, Chen D, Tian G, Zheng P, Mao X, Yu J, et al. Effects of soluble and insoluble dietary fiber supplementation on growth performance, nutrient digestibility, intestinal microbe and barrier function in weaning piglet. *Anim Feed Sci Technol.* (2020) 260:114335. doi: 10.1016/j.anifeedsci.2019.114335
82. Fuller MF, Chamberlain AG. Protein requirements of pigs. In: Haresign W, editor. *Recent Advances in Animal Nutrition.* London: Butterwoths (1982). p. 175–186. doi: 10.1016/B978-0-408-71015-2.50012-2
83. Fuller MF. *The Encyclopedia of Farm Animal Production.* Wallingford: CABI Pub (2004). doi: 10.1079/9780851993690.0000
84. Noblet J, Quiniou N. Principaux facteurs de variation du besoin en acides aminés du porc en croissance. *Techni-Porc.* (1999) 22:9–16.
85. Knap PW. Stochastic simulation of growth in pigs: relations between body composition and maintenance requirements as mediated through protein turn-over and thermoregulation. *Anim Sci.* (2000) 71:11–30. doi: 10.1017/S1357729800054850
86. Vautier B, Quiniou N, Van Milgen J, Brossard L. Accounting for variability among individual pigs in deterministic growth models. *Animal.* (2013) 7:1265–73. doi: 10.1017/S1751731113000554
87. Van Milgen J, Noblet J. Partitioning of energy intake to heat, protein, and fat in growing pigs. *J Anim Sci.* (2003) 81(E Suppl 2):E86–93. doi: 10.2527/2003.8114_suppl_2E86x
88. Ferguson NS, Gous RM, Emmans GC. Preferred components for the construction of a new simulation model of growth, feed intake and nutrient requirements of growing pigs. *S Afr J Anim Sci.* (1994) 24:10–7.
89. Mahan DC, Shields RG. Essential and nonessential amino acid composition of pigs from birth to 145 kilograms of body weight, and comparison to other studies. *J Anim Sci.* (1998) 76:513–21. doi: 10.2527/1998.762513x
90. Noblet J, Le Goff G. Effect of dietary fibre on the energy value of feeds for pigs. *Anim Feed Sci Technol.* (2001) 90:35–52. doi: 10.1016/S0377-8401(01)00195-X
91. Noblet J, Van Milgen J. Energy value of pig feeds: effect of pig body weight and energy evaluation system. *J Anim Sci.* (2004) 82:E229–238. doi: 10.2527/2004.8213_supplE229x
92. Cloutier L, Pomar C, Letourneau Montminy MP, Bernier JF, Pomar J. Evaluation of a method estimating real-time individual lysine requirements in two lines of growing-finishing pigs. *Animal.* (2015) 9:561–8. doi: 10.1017/S1751731114003073
93. Conde-Aguilera JA, Barea R, Le Floc'h N, Lefaucheur L, Van Milgen J. A sulfur amino acid deficiency changes the amino acid composition of body protein in piglets. *Animal.* (2010) 4:1349–58. doi: 10.1017/S1751731110000340
94. Conde-Aguilera JA, Cobo-Ortega C, Mercier Y, Tesseraud S, Van Milgen J. The amino acid composition of tissue protein is affected by the total sulfur amino acid supply in growing pigs. *Animal.* (2014) 8:401–9. doi: 10.1017/S1751731113002425
95. Conde-Aguilera JA, Cholet JCG, Lessire M, Mercier Y, Tesseraud S, Van Milgen J. The level and source of free-methionine affect body composition and breast muscle traits in growing broilers1. *Poult Sci.* (2016) 95:2322–31. doi: 10.3382/ps/pew105
96. Mitchel HH. *Comparative Nutrition of Man and Domestic Animals*. New York, NY: Academic Press (1962).
97. Gloaguen M, Le Floc'h N, Brossard L, Barea R, Primot Y, Corrent E, et al. Response of piglets to the valine content in diet in combination with the supply of other branched-chain amino acids. *Animal*. (2011) 5:1734–42. doi: 10.1017/S1751731111000760
98. Gloaguen M, Le Floc'h N, Primot Y, Corrent E, Van Milgen J. Response of piglets to the standardized ileal digestible isoleucine, histidine and leucine supply in cereal-soybean meal-based diets. *Animal*. (2013) 7:901–8. doi: 10.1017/S1751731112002339
99. Cemin HS, Tokach MD, Vier CM, Dritz SS, Woodworth JC, Derouchey JM, et al. 176 effects of standardized ileal digestible histidine:lysine ratio on growth performance of 7 to 11 Kg pigs. *J Anim Sci.* (2018) 96:93. doi: 10.1093/jas/sky073.173
100. Soto JA, Tokach MD, Dritz SS, Woodworth JC, Derouchey JM, Goodband RD, et al. Optimal dietary standardized ileal digestible lysine and crude protein concentration for growth and carcass performance in finishing pigs weighing greater than 100 kg1,2. *J Anim Sci.* (2019) 97:1701–11. doi: 10.1093/jas/skz052
101. Jansman AJM, Cirot O, Corrent E, Lambert W, Ensink J, Van Diepen JTM. Interaction and imbalance between indispensable amino acids in young piglets. *Animal.* (2019) 13:941–9. doi: 10.1017/S175173111800263X
102. Gloaguen M, Le Floc'h N, Corrent E, Primot Y, Van Milgen J. Providing a diet deficient in valine but with excess leucine results in a rapid decrease in feed intake and modifies the postprandial plasma amino acid and α-keto acid concentrations in pigs. *J Anim Sci*. (2012) 90:3135–42. doi: 10.2527/jas.2011-4956
103. Wu G. Amino acids: metabolism, functions, and nutrition. *Amino Acids.* (2009) 37:1–17. doi: 10.1007/s00726-009-0269-0
104. Rodrigues LA, Wellington MO, González-Vega JC, Htoo JK, Van Kessel AG, Columbus DA. Functional amino acid supplementation, regardless of dietary protein content, improves growth performance and immune status of weaned pigs challenged with Salmonella Typhimurium. *J Anim Sci.* (2021) 99:365. doi: 10.1093/jas/skaa365
105. De Ridder K, Levesque CL, Htoo JK, De Lange CFM. Immune system stimulation reduces the efficiency of tryptophan utilization for body protein deposition in growing pigs. *J Anim Sci.* (2012) 90:3485–91. doi: 10.2527/jas.2011-4830
106. Wu G. Functional amino acids in growth, reproduction, and health. *Adv Nutr.* (2010) 1:31–7. doi: 10.3945/an.110.1008
107. Le Floc'h N, Wessels A, Corrent E, Wu G, Bosi P. The relevance of functional amino acids to support the health of growing pigs. *Animal Feed Sci Technol.* (2018) 245:104–16. doi: 10.1016/j.anifeedsci.2018.09.007
108. Le Floc'h N, Gondret F, Matte JJ, Quesnel H. Towards amino acid recommendations for specific physiological and patho-physiological states in pigs. *Proc Nutr Soc.* (2012) 71:425–32. doi: 10.1017/S0029665112000560
109. Rakhshandeh A, Htoo JK, Karrow N, Miller SP, De Lange CF. Impact of immune system stimulation on the ileal nutrient digestibility and utilisation of methionine plus cysteine intake for whole-body protein deposition in growing pigs. *Br J Nutr*. (2013) 111:101–10. doi: 10.1017/S0007114513001955
110. Pastorelli H, Van Milgen J, Lovatto P, Montagne LJA. Meta-analysis of feed intake and growth responses of growing pigs after a sanitary challenge. (2012) 6:952–61. doi: 10.1017/S175173111100228X
111. Remus A, Hauschild L, Andretta I, Kipper M, Lehnen CR, Sakomura NK. A meta-analysis of the feed intake and growth performance of

broiler chickens challenged by bacteria. *Poult Sci.* (2014) 93:1149–58. doi: 10.3382/ps.2013-03540

112. Reckmann K, Krieter J. Environmental impacts of the pork supply chain with regard to farm performance. *J Agric Sci.* (2015) 153:411–21. doi: 10.1017/S0021859614000501
113. Cadéro A, Aubry A, Dourmad JY, Salaün Y, Garcia-Launay F. Effects of interactions between feeding practices, animal health and farm infrastructure on technical, economic and environmental performances of a pig-fattening unit. *Animal.* (2020) 14:s348–59. doi: 10.1017/S1751731120000300

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REVIEW
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Dietary Phosphorus and Calcium Utilization in Growing Pigs: Requirements and Improvements

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The sustainability of animal production relies on the judicious use of phosphorus (P). Phosphate, the mined source of agricultural phosphorus supplements, is a non-renewable resource, but phosphorus is essential for animal growth, health, and well-being. P must be provided by efficient and sustainable means that minimize the phosphorus footprint of livestock production by developing precise assessment of the bioavailability of dietary P using robust models. About 60% of the phosphorus in an animal's body occurs in bone at a fixed ratio with calcium (Ca) and the rest is found in muscle. The P and Ca requirements must be estimated together; they cannot be dissociated. While precise assessment of P and Ca requirements is important for animal well-being, it can also help to mitigate the environmental effects of pig farming. These strategies refer to multicriteria approaches of modeling, efficient use of the new generations of phytase, depletion and repletion strategies to prime the animal to be more efficient, and finally combining these strategies into a precision feeding model that provides daily tailored diets for individuals. The industry will need to use strategies such as these to ensure a sustainable plant–animal–soil system and an efficient P cycle.

Keywords: phosphorus, calcium, mitigation, requirements, environment, swine

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1. INTRODUCTION

Phosphorus (P) is an essential element for all living beings, as it is a key component of nucleic acids and energy transfer molecules (adenosine triphosphate, creatine phosphate) and a major mineral component of bone (1). The element P is found in animals as orthophosphates. This is the circulating form of P. Adequate amounts must be provided in livestock diets to ensure animal growth and health. To date, producers have used inorganic phosphate, a limited and non-renewable resource that will be depleted within 100–200 years at current rates of extraction (2). As a commodity mineral, its price is volatile (3). Of greater concern is that P is not absorbed completely from any diet, and in the case of monogastric livestock farming, phosphorus-laden run-off can pollute and cause eutrophication of waterways, which can lead to growth of toxic nitrogen-fixing algae or cyanobacteria (4). This compromises the sustainability of pig farming, which has become highly concentrated in certain regions of several pork-producing countries. In swine production, to avoid an excess of P, the cost of transporting P-rich manure for use as crop field fertilizer can be high and the cost of treating it can be prohibitive; rational and efficient use of P is therefore essential.

Calcium (Ca) is the most abundant mineral in the body (1, 5) and is indispensable for bone mineralization, muscle contraction, and nerve impulse propagation. It is not an expensive element in livestock feed, it is abundant, and it does not represent a threat to the environment. However, as absorption and utilization of P in growing pigs is related to that of Ca, P and Ca requirements must be studied together. Insoluble and indigestible Ca–P complexes can form in the intestines (6, 7). Ca and P deposits in bone are co-dependent. If discharges of phosphorus are to be minimal and its efficiency of utilization must be maximized, its supply must be matched as closely as possible to the requirements of the animals. To achieve this, the actual usable P content of feedstuffs and the animal physiological requirement both must be estimated accurately and precisely. Both global and factorial methods have been used to estimate the Ca and P requirement.

P and Ca requirements can be estimated to maximize growth performance, keep P rejection minimal and/or maximize bone mineralization. Novel approaches in development aim to improve the digestive and/or metabolic utilization of P, thereby decreasing P excretion. The best-known example is the use of phytases, which facilitate the digestion of plant P as phytic acid, the phosphoric ester of inositol, a compound found in many plants and poorly absorbable by pigs. The new generation of phytases makes this strategy even more attractive. The depletion–repletion, a strategy less well known, consists of reducing P and Ca input below the animal's requirements over some period of growth and then increasing the supply as needed (8). This strategy can increase the animal's P digestive efficiency and metabolic utilization in growing pigs; thus it overall decreases in P intake and excretion while maintaining growth and bone mineralization (9, 10). Finally, a mechanistic model approach predicts bone ash and then P and Ca requirement (11, 12) and does not estimate the P and Ca requirement for bone directly from protein. This is an interesting multicriteria approach to mitigate P impact that will be essential for P precision feeding (13). The objective of this paper is to review the latest P and Ca assessment of bioavailability methods for evaluating the nutritional values of feed ingredients for pigs and estimating precisely P requirements, as well as, describing innovations and promising strategies to decrease the P excretions by growing pigs.

2. PRECISELY ASSESS BIOAVAILABILITY AND EVALUATING THE NUTRITIONAL VALUES OF FEED INGREDIENTS

2.1. Dietary Forms of Phosphorus and Calcium

2.1.1. Plant Phosphorus and Calcium

Phytic acid is synthesized in plants by phosphorylating inositol in any or all possible positions. It can thus bear 6 phosphate groups (IP_6) as shown in **Figure 1**, or a smaller number (IP_5-IP_1). The main form found in feed ingredients of plant origin is IP_6 (15, 16). Phytic acid plays a key role in plant metabolism by constituting a reserve of P and chelating other minerals, whereas inositol is used in cell wall formation (17). Phytic acid is present in all plant-based ingredients (18), in which it accounts for 50–80% of the P content (19, 20) and is found almost entirely in the form of salts called phytates, primarily with Ca, Fe, Zn, Mg, K, and Mn. Phytates are solubilized at gastric pH, whereas the higher pH of the small intestine is conducive to their re-formation or *de novo* complexation thus decreasing the absorption of minerals and trace elements (21, 22). *In vitro*, phytic acid forms its most stable salts with Cu, Zn, Ni, Co, Mn, Fe, and then Ca (23, 24). Ca rarely makes up more than 1% of plant dry matter (20) and its absorption is decreased by phytate formation, but this can be countered somewhat by using phytases (see section 4.2), which break down phytates that are in solution (25–27). The cation-binding ability of phytic acid declines as phosphate groups are removed (21). Phytates form insoluble complexes also with proteins, amino acids, and starch and thereby decrease the digestibility in the small intestine and utilization of these nutrients (18).

2.1.2. Mineral Phosphorus and Calcium

P is usually added to pig diets as dicalcium phosphate, which represents 60% of the feed phosphates used in the European Union; monocalcium and monodicalcium phosphates are also used (28). Magnesium, calcium-magnesium, ammonium, and sodium phosphates are also available for use in livestock feed (28–30). To minimize excreted phosphate, which becomes pollution, the most digestible phosphates are preferred, although price also is considered. The first and foremost criterion is to meet narrow technical specifications in terms of composition and physicochemical stability. Phosphates can be classified according to their solubility in 2% citric acid solution. This test does not indicate real digestibility but makes it possible to rank different products (29). A feed-grade phosphate must be at least 95% soluble in 2% citric acid and in alkaline ammonium citrate (28, 31). For monocalcium phosphates, the solubility in water must be greater than 80%, and for monodicalcium phosphate, greater than 50% (28). Monocalcium phosphate is more digestible than monodicalcium phosphate, which is more digestible than dicalcium phosphate (28, 29). Dicalcium phosphate dihydrate is more digestible than the anhydrous form. The final criterion for judging the quality of a feed-grade phosphate is its level of undesirable substances such as arsenic, cadmium, lead, fluorine, or mercury, and dioxins (28, 29).

The inorganic Ca supplements most used in pig farming are calcium phosphates (32) and carbonates supplied in the form of limestone, a mineral that contains calcium carbonate and dolomite and which varies in Ca content (35–38% Ca; (19)). The bioavailability of the Ca in these sources is in the 90–100% range of calcium carbonate ($CaCO_3$) used as reference (19, 20, 30). Unlike in poultry, carbonate particle size appears to have no significant effect on apparent or standardized Ca digestibility in growing pigs, based on tests with animals in the 10–20 kg live weight range (33, 34). Calcified red algae has been studied due to its solubility at gastric pH. It is 32% more soluble than calcareous Ca at pH 6.7 and 34% more soluble at pH 3.0 (35). Limestone is 100% calcite, whereas CeltiCal (Celtic Sea Minerals) is 65% calcite, 23% aragonite, and 12% valterite (polymorphs of calcite). The greater solubility does not make

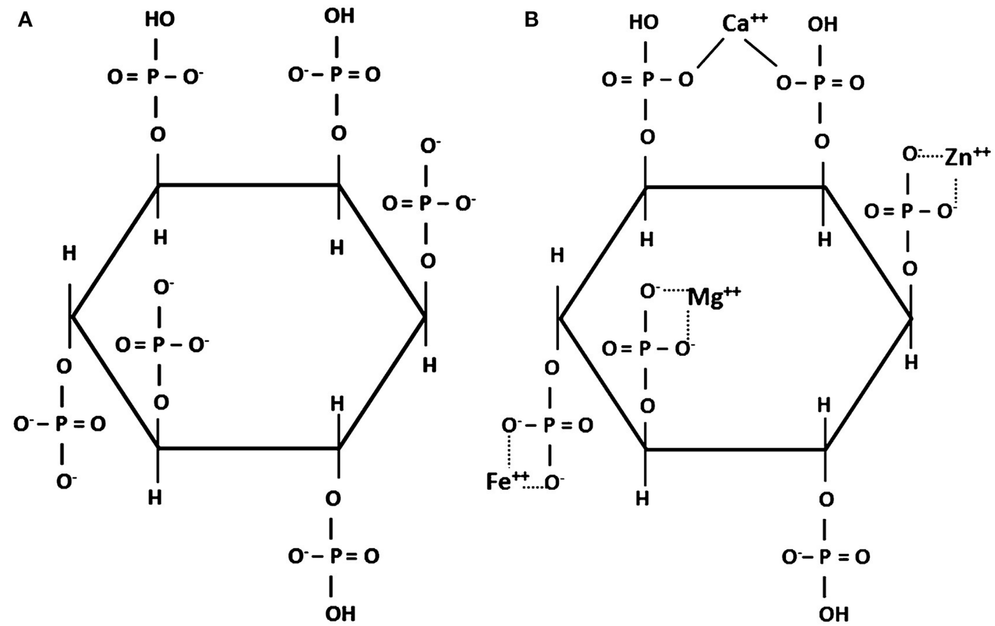

FIGURE 1 | (A) Structure of phytic acid at neutral pH (14); **(B)** phytate chelate with different cations. (14).

the Celtic Sea product more digestible than calcium carbonate. Its digestibility in pigs is at best equivalent to that of calcium carbonate [apparent total tract digestibility [ATTD] Ca of 46.7% and 51.2% for calcium carbonate and CeltiCal, respectively (36), 64% for both sources (37), and may be lower: 46.9% of ATTD Ca and 30.5% for calcium carbonate and CeltiCal (38)]. Marine Ca is absorbed poorly in the upper parts of the gut (38); the higher concentration of dissolved cations moreover makes it precipitate more with phosphate or phytic acids, decreasing P digestibility, bone mineralization, and animal growth (35, 36). A highly soluble Ca to P ratio makes precipitation more likely. Nevertheless, adding marine Ca in smaller amounts and using phytase allows proper balancing of the soluble Ca:P ratio and growth performance equivalent to the control group, and quantitatively superior bone mineralization, at least in broiler chicken studies (35). These results show, above all, that Ca and P interact strongly in the digestive system, and how much further study, especially of the soluble Ca:P ratio, is needed to optimize their utilization.

2.1.3. Phosphorus and Calcium of Animal Origin

In addition to inorganic phosphates, meat and bone meal from the rendering industry is also used as a source of P and Ca. Except in Europe, where it is prohibited in livestock feed other than for fish, these by-products are commonly included in poultry diets. Meat and bone meal can be made up of bones and soft tissues but not blood, hair, hoof, horn, skin/leather, stomach and ruminal contents, or excrement. Most meat and bone meal in North America is a mixture of cattle, pig, and poultry by-products (39). It must contain at least 4.0% of P, and the Ca:P ratio must not exceed 2.2 [AAFCO 2011, cited in Sulabo and Stein (40)]. Meat and bone meal is a source of highly available Ca and P (41, 42) but has unpredictable quality and Ca:P ratios, due to differences in raw materials and processes (39). Depending on the source, the P and Ca contents may vary 2–4 times as much as the protein content, the coefficients of variance being, respectively, 20, 22, and 6.2% (40). A negative correlation exists between protein concentration and P and Ca concentration, due to variations in the proportions of soft tissue and bone (40). The most important sources of variation in the composition of meat and bone meal are therefore the origin of the by-products used and the ratio of soft tissue to bone.

When meat and bone meal is fed to pigs, it provides much of the Ca and P in the diet. It is therefore necessary to have a supplier that uses controlled processes and can guarantee Ca and P content. The standardized digestibility of P in meat and bone meal ranges from 55 to 84% and averages 70% (20, 40), falling between the values for inorganic P and materials of plant origin. Standardized Ca digestibility in meat and bone meal is estimated at 77% but can be 82% for poultry meal (43). The apparent Ca digestibility ranges from 55 to 84% (40). The digestibility of P and Ca in meat and bone meal does vary somewhat, due mostly to the P concentration: the higher the P concentration, the lower the Ca and P digestibility. Since the P concentration depends mainly on the proportion of bone in the meal, it may be presumed that the higher the bone-to-meat ratio, the lower the Ca and P digestibility. The apparent digestibility of P in bone meal is in fact about 68 vs. 80% in meat-and-bone meal and 85% in meat meal (44). Hydroxyapatite, therefore, seems to be a less digestible form of P and Ca. This has been validated for P by comparing diets containing different forms of bone meal. The pre-cecal digestibility of P in chickens is lower when it is still in the form of hydroxyapatite than when it has been previously dissolved (45). Although its composition may vary widely, meat and bone meal offers the possibility of recycling, providing sufficient P to livestock without inorganic P from non-renewable sources. At least one study suggests that heat and pressure treatment of bone meal and removal of gelatin may improve P digestibility (45).

2.2. Precisely Estimates of Dietary Phosphorus and Calcium Values of Feedstuffs

2.2.1. Total Analyzable Value

Total dietary Ca and P content in feed ingredients are routinely measured by chemical analysis. However, these numbers do not indicate what portion the animals digest or retain or how much will be excreted. Although this method has its drawbacks, it is still the preferred method for Ca, mainly because of the lack of knowledge on Ca bioavailability. Recent work underway is expected to provide a more accurate Ca bioavailability assessment method with standardized digestibility measurements (46). The P system is more precise with different expression modes, which will be described in the following sections.

2.2.2. Relative Bioavailability

Bioavailability, also called availability, was added in the ninth edition of NRC (47). Availability is an indicator of the use of a nutrient based on a predefined criterion, for example, in the case of P and Ca, bone mineralization measured in terms of mineral (ash) content or a biomechanical property such as breaking strength (48, 49). The value is obtained by comparison with a reference that is considered 100% bioavailable, usually monocalcium phosphate. The relative bioavailability of a nutrient in an ingredient is generally expressed as the slope ratio, which is obtained from linear regressions of the criterion vs. nutrient ingested (48). The main disadvantage of this method is that it is not standardized and thus the bone and parameter measured (e.g., ash content and break strength) may differ between studies, so studies are not comparable (46).

2.2.3. Digestibility

The digestibility concept was first used to assess P content of feedstuffs as ATTD in the Netherlands (50) and then in France (19). In 2012, the National Research Council (NRC) proposed another method, like the one used for amino acid and that should be more precise, the standardized total tract digestibility (STTD). Digestibility refers to the quantity of nutrient that is not found in the feces and therefore must have been digested, or, at least, has disappeared from the digestive tract, a definition that must be nuanced according to whether endogenous losses are considered. Unlike other nutrients, the digestibility of P (and of Ca to a lesser extent) is estimated over the entire digestive tract as fecal digestibility. Two reasonable assumptions justify this: (1) P and Ca are absorbed in the cecum and the colon, respectively (51). These play a homeostatic role in maintaining serum P and Ca under conditions of low intake, and (2) for P and Ca in most dietary supplies, there are no difference between fecal and ileal digestibility for true and apparent P digestibility (52, 53), or apparent and standardized Ca digestibility (38) and therefore no interest in estimating ileal digestibility, which is much more difficult and expensive than measuring fecal digestibility (20).

Apparent total tract digestibility (ATTD) of a nutrient in a feed is the difference between the total intake of the nutrient in question and the amount found in the feces (54, 55):

$$ATTD_{Ca\ or\ P}(\%) = [(Ca\ or\ P_{intake} - Ca\ or\ P_{feces})/Ca\ or\ P_{intake}] \times 100 \quad (1)$$

The methods most used to determine apparent digestibility are total feces collection or partial collection in conjunction with an indigestible marker. Apparent P digestibility is still used widely but has the disadvantage of not being additive in feeds composed of several ingredients (55, 56).

STTD considers basal endogenous losses, which represent the minimal loss of a nutrient, independent of feed composition but influenced by dry matter intake (49, 54). These losses were first estimated by regression with extrapolation to zero ingestion of the studied mineral (57). They are now measured by analyzing feces of animals fed a diet free of P or Ca (34, 37, 58–60). Critics of this method point out that Ca metabolism is well known to be regulated through absorption and thus reabsorption of endogenous losses, leading to underestimation of basal endogenous losses (61). Likewise, a P imbalance due to a P-free but Ca-containing feed would probably affect endogenous P losses (62). Further trials are needed to determine whether endogenous P losses should be measured with a P-free and Ca-free diet, or if it is better to measure P losses with some Ca to minimize interference by regulation. Basal endogenous losses of P and Ca fall, respectively, into the ranges of 139–252 mg and 123–670 mg/kg of dry matter intake (DMI) (37, 38, 63, 64). Basal endogenous P losses in pigs have been estimated at 190 mg/kg of DMI by (20) and 6 mg/kg of live bodyweight (BW) by Bikker and Blok (65). Standardized digestibility can then be calculated using the following equation (55):

$$STTD_{Ca\ or\ P}(\%) = [(Ca\ or\ P_{intake} - (Ca\ or\ P_{feces} - Basal\ endogenous\ losses))/Ca\ or\ P_{intake}] \times 100 \quad (2)$$

Standardized digestibility values are considered additive in feeds composed of several raw materials (20, 46). According to this equation and the use of a constant basal P loss of 190 mg/kg of DMI, it is simple to convert values of ATTD digestibility values into STTD values.

True digestibility accounts for total endogenous losses, which include basal and specific endogenous losses. The latter represents the losses above basal endogenous ones, due to specific characteristics of the feed, such as the level of anti-nutritional factors and fiber content (54). No method of direct measurement of true digestibility exists, except the use of radioisotopes that are now banned in many countries. It is therefore determined by regression, using apparent digestibility and ingested quantity of the nutrient (46, 49):

$$Ca\ or\ P_{absorbed} = (TTTD \times Ca\ or\ P_{intake}) - Total\ endogenous\ losses \quad (3)$$

The negative intercept corresponds to total endogenous loss, while the slope of the regression represents true digestibility. Critics of this method point out that for P and Ca, estimates are highly variable, dependent on individuals, often intercept is not different from 0 (53, 66, 67) and influenced by the amount ingested, in violation of the basic assumption of the regression method (66, 67).

Although several studies about Ca digestibility have been completed (34, 37, 40, 52, 59, 66, 68), the Ca requirement continues to be generally expressed as a total requirement (20, 46) due to the lack of data on digestibility in specific feed ingredients. To overcome the non-additivity of apparent digestibility in a mixed feed, most recent studies have focused on standardized digestibility (59). However, basal endogenous Ca losses measured so far are highly variable and appear to depend on feed composition (59). In addition, components such as fiber may have a direct and proportionate positive effect on standardized Ca digestibility (59), as shown in rat studies (69). These last considerations show the interest in evaluating the Ca digestibility of raw materials under the specific conditions in which they will be used, as recommended in chicken for P (70).

2.2.4. Mechanistic Modeling and Meta-Analysis Approach

All the methods described earlier give a unique P and Ca value for each feedstuff regardless of the interactions with other components of the diet. With the objective of precisely estimate the digestibility of dietary P in a complete diet, two approaches have been used by Létourneau-Montminy et al. (71, 72) based on available literature. First, a mechanistic research mathematical model that simulates the fate of dietary P forms, phytate P (PP) and non-phytate P (NPP) from plant, mineral and animal origin, in the gastro-intestinal-tract was developed and evaluated by Létourneau-Montminy et al. (71). The proposed model integrates and predicts the impact of the most relevant physiological processes involved in P digestion and absorption, including P dietary forms, the presence of exogenous phytase, and the dietary concentration of Ca. It also predicts the impact of transit time and pH of the different dietary sections. The output is the standardized P and Ca absorbed. It can be used as a prospective tool to study P digestibility for different feedstuffs and feeding strategies, as well as the effect of specific digestive processes on P digestive utilization. Second, given the large number of publications on P digestibility in pigs, meta-analysis, a statistical method relevant for summarizing and quantifying knowledge acquired through previously published research (73, 74), was chosen to predict P digestibility considering dietary P forms, Ca, and exogenous phytases. Dietary forms are PP and NPP from plant, mineral, and animal (72). This study provided a generic response of ATTD P (g/kg) to variation of PP, NPP, and phytase. Results showed a linear relationship between NPP and digestible P. Both NPP from mineral and animal feedstuffs and NPP from plant are highly digestible (78 and 73%, respectively). A digestibility coefficient of 21% was also found for PP showed that part of the PP is available for absorption without any exogenous phytase supply (75–77). Then microbial phytase improved digestible P given hydrolysis was simulated with a classic enzyme equation, the Michaelis–Menten. Its response depends on PP quantity, its substrate. The addition of 500 FTU of microbial phytase per kg of feed to a diet with 2 g of PP/kg, increased the amount of digestible P by 0.60 g/kg. With 3 g of PP/kg, the amount of digestible P increased by 0.67 g/kg. It is worthy to note that the amount of PP varies little in swine ingredients. Finally, dietary Ca linearly decreases digestible P independently of phytase supply as previously shown when testing different concentrations of dietary Ca crossed with different levels of phytase (78, 79). This simple method allows a prediction of true P digestibility based on chemical analysis of the diet in total P, PP, Ca, and microbial phytase, while NPP is the difference between total P and PP as used in broilers (80).

3. PRECISELY ASSESS PHOSPHORUS AND CALCIUM REQUIREMENTS

According to the FAO and WHO (81), a nutrient requirement is defined as the intake level that will meet specified criteria of adequacy without risking deficit or excess. These criteria include an array of biological effects associated with the nutrient. In livestock production, a requirement is defined as the quantity necessary to maximize a production factor such as body growth or bone mineralization. In practice, growth alone is often a poor indicator of mineral status. Tissue analyses should always accompany growth and feed intake data when evaluating mineral adequacy (82). Bone mineralization has long been the standard, but environmental issues have led several countries to review this, giving rise to the notion of growth performance (20). Ca and P requirements may be defined as facilitating growth according to genetic potential while ensuring optimal bone mineralization and keeping environmental risks minimal. In other words, a multicriteria approach to setting nutrient requirements is needed. To respond to these different objectives, global and factorial approaches, and increasingly mechanistic models simultaneously consider the most important variables, including genetics, live weight, and sex.

3.1. Global Approach

This method consists of measuring different performance criteria (growth rate, feed conversion ratio, etc.) in herds that have been fed with increasing levels of the tested nutrient. If all the criteria are not satisfied simultaneously, the proper intake is then considered to be the one that optimizes the most important criterion (83). At this time, the digestibility of nutrients was not considered. This approach presents two main disadvantages. The first one is that it is difficult to compare the estimation of nutrient requirements by this approach with the availability or digestibility of the raw material. The second is that, like nutrient availability, the approach does not consider the portions of P and Ca effectively used and does not allow differentiation between the portions released in feces and urine. Global approaches were replaced by factorial approaches in the 1990s.

3.2. Factorial Approaches

A more advanced method is the factorial approach, which consists of quantification and the addition of the requirements

TABLE 1 | Estimates of P and Ca requirements for growing pigs according to different models.

Bodyweight	30 kg				50 kg				70 kg				100 kg			
ADG	0.96 kg				1.11 kg				1.17 kg				1.12 kg			
Feed intake	1.36 kg				2.14 kg				2.71 kg				3.22 kg			
Body protein	4.68 kg				7.08 kg				11.09 kg				15.03 kg			
	CVB[a]	**NRC[b]**	**INRAe[c]**	**Lautrou[d]**	**CVB[a]**	**NRC[b]**	**INRAe[c]**	**Lautrou[d]**	**CVB[a]**	**NRC[b]**	**INRAe[c]**	**Lautrou[d]**	**CVB[a]**	**NRC[b]**	**INRAe[c]**	**Lautrou[d]**
STTD P (g/kg)	3.77	4.2	-	4.07	2.83	3.07	-	2.85	2.39	2.45	-	2.38	2.01	1.83	-	2.12
ATTD P (g/kg)	-	-	4.0	3.9	-	-	2.98	2.68	-	-	2.5	2.21	-	-	2.1	1.95
Total Ca (g/kg)	9.96	9.03	11.61	8.16	7.53	6.6	8.65	5.9	6.38	5.27	7.26	5.2	5.4	3.93	6.09	5.06
Total Ca:STTD P	2.64	2.15	-	2.00	2.66	2.15	-	2.07	2.67	2.15	-	2.18	2.69	2.15	-	2.39
Total Ca:ATTD P	-	-	2.90	2.09	-	-	2.90	2.20	-	-	2.90	2.35	-	-	2.90	2.59

[a] *Estimated according to Bikker and Blok (65).*
[b] *Estimated according to NRC (20).*
[c] *Estimated according to Jondreville and Dourmad (84).*
[d] *estimated according to Lautrou et al. (12).*

for each physiological function (e.g., for maintenance and growth). With the emergence of this method came the consideration of the intestinal absorption of minerals. Several factorial methods estimating P and Ca requirements had been proposed such as Jondreville and Dourmad (84), NRC (20), and Bikker and Blok (65). The first of these methods is based partly on studies conducted years ago in France (83, 85) and the Netherlands (86) and is applied widely in France and Europe. The second one is popular in North America. The third one is in fact an update of the Jongbloed et al. (86) method based on data published since then. The P requirements estimated by these methods are presented in **Table 1**.

In Jondreville and Dourmad (84)'s model, estimation of P and Ca requirements aims for a bone mineralization of 100%. The maintenance requirement corresponds to obligatory urinary losses, because the P requirements are expressed on an ATTD basis, and endogenous fecal losses are already considered. The maintenance P requirements are estimated at 10 mg/kg of BW (85). The requirement for growth is assessed based on the average daily gain. Finally, the total Ca requirement is estimated according to a fix ratio of 2.9 with the ATTD P requirement.

The NRC (20) considers that P and N retention are highly correlated, and that this correlation is affected little by animal genetics or sex. According to their model, maximal P retention in growing pigs is dependent on body protein. Endogenous basal losses in the gastrointestinal tract are estimated at 190 mg/kg of DMI, to express the P requirements in STTD, and minimal urinary loss at 7 mg/kg of BW. Finally, growth performance is maximized by considering the standardized digestible P requirement to be 85% of the level that maximizes body P retention. The total Ca requirement is set at 2.15 times the standardized P requirement.

In Bikker and Blok (65), Ca and P requirements are estimated independently and aim for a bone mineralization of 100%. The requirement is the sum of the Ca or P retention and the maintenance requirement. An allometric relationship links body Ca and P retention to animal empty body weight gain. The maintenance requirement includes the obligatory urinary loss

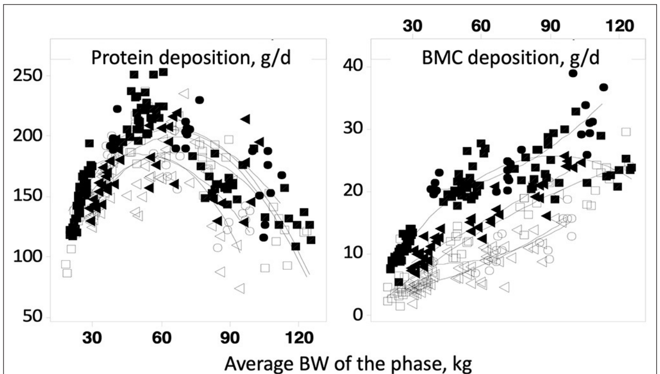

FIGURE 2 | Evolution of protein and bone mineral content deposit as a function of average weight (89).

and the minimal endogenous loss. Basal fecal endogenous losses of P and Ca are set at 6 mg and 8 mg/kg of BW, respectively, and obligatory urinary losses are estimated to be 1 mg and 2 mg/kg of BW. These unavoidable losses are low under conditions of low P or Ca supply, and become greater as the supply increases. The utilization efficiency of the absorbed P and Ca is therefore set at 98%. The Ca and P requirements are first estimated as standardized before applying a digestibility coefficient of 58% to the Ca requirement for expression as total Ca. In this model, as in Sauveur and Perez (83) Ca requirement is estimated according to a factorial approach based on digestible Ca and expressed on a total basis assuming 45–50% Ca digestibility. This permits adaptation of the Ca:digestible P requirement according to animal weight and performance. The same approach was recently used for sows by Gauthier et al. (87) and Gaillard et al. (88).

In all these models, ash deposition strongly correlates with soft tissue gain. However, recent feed trials have shown that this is not the case in growing pigs (**Figure 2**; 89). Protein deposition increases linearly up to a body weight of about 60 kg, then decreases while bone mineral content deposition increases until the pigs reach slaughter weight (120 kg). These two variables are, therefore, measurements of different physiological processes. In the Jondreville and Dourmad (84) and NRC (20) models, the Ca requirement and the digestible P requirement form a fixed ratio throughout the life of the pig. However, as seen previously, bone growth and soft tissue growth are dissociated, which logically results in a Ca:P requirement ratio that is not the same throughout the life of the animal.

4. STRATEGIES TO REDUCE PHOSPHORUS EXCRETION

4.1. Improved Mechanistic Models to Assess Phosphorus and Calcium Requirements

The factorial approach can be integrated in a mechanistic model. The mechanistic models aim to represent the mechanisms of a system. In fact, they connect the underlying mechanisms that control operation of a system. It is, therefore, a matter to meet the conventional notion of requirements (homeorhesis, long-term response) with the response of the animals to inputs [homeostasis, short time scales, (90)]. In a more recent model (11), because of a lack of data, the potential Ca and P depositions were driven by potential protein deposition. But as seen previously, the protein and ash bone depositions are not correlated (89). Consequently, the assessment of Ca and P requirements must consider the fact that changes in skeletal tissue are not directly proportional to lean growth. This is clear when looking at the capacity of P- and Ca-depleted pigs to rapidly replace bone mass through compensatory bone mineralization (see section 4.3). This model has been revised (12) to rectify the no dependency of bone mineral deposition on protein gain by establishing a potential for Ca deposition independent of soft tissue gain, thus allowing P and Ca requirements for soft tissue growth and bone growth to be predicted independently (**Figure 3**).

This new model estimates apparent digestible Ca and P requirements, which can be converted to STTD or total requirements. The only input required is the initial body weight, from which body protein, lipid, water, and ash (soft tissue and bone) are estimated. Soft tissue growth is currently estimated by applying the principles of van Milgen et al. (91), although other models such as NRC (20) or even user-specific equations tailored to animal growth in a specific setting may be adequate. Estimated protein and lipid gains can be used to assess P and Ca retention in soft tissues (92). In parallel, the Ca requirement for bone is estimated with the bone Ca potential deposition curve presented by the same authors (12). The deposition of P in bone is estimated at a fixed ratio of 2.16 to Ca deposition. The maintenance requirements, equivalent to the obligatory urinary losses, are set at 0.5 mg for P and 2 mg for Ca, per kg of body weight. The sum of the maintenance and growth requirements (of soft tissue and bone) thus provides apparent digestible P and Ca requirements. In fact, the ratio increased with body weight because protein deposition that represents about 30–40% of the body P decreases while bones continue to grow after 70 kg of BW. These can be converted to standardized or total requirements.

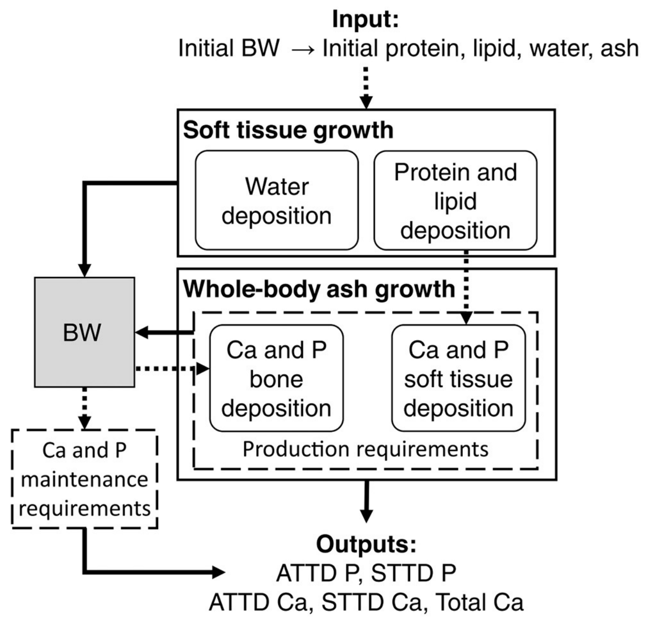

FIGURE 3 | General layout of the proposed mechanistic model predicting total calcium (Ca) and apparent and standardized digestible phosphorus (ATTD and STTD P) requirements of growing pigs (12).

Results confirm the need for a non-fixed Ca:P requirement ratio (**Table 1**). This model has the additional advantage of being adaptable to different production objectives such as 100% or 85% mineralization, without decreasing the share of Ca or P destined for soft tissues. Although a single deposition potential has been established, it will become necessary to consider animal genetics (93, 94) and/or sex in further validations of the model. The sensitivity analysis of the model showed that protein deposition influenced ATTD-P variance by 15% for pigs at 30 kg, 6% at 60 kg, and 1% at 120 kg based on protein deposition variation in previous trials (12). The decrease in the influence of protein deposition on P with BW increase coincides with the linear increase in bone deposition. Moreover, the ATTD-P variance associated with protein deposition at 30 kg shows that animal growth will have a major impact on P recommendations.

4.2. Toward More Efficient Degradation of Phytate Phosphorus

4.2.1. Description of Phytases

Phytases, or myo-inositol hexaphosphate phosphohydrolases, are enzymes that hydrolyze phytic acids and release the phosphate groups (55). In growing pigs, there are 4 sources of phytase: (1) the mucosa of the small intestine, (2) microorganisms in the large intestine, (3) ingested plant matter, and (4) exogenous phytase added to the feed. A unit of phytase activity is defined as the release of 1 μmol of inorganic P per minute in a solution containing 5.1 mmol of sodium phytate per liter at pH 5.5 and 37°C (95). Low endogenous phytase activity is observed in the proximal part of the pig intestine, but about 20% of the phytic P would nevertheless be potentially absorbable (72, 76, 77). Some

TABLE 2 | Characteristics of some commercial microbial phytases.

Product	Origin	Expression	Type[a]	pH optima	IP_6 degradation[b]	Year[c]
Natuphos®	A. Niger	A. Niger	3	2; 5–5.5	503	1990
Allzyme® SSF	A. Niger	A. Niger,	3	6		
Finase® P/L	A. Niger	Trichoderma reesei	3	2.5		
Ronozyme® P	Peniophora lycii	Aspergillus oryzae	6	4–4.5	480	2002
Phyzyme® XP	Escherichia coli	Schizosaccharomyces pombe (ATCC 5233)	6	4.5	140	2003
OptiPhos®	Escherichia coli	Pichia pastoris	6	3.4; 5.0		2006
Quantum™	Escherichia coli	Pichia pastoris	6	4.5	148	2007
Ronozyme® Hiphos	Citrobacter braakii	Aspergillus oryzae	6	4–5	269	2010
Quantum® Blue	Escherichia coli	Trichoderma reesei	6	4–5	211	2012
Axtra® PHY	Buttiauxella sp.	Trichoderma reesei	6	3.5–4.5	129	2013
Natuphos® E	Hybrid phytase (Hafnia sp., Yersinia sp. et Buttiauxella sp)	A. Niger	6	4–5		2016

***Table 2** is not an exhaustive list and represents only a few of the currently available commercial phytases, adapted from Dersjant-Li et al. (95, 97), and Lei et al. (98).*
[a] *3 or 6 phytase.*
[b] *Phytase activity needed to achieve 50% reduction in IP6, with high buffer volume.*
[c] *Year of the commercial launch.*

plant raw materials have their own phytasic activity. This one is more or less important according to the ingredient and the part used (14, 84). Phytasic activity is higher in some cereals such as rye, triticale, wheat, or barley than in cereals richer in proteins (19). Plant phytase is sensitive to heat (more than microbial phytases), since its activity is partially or completely inactivated after high temperature treatment (>70°C) such as those for pelleting (18, 84, 96). It is why in INRA-AFZ feed tables (19) two values are given for P digestibility, one which takes account of the effect of endogenous phytase to be used when feed is given as meal, and a second without considering the effect of endogenous phytase to be used when the feed is pelleted. Therefore, the most promising phytase sources remains the exogenous phytase.

4.2.2. New Generations of Exogenous Phytases

The first exogenous phytase was marketed in 1991 in the Netherlands, the first country to introduce strict regulations intended to limit P discharges from pig and poultry farming. The use of phytases then accelerated following the introduction of similar legislation in other countries and the ban on the use of animal byproducts in Europe (18). These enzymes were isolated first from fungi (**Table 2**), then new techniques allowed the production of phytases by bacteria and yeast, leading to the second generation of phytases. The common commercial phytases are obtained from cultures of *Aspergillus niger*, *Peniophora lycii* (fungi, 3-phytase), and *Escherichia coli* (bacteria, 6-phytase). In pigs, bacterial phytase has been found to be more effective than fungal phytase (78, 99). This explains why fungial phytases were supplanted in the early 2000s by 6-phytases produced by *Escherichia coli*. Other second-generation phytases obtained from cultures of *Citrobacter braakii*, *Buttiauxella spp.* and even hybrid forms soon followed (**Table 2**). Third generation phytases with up to 8 amino acid substitutions in the *E. coli* enzyme have better thermostability (100). The presence of plant phytase reduces the response to added exogenous phytase (18). New generation phytases developed through genetic engineering release more P (101). Exogenous phytases also increase Ca availability (32) but the underlying mechanism remains to be determined. P and Ca digestion in pigs has been modeled, integrating interactions, the different chemical forms, and the effect of phytase (71). Dissociation of Ca phytates at gastric pH is presumed in this model has showed *in vitro* (102). By increasing the proportion of phytate degraded by phytase in the upper digestive tract, less Ca should form insoluble complexes with phytate in the small intestine where pH is favorable and therefore more should remain available for absorption. However, we have not seen validation of this hypothesis *in vivo* and the exact mode of action of phytase on Ca remains unclear, but undoubtedly have an impact *in vivo.* Phytases preferentially release the position 5 and 6 phosphates, which have the highest affinity for cations such as Ca, rather than dephosphorylating phytate completely (103). As a result, the phytase doses that are now commonly used would increase Ca availability more than P availability, at a ratio of about 2, whereas high doses would sustain P release while Ca release reached an asymptote (103).

4.2.3. Factors Influencing the Efficiency of Exogenous Phytases

For optimal action, phytic acid must be hydrolyzed upstream from the sites of absorption of P and other minerals such as Ca, Zn, and Fe. P is absorbed mostly in the upper small intestine (5, 51). Hydrolysis in the stomach is therefore ideal, meaning that the enzyme must be sufficiently active at gastric pH (3.5 in young pigs and lower in older animals (104)). Phytase from *A. niger* works well at pH 2 or 5–5.5, but poorly at porcine gastric pH. The optimal pH range of new generation phytases has been lowered and in some cases broadened [**Figure 4**; (105–107)].

To limit the loss of activity, phytases must be made resistant to digestive proteases. Second-generation phytases were better in this sense (*P. Lycii vs. E. coli*, **Figure 5**, 106). After 2 h in contact

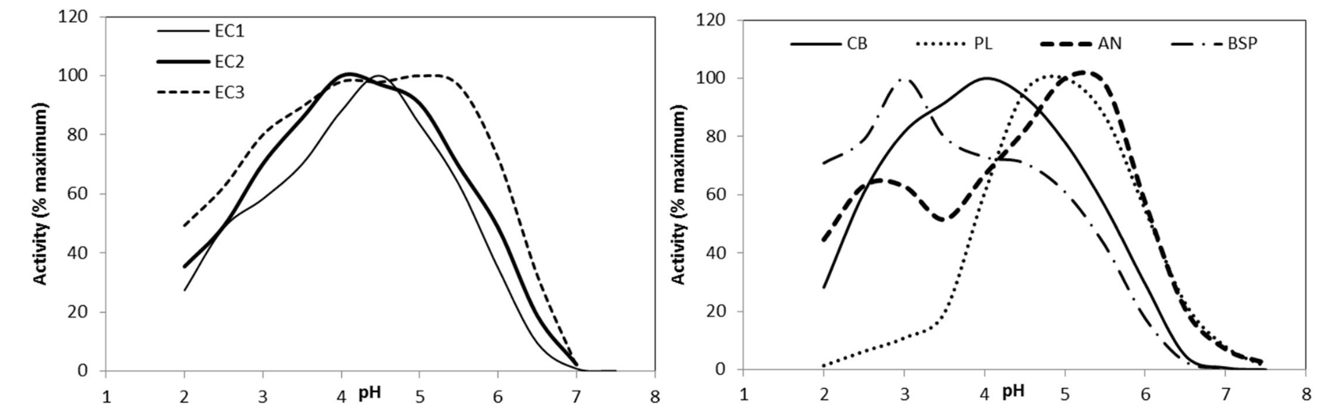

FIGURE 4 | Effect of pH on phytase activity of the phytase products used in the *in vitro* degradation model with EC1: Quantum (AB Vista), EC2: Quantum Blue (AB Vista), EC3: Phyzyme XP (Danisco), CB: Ronozyme Hiphos (DSM), PL: Ronozyme NP (DSM), AN: Natuphos (BASF), BSP: AxtraPHY (Danisco). Reprinted with permission from (105). Copyright 2015 American Chemical Society.

with pepsin, *E. coli* phytases retained 77% of their initial activity compared to 31% for an *A. niger* phytase (95).

The ideal temperature of activation of the phytase is between 50 and 60°C. On the other hand, high temperature treatments (> 70°C) decrease the phytase activity of the feed (108–110). The second-generation *E. coli* phytases lost thermostability compared to the fungal phytases (98), except for a third-generation phytase from *E. coli* (Phy9X), which is resistant to higher temperatures, up to 75°C (108, 111). On the other hand, increasing the resistance temperature of phytases can lead to a higher optimal temperature and thus potentially decrease their efficacy at normal pig body temperature (around 39°C) (112).

To be the most useful, phytases must preferentially degrade IP_6 and IP_5 phytates as quickly as possible. They must, therefore, have a high affinity for the preferred substrate. Second-generation phytases were improved in this sense (112), at least in terms of initial reaction velocity (V_{max}) *in vitro* with IP_6 and IP_5 substrates (105).

Despite the improvement in phytases, it is important to understand that they act on soluble phytates. Therefore, the factors that influenced phytate solubility must be control. Certain minerals interfere with phytates. Cations have an inhibitory power related to their affinities for phytic acid but also the insolubility of the complexes they formed. This is measurable as the amount of mineral that causes phytates hydrolysis to drop by 50% at a given pH (102). The smaller the amount, the more inhibitory. On this basis, the following ranking has been established (102): at pH 6: Fe^{2+} > Zn^{2+}= Fe^{3+} >Mn^{2+} >> Ca^{2+}>Mg^{2+} At pH 5: Fe^{3+} > Fe^{2+}> Zn^{2+} >> Mn^{2+} > Ca^{2+} >> Mg^{2+}, representatives of the gut pH in pigs. This inhibitory power represents the affinities of the minerals for phytic acid but also the insolubility of the complexes formed. Reducing the pH to 4, which corresponds to gastric pH, strongly reduces the power of all minerals tested. Iron has the greatest potential for inhibition, but to our knowledge, no study of its effect on phytase effectiveness in animal feed has been published. Regarding Zn and Cu, their supply can be high in piglets with so-called pharmacological levels (2,500 ppm) when used as a

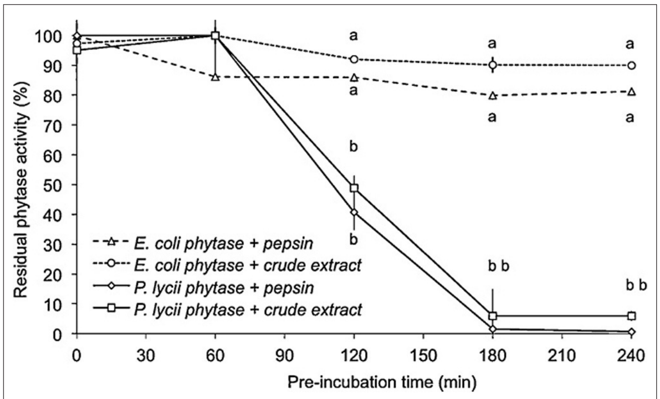

FIGURE 5 | Residual phytase activity of *E. coli* and *P. lycii* phytase after pepsin or gastric crude extract from trout stomach hydrolysis throughout incubation time (0, 60, 120, 180, and 240 min). The incubation was performed by adding 1 FTU phytase to a protease solution with 5000 U from porcine pepsin or gastric crude extract from fish, performed at pH 2.0 (HCl), 16 °C. The results are plotted as the mean ± SE (triplicates). Different letters, for each time, indicate significant differences (P<0.05) between phytases (106).

growth factor to reduce diarrhea. Zn has a high complexing power, a single Zn cation being capable of binding to two phytic acid molecules (113). The effect of Zn on phytase efficiency has been studied in weaned pigs (6–20 kg). With 1,000 and 3,000 FTU in the diet, zinc oxide at 3,000 mg/kg decreased the Ca ATTD by 6 and 9%, respectively, and the P ATTD by 10 and 16% in pigs weighing 15–20 kg (114). In young pigs weighing 7–13 kg, P release by phytase was reduced by 30% when the dietary Zn content was 1,500 mg/kg (115). The effect of Cu on phytase is less clear. Cu phytates appear to be soluble at neutral pH (113), suggesting no effect. An *in vitro* study of Cu at 62.5 mg/kg and pH 5.5 suggests that P release from phytase may decrease by 2–30% depending on the source of the Cu (116). At 500 mg/kg, the decreases ranged from 5 to 75%. At pH 6.5, the decreases were even more marked but were almost non-existent at pH 2.5. The most likely explanation for these

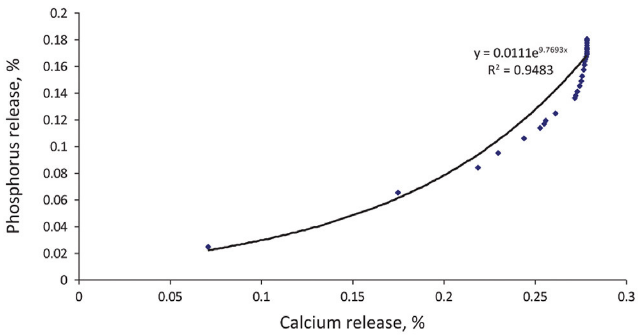

FIGURE 6 | Theoretical relationship between P release from phytate and associated Ca value showing disproportionate extra phosphoric effect with initial destruction of the higher esters (103).

observations would be formation of insoluble phytic acid–Cu complexes at higher pH, which is of some concern given the pH of the porcine gut (116–118). In pigs weighing 6–22 kg, P digestibility was greater with methionine-chelated Cu than with Cu sulfate (118). Chelated Cu would be more stable in the upper gastrointestinal tract and less available to form complexes with phytic acid; thus there would be a better release of absorbable phosphate (116, 119). Tests of the effect of Zn and Cu form and concentration on Ca and P digestibility in pigs weighing 6–22 kg showed that the form of Cu had no effect, while the form of Zn did (117).

Ca is ranked as less inhibitory but is incorporated into feed at much higher concentrations than Zn, Cu, or Fe. As a result, Ca forms a significant proportion of insoluble phytates, frequently with Zn (120). Because the recent phytases have a higher affinity for IP_6 and IP_5, which have higher affinities for Ca, the ratio of Ca:P released using second and third generation phytases is around 2 at 500 FTU/kg (**Figure 6**) and decreases as phytase activity increases (103, 121, 122). The phytase levels practiced in the field may therefore lead to an increase in the digestible Ca:P ratio. Trials have shown phytase effectiveness to decrease as the Ca:P ratio increases in the feed (123–125) albeit without comparison to phytase-free diets, making it impossible to know whether the effect of Ca was on phytase or on P absorption (126). When the ratio of Ca to total P was increased from 1.2 to 1.8, pigs grew more slowly regardless of the presence of phytase, suggesting a specific effect due to Ca rather than an influence on phytase efficacy in releasing P (78). In trials conducted with P at requirement levels, P digestibility decreased slightly as the Ca:P ratio increased from 1.2 to 1.9 but was indifferent to phytase (79). Furthermore, urinary excretion of P was 5-fold higher at a Ca:P ratio of 1.2, due to the lack of Ca for deposition of P in bone. Nor was any effect of Ca on phytase efficacy found when animals were fed above the P requirement (127). A high Ca:P ratio therefore does not seem to have a direct effect on phytase efficacy in releasing P but rather on P absorption and retention, possibly going so far as to cause a P deficiency and ultimately poorer growth regardless of the presence of phytase (18, 79).

4.3. Depletion–Repletion Strategy

Animals have a survival strategy to overcome some mineral deficiencies by enhancing digestion and increasing the efficiency of utilization of the deficient nutrient (128). In several species, dietary restriction of Ca and P results in increased intestinal absorption, renal reabsorption, and deposition and mobilization in bone tissue (1). The effects of dietary Ca restriction and recovery processes on bone metabolism were studied decades ago in rats (129–131) and humans (132). The findings suggest that bone has ways of replenishing losses due to the use of mineral reserves and that parathyroid hormone and vitamin D play a role in the mechanisms. Bone accretion, intestinal absorption, and renal reabsorption of minerals are under hormonal regulations described in **Figure 7**.

The Ca depletion–repletion strategy is already used to prime dairy cows for high Ca demand during early lactation (133) and to prevent milk fever. By feeding a Ca-deficient ration for a few weeks before the start of lactation, regulatory mechanisms that maintain blood Ca levels (increased intestinal absorption and renal reabsorption) are activated (133, 134). A few days before calving, when the demand for Ca becomes very high, the cows then receive more Ca (134), and the shortfall between the requirement and Ca absorbed is smaller because of the effect of priming on parathyroid hormone. The animal is also better prepared to draw upon bone Ca as needed to maintain blood Ca levels and thus prevent milk fever. Similar regulatory mechanisms allow maintenance of P levels, and these can be exploited to increase dietary P utilization in growing animals and hence the sustainability of livestock farms from the environmental perspective. The idea underlying the depletion–repletion strategy is therefore to trigger regulatory mechanisms during the depletion phase to induce an increase in P utilization efficiency without affecting growth performance (1). In the case of P and Ca, the mineral content of the body or of a specific bone is monitored using X-ray absorptiometry [DXA, (135)]. During depletion in growing pigs, body bone mineral content continues to increase, but bone accretion is decreased compared to control pigs, leading to reduced bone mineral content.

When P supply is intentionally below the estimated requirement of the animals, the level of Ca is generally decreased at the same time to avoid the deleterious effects of a high digestible Ca:P ratio on P absorption. When animals thus primed are fed the repletion diet, which provides P at least at the requirement level, the deficit overcome. This allows an overall reduction in dietary P intake during the rearing phase. Depletion–repletion studies of growing animals such as pigs (8, 10, 136) and chickens (137–141) led to effectively increasing P utilization and limiting excretion without compromising animal well-being and performance. Some authors (9, 142) have focused on improvements to bone health; these studies have led to better understanding of the deleterious effects of short-term dietary Ca deficits during growth on long-term bone mineralization. The main trials performed with pigs are summarized in **Table 3**.

When Ca is deficient, the Ca regulation calls for parathyroid hormone, which is a hypercalcemic hormone that increases dietary Ca utilization, but with a concomitant hypophosphoremic effect due to renal excretion of P (144).

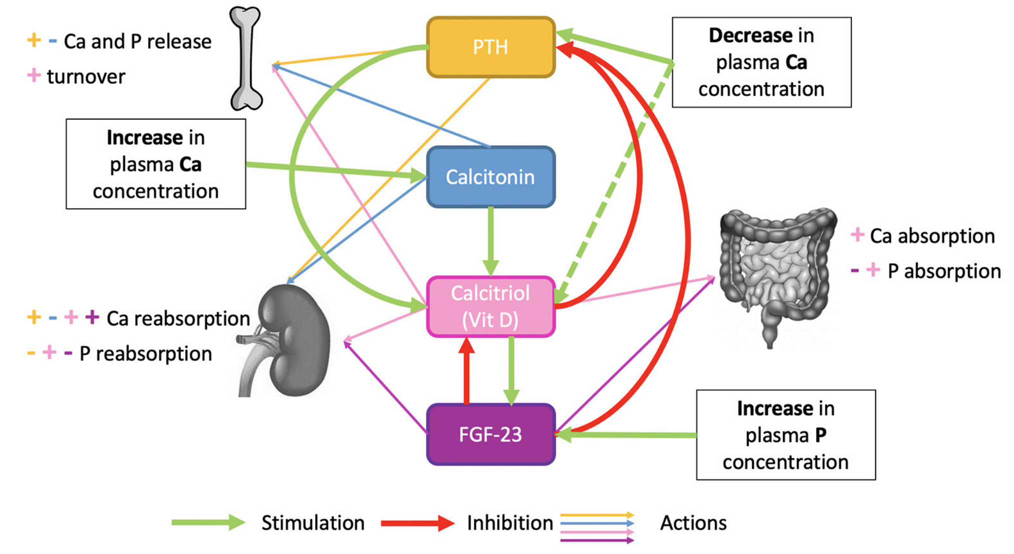

FIGURE 7 | Hormonal regulation of phosphocalcic metabolism.

Ca deficiency must therefore be avoided. In growing pigs, it has been found to reduce the expression of genes related to P reabsorption in the kidney, favoring P excretion in urine (145). P depletion in the range of 30–40% and slightly lower for Ca induces demineralization of the same order in the whole body and vertebrae as measured by DXA (**Table 3**). The trial conducted by Aiyangar et al. (9) shows greater demineralization with a higher Ca deficiency. The metacarpus appears to demineralize less (5–10%) than the whole body or vertebrae (10), whereas the femur responds like the whole body. Bone reserve depletion measured thus depends primarily on the degree of dietary depletion and on the bone region studied.

Several studies have shown that this strategy works and increases bone mineral content (BMC) gains and digestible P utilization when animals are fed a repletion diet (at requirement or above). The gain of BMC in L2 to L4 vertebrae in depleted animals exceeded those in non-depleted control pigs by 56% during a first 28-day repletion phase and 15% during a second repletion of the same length (8) and by 29% after a 28-day repletion and 11% after a 56-day repletion in another study (**Figure 8**; 136). The corresponding increases in digestible P utilization estimated as deposition vs. intake were 20–50% with bone deficit recovery in 28–56 days for the whole body and in 28 days for vertebrae. The shorter time for vertebrae could be due to their high percentage of trabecular tissue, which is more sensitive than cortical tissue to mineral deficiencies (146). Furthermore, in pigs, bone mineralization is faster in the trunk from 3 to 30 kg of BW than in other parts of the skeleton (147). In a study using the common dosage of 750 FTU/kg without phosphate, thus 40% below the requirement, the deficit was recovered in 27 days on the repletion diet with a 47% increase in whole body BMC gain (143). Overall, the depletion–repletion strategy reduced dietary phosphate use and P release by about 40%. In contrast, the repletion diet has failed to restore bone mineralization in at least two porcine studies (9, 142).

Phosphocalcic regulations occur in the gut, kidney, and bone (32, 148). Ca absorption may increase by 27% upon repletion compared to control animals receiving the same feed (8). Osteocalcin, derived from newly synthesized bone and thus an indicator of osteoblastic activity and hence increased bone accretion (149), has been found to increase during the repletion phase (10). The physiological mechanisms underlying animal responses to the depletion–repletion strategy remain poorly understood. It, nevertheless, appears that adequate bone mineralization and growth performance can be achieved at decreased P intake and excretion through improved P utilization.

5. MITIGATION STRATEGIES: COMPARISON AND PERSPECTIVES

The mitigation of the environmental footprint of P in pig production both refers to the optimization of the use of phosphates, which are a non-renewable resource that must be extracted and transported, and to the minimization of its excretion especially in regions with high production density. Some strategies of mitigation have been proposed in the previous sections.

The potential for decreasing P excretion with phytase is well known (18). Its potential depends mainly on a precise nutritional matrix. First, a precise estimation and utilization of the P matrix is crucial. The Ca matrix has recently been shown to be of great importance because, on the one hand, an excess of soluble Ca can decrease the P digestibility (78) and, on the other hand, in case of Ca deficiency, the P is not retained and is excreted in the urine (79). In recent trial with microbial phytases (500 FTU), Lagos et al. (150) showed a drop of 37% in the total P excretion,

TABLE 3 | Effect of depletion–repletion strategy on bone mineralization of growing pigs.

			Depletion period										Repletion period						
Article[a]	Measurement	Phase	Sequence[b]	BW, kg	*p*-value[c]	Days[d]	P[e], %	Ca[e], %	Bone[f], %	*p*-value[c]	Bone accretion[g], %	*p*-value[c]	Sequence[b]	Days[d]	BW, kg	*p*-value[c]	Bone[f], %	*p*-value[c]	Bone accretion[g], %	*p*-value[c]
1	BMC total body (DXA)	1	L	34	ns	28	-31	-39	-34	<0.001	-62	<0.001								
		2	CL	67		28	-42	-22	-25		-48		LC	28	64	0.03	-17	<0.001	+2	ns
			LL	66	ns	56	-42	-22	-25	<0.001	-45	<0.001								
		3	CCL	102	ns	28	-34	-13	-14	0.002	-31	<0.001	CLC	28	100	ns	-13	0.006	+8	ns
			LLL	100		84	-34	-13	-33		-23									
		4											CLCC	56	129		-3		+11	
													CCLC	28	134	ns	+1	ns	+29	<0.001
2	BMC of the L2 to L4 vertebrae	1	Low	46	ns	28	-40	-30	-29	<0.001										
		2	Con-Low	72		28	-40	-46	-24		-53		Low-Con	28	70	<0.01	-9	0.007	+17	0.005
			Low-Low	77	ns	56	-40	-46	-30	<0.001	-36	<0.001								
		3	Con-Con-Low	104		28	-40	-33	-2		-18									
			Con-Low-Low	101		56	-40	-33	-16		+1		Low-Con-Con	56	99		-1		+15	
			Low-Low-Low	106	ns	84	-40	-33	-18	<0.001	0	ns	Low-Low-Con	28	103	ns	-7	ns	+56	<0.001
3	Ash of the 3rd and 4th metacarpus	1	L	48	<0.001	59	-47	-29	-9	<0.05										
		2	LL	91	<0.05	131	-45	-30	-7	<0.05			LH	72	99	ns	-1	ns		
			HL	100	ns	72	-45	-30	-1	ns										
4	BMC total body (DXA)	1	L			28		-54	-66	<0.01										
		2	LL			71		-54	-62	<0.01	-60	<0.01	LH	43					+3	
			HL			43		-54			-59	<0.01								
5	Femur ash	1	DD-	12	ns	10	-60	-53	-19	<0.01										
		2	DD+ HCaPhyt-	21		25	-32	0	-10				DD- HCaPhyt+	25	21		1			
			DD+ LCaPhyt-	21		25	-32	-37	-7											
			DD- LCaPhyt+	21		35	0	-34	-1											
			DD- HCaPhyt-	21		35	-32	0	-17											
			DD- LCaPhyt-	21		35	-32	-37	-19											
6	BMC total body (DXA)	1	Phyt	71	<0.05	39	-40	-40	-17	<0.001	-23	<0.01								
		2											Phyt-Phyt	27	108	ns	+3	ns	+47	<0.05
		3											Phyt-Phyt-Phyt	55	130	ns	+7	ns	+4	ns

[a] *Article 1 : Gonzalo et al. (136), article 2 : Létourneau-Montminy et al. (8), article 3 : Varley et al. (10), article 4 : Aiyangar et al. (9), article 5 : Létourneau-Montminy et al. (79), article 6 : Lautrou et al. (143).*

[b] *Sequences of depletion and repletion as named in the original articles.*

[c] *p-value of the statistical analysis of the control vs. the studied group, for the variable of the previous column.*

[d] *Duration of the depletion or repletion.*

[e] *P or Ca depletion against the control.*

[f] *Difference of the state of the bone at the end of the phase between the control vs. the studied group, according to the measurement.*

[g] *Difference of the bone accretion measurement between the control and the studied group.*

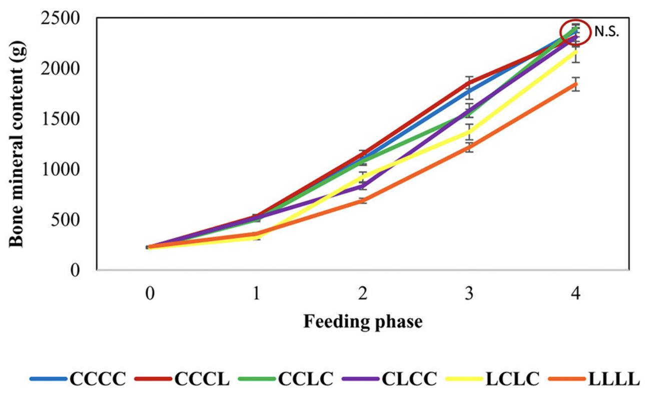

FIGURE 8 | Body bone mineral content of growing pigs feeding depletion–repletion diets (145).

and a reduction of 6.1 g/kg monocalcium phosphate supply, for 60 kg BW pigs. Besides, Almeida and Stein (55) showed that the total P excretion decreases up to 51% with 685 FTU at 15 kg of BW, and monocalcium phosphate supply is reduced up to 8 g/kg.

The depletion–repletion strategy also led to a decrease of the phosphate input and the P excretion. In Gonzalo et al. (136)'s trial, a depletion period of 28 days (L) resulted in a decrease of P supply from 8 to 12%, and 2 separate depletion periods of 28 days (LCLC, C being a 28 days phase of feeding control diet) resulted in a reduction of 12% of P input. The excretion of P in the CLCC, CCLC, and LCLC groups decreased of 15, 13, and 16%, respectively, compared to the control. The decrease of P excretion was greater than the decrease of P input thanks to animals utilizing P more efficiently during the depletion and the repletion periods. With P total collection of feces and urine, Létourneau-Montminy et al. (8) showed that a depletion of 56 days can lead to a P excretion decrease of 19% with a diminution of P intake of 23%. As seen previously, few authors tested depletion–repletion strategies on growing pigs and the results differ in terms of bone mineralization compensation (145). There is a lack of data to precisely defined an ideal strategy of depletion repletion (depletion duration, age, intensity). The study of underlying mechanisms, such as hormonal regulations, will certainly help to reach this objective to reduce phosphate input without compromising bone mineralization and to apply this strategy on farm. Nevertheless, a reduction of 15–20% of both phosphate use and P excretion may be achieved with depletion–repletion strategy.

The strategy of depletion–repletion can also be combined with phytase. Lautrou et al. (143) tried to evaluate the effect of a zero phosphate diet on the growth of pigs and the environment. The use of phytase did not meet the full extent of the P requirements for pigs during the first growing phase of 39 days, but phytase provided enough P during the 2 next phases. This strategy led to a drop of 66% in P excretion during the 2 first phases (the data are not available for the last one), and a reduction in monocalcium phosphate supply of 18.71, 9.52, and 7.17 g/kg in Phases 1, 2, and 3, respectively. This trial showed that there is an opportunity to feed growing pigs from 30 to 130 kg without adding any mineral phosphates. This success has to be confirmed and always requires a well-defined phytase matrix, particularly to mitigate the risks associated with the depletion phase.

In a simulation, Pomar et al. (151) showed that precision feeding, a strategy in development that allow to feed pigs with diets tailored daily to each individual's nutrient requirements, could reduce P excretion by 38%. A recent study compared the P excretion of pigs under conventional or precision feeding (152). The individual and daily feeding system (based on estimated lysine requirement) led to a decrease of 27% in P excretion compared to the group phase feeding system. In this trial, phytase was used but not compared with a control without phytase. The combination of precision feeding with phytase and a depletion–repletion strategy has not been tested yet, but after the synergy observed with the phytase and depletion–repletion strategy, combining these 3 methods seems a promising strategy that could lead to an even greater reduction of P excretion.

6. CONCLUSIONS

This review has shown that it is still possible to improve P utilization in swine and to improve the sustainability of the industry by mitigating phosphorus' impact on the environment. The first step is to precisely estimate the P and Ca content of feedstuffs and each animal's total diet. The second step is to use a robust multicriteria modeling approach to establish animal requirements. The new generation of phytases may provide a strategy to increase P utilization by pigs by providing a precise estimation of the equivalences and interfering factors and maximizing the solubility of phytates. A depletion–repletion strategy to prime animals to make them more efficient is also promising, but still requires testing to refine it and better understand the underlying mechanisms. Finally, precision feeding, a strategy in development that permits feeding pigs with diets that are tailored daily to each individual's nutrient requirements, shows possibilities to reduce more P excretion, and will undoubtedly be employed once the P requirements will be well defined by a robust modeling approach.

AUTHOR CONTRIBUTIONS

ML wrote the original draft. AN, J-YD, CP, PS, and M-PL reviewed the article and add new ideas and participate to improve the structure of the document. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Suttle NF. *Mineral nutrition of livestock, 4th ed*, Suttle N, editor. Wallingford: CABI (2010). Available online at: http://www.cabi.org/cabebooks/ebook/20103291114
2. Van Enk RJ, Acera LK, Schuiling RD, Ehlert P, De Wilt JG, De Vries CK, et al. *The Phosphate Balance: Current Developments and Future Outlook*. (2011). Available online at: http://www.innovatienetwerk.org/sitemanager/downloadattachment.php?id=TnV9h_IU2899_OkhJmHC1
3. Heckenmüller M, Narita D, Klepper G. Global availability of phosphorus and its implications for global food supply: An economic overview. Kiel Working Paper, No. 1897, Kiel Institute for the World Economy (IfW), Kiel (2014).
4. Yang XE, Wu X, Hao HL, He ZL. Mechanisms and assessment of water eutrophication. *J Zhejiang Univ*. (2008) 9:197–209. doi: 10.1631/jzus.B0710626
5. Crenshaw TD. Calcium, phosphorus, vitamin d, and vitamin k in swine nutrition. In: Lewis AJ. Southern LL, editors. *Swine Nutrition, 2nd ed*. New York, NY: CRC Press (2001). p. 187–212.
6. Heaney RP. Phosphorus nutrition and the treatment of osteoporosis. *Mayo Clin Proc*. (2004) 79:91–97. doi: 10.4065/79.1.91
7. Selle PH, Cowieson AJ, Ravindran V. Consequences of calcium interactions with phytate and phytase for poultry and pigs. *Livestock Sci*. (2009) 124:126–41. doi: 10.1016/j.livsci.2009.01.006
8. Létourneau-Montminy MP, Pomar C, Lovatto PA. Apparent total tract digestibility of dietary calcium and phosphorus and their efficiency in bone mineral retention are affected by body mineral status in growing pigs. *J Anim Sci*. (2014) 92:3914–24. doi: 10.2527/jas.2013-7320
9. Aiyangar AK, Au AG, Crenshaw TD, Ploeg HL. Recovery of bone strength in young pigs from an induced short-term dietary calcium deficit followed by a calcium replete diet. *Med Eng Phys*. (2010) 32:1116–23. doi: 10.1016/j.medengphy.2010.08.001
10. Varley PF, Sweeney T, Ryan MT, O'Doherty JV. The effect of phosphorus restriction during the weaner-grower phase on compensatory growth, serum osteocalcin and bone mineralization in gilts. *Livestock Sci*. (2011) 135:282–8. doi: 10.1016/j.livsci.2010.07.025
11. Létourneau-Montminy MP, Narcy A, Dourmad JY, Crenshaw TD, Pomar C. Modeling the metabolic fate of dietary phosphorus and calcium and the dynamics of body ash content in growing pigs. *J Anim Sci*. (2015) 93:1200–17. doi: 10.2527/jas.2014-8519
12. Lautrou M, Pomar C, Dourmad JY, Narcy A, Schmidely P, Létourneau-Montminy MP. Phosphorus and calcium requirements for bone mineralisation of growing pigs predicted by mechanistic modelling. *Animal*. (2020) 14:s313–22. doi: 10.1017/S1751731120001627
13. Pomar C, Pomar J, Rivest J, Cloutier L, Létourneau-Montminy MP, Andretta I, et al. Estimating real-time individual amino acid requirements in growing-finishing pigs: towards a new definition of nutrient requirements in growing-finishing pigs? In: Sakomura NK, Gous R, Kyriazakis I, Hauschild L, editors. *Nutritional Modelling for Pigs and Poultry*. Wallingford, UK: CAB International (2015). p. 157–74. doi: 10.1079/9781780644110.0157
14. Humer E, Schwarz C, Schedle K. Phytate in pig and poultry nutrition. *J Anim Physiol Anim Nutr*. (2015) 99:605–25. doi: 10.1111/jpn.12258
15. Pointillart A. Phytates, phytases : leur importance dans l'alimentation des monogastriques. *INRAE Prod Anim*. (1994) 7:29–39. doi: 10.20870/productions-animales.1994.7.1.4155
16. Pontoppidan K, Pettersson D, Sandberg AS. The type of thermal feed treatment influences the inositol phosphate composition. *Anim Feed Sci Technol*. (2007) 132:137–47. doi: 10.1016/j.anifeedsci.2006.03.008
17. Feil B. Phytic acid. *J New Seeds*. (2001) 3:1–35. doi: 10.1300/J153v03n03_01
18. Selle PH, Ravindran V. Phytate-degrading enzymes in pig nutrition. *Livestock Sci*. (2008) 113:99–122. doi: 10.1016/j.livsci.2007.05.014
19. Sauvant D, Perez JM, Tran G. *Tables INRA-AFZ de Composition et de Valeur Nutritive des matières premières destinées aux animaux d'élevage: 2ème édition*. Versailles: INRA Editi Edition (2004).
20. NRC. *Nutrient Requirements of Swine, 11th Rev*. Washington, DC: National Academies Press (2012).
21. Wise A. Dietary factors determining the biological activities of phytase. *Nutr Abstr Rev*. (1983) 53:791–806.
22. Angel R, Tamim NM, Applegate TJ, Dhandu AS, Ellestad LE. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. *J Appl Poultry Res*. (2002) 11:471–80. doi: 10.1093/japr/11.4.471
23. Vohra P, Gray GA, Kratzer FH. Phytic acid metal complexes. *Proce Soc Exp Biol Med*. (1965) 120:447. doi: 10.3181/00379727-120-30559
24. Vasca E, Materazzi S, Caruso T, Milano O, Fontanella C, Manfredi C. Complex formation between phytic acid and divalent metal ions: a solution equilibria and solid state investigation. Anal Bioanal Chem. (2002) 374:173–8. doi: 10.1007/s00216-002-1469-6
25. Taylor TC. The availability of the calcium and phosphorus of plant materials for animals. *Proc Nutr Soc*. (1965) 24:105–12. doi: 10.1079/PNS19650017
26. O'Dell BL, De Boland A. Complexation of phytate with proteins and cations in corn germ and oil seed meals. J Agric Food Chem. (1976) 24:804–8. doi: 10.1021/jf60206a034
27. Greiner R, Konietzny U. Update on characteristics of commercial phytases. *Int Phytase Summit*. (2012) 96–107.
28. Bleukx W. Production et qualité nutritionnelle des phosphates alimentaires. *Prod Anim*. (2005) 18:169–73. doi: 10.20870/productions-animales.2005.18.3.3521
29. Viljoen J. Quality of feed phosphate supplements for animal nutrition. *S Afr J Anim Sci*. (2001) 2:13–19.
30. De Groote G, Lippens M, Jongbloed AW, Meschy F. *Study on the Bioavailability of Major and Trace Minerals*. Technical report (2002).
31. IFP. *Evaluating Feed Phosphates*. (2021). Available online at: https://www.feedphosphates.org/index.php/guides/11-guides/13-evaluating-feed-phosphates
32. González-Vega JC, Stein HH. - Invited review - calcium digestibility and metabolism in pigs. *Asian Aust J Anim Sci*. (2014) 27:1–9. doi: 10.5713/ajas.2014.r.01
33. Ross RD, Cromwell GL, Stahly TS. Effects of source and particle size on the biological availability of calcium in calcium supplements for growing pigs. *J Anim Sci*. (1984) 59:125–34. doi: 10.2527/jas1984.591125x
34. Merriman LA, Stein HH. Particle size of calcium carbonate does not affect apparent and standardized total tract digestibility of calcium, retention of calcium, or growth performance of growing pigs. *J Anim Sci*. (2016) 94:3844–50. doi: 10.2527/jas.2015-0252
35. Walk CL, Addo-Chidie EK, Bedford MR, Adeola O. Evaluation of a highly soluble calcium source and phytase in the diets of broiler chickens. *Poultry Sci*. (2012) 91:2255–63. doi: 10.3382/ps.2012-02224
36. Schlegel P, Gutzwiller A. Effect of dietary calcium level and source on mineral utilisation by piglets fed diets containing exogenous phytase. *J Anim Physiol Anim Nutr*. (2017) 101:e165–74. doi: 10.1111/jpn.12582
37. González-Vega JC, Walk CL, Stein HH. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. *J Anim Sci*. (2015) 93:2255–64. doi: 10.2527/jas.2014-8215
38. González-Vega JC, Walk CL, Liu Y, Stein HH. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility. *Arch Anim Nutr*. (2014) 68:126–42. doi: 10.1080/1745039X.2014.892249
39. Garcia RA, Rosentrater KA, Flores RA. Characteristics of North American meat and bone meal relevant to the development of non-feed applications. *Appl Eng Agric*. (2006) 22:729–36. doi: 10.13031/2013.21989
40. Sulabo RC, Stein HH. Digestibility of phosphorus and calcium in meat and bone meal fed to growing pigs. *J Anim Sci*. (2013) 91:1285–94. doi: 10.2527/jas.2011-4632
41. Perez JM, Bories G, Aumaitre A, Barrier-Guillot B, Delaveau A, Gueguen L, et al. Conséquences en élevage et pour le consommateur du remplacement des farines et des graisses animales. *Prod Anim*. (2002) 15:87–96. doi: 10.20870/productions-animales.2002.15.2.3689
42. Traylor SL, Cromwell GL, Lindemann MD. Bioavailability of phosphorus in meat and bone meal for swine. *J Anim Sci*. (2005) 83:1054–61. doi: 10.2527/2005.8351054x
43. Merriman LA, Walk CL, Stein HH. The effect of microbial phytase on the apparent and standardized total tract digestibility of calcium in feed ingredients of animal origin. *J Anim Sci*. (2016) 94(E-Suppl):110. doi: 10.2527/msasas2016-240

44. Jongbloed AW, Kemme PA. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 1. Digestible phosphorus in feedstuffs from plant and animal origin. *Neth J Agric Sci*. (1990). 38:567-575. doi: 10.18174/njas.v38i3B.16579
45. Van Harn J, Spek JW, Van Vuure CA, Van Krimpen MM. Determination of pre-cecal phosphorus digestibility of inorganic phosphates and bone meal products in broilers. *Poultry Sci*. (2017) 96:1334–40. doi: 10.3382/ps/pew458
46. Lee SA, Lagos LV, Stein HH. Digestible calcium and digestible phosphorus in swine diets. In: Smith JH, Eastwood L, editors. *Proceedings of London Swine Clonference.* (2019). p. 63–72.
47. NRC. *Nutrient Requirements of Swine*. Washington, DC: National Academies Press (1998).
48. Petersen GI, Pedersen C, Lindemann MD, Stein HH. Relative bioavailability of phosphorus in inorganic phosphorus sources fed to growing pigs. *J Anim Sci*. (2011) 89:460–6. doi: 10.2527/jas.2009-2161
49. She Y, Li D, Zhang S. Methodological aspects of determining phosphorus digestibility in swine: a review. *Anim Nutr*. (2017) 3:97–102. doi: 10.1016/j.aninu.2017.02.003
50. CVB Centraal Veevoederbureau. *Veevoedertabel*. Lelystad (2000).
51. Liu J, Bollinger DW, Ledoux DR, Veum TL. Effects of dietary calcium:phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum, and colon of pigs. *J Anim Sci*. (2000) 78:106–9. doi: 10.2527/2000.781106x
52. Bohlke RA, Thaler RC, Stein HH. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. *J Anim Sci*. (2005) 83:2396–403. doi: 10.2527/2005.83102396x
53. Dilger RN, Adeola O. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing pigs fed conventional and low-phytate soybean meals. *J Anim Sci*. (2006) 84:627–34. doi: 10.2527/2006.843627x
54. Stein HH, Fuller MF, Moughan PJ, Sève B, Mosenthin R, Jansman AJM, et al. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. *Livestock Sci*. (2007) 109:282–5. doi: 10.1016/j.livsci.2007.01.019
55. Almeida FN, Stein H. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. *J Anim Sci*. (2010) 88:2968–77. doi: 10.2527/jas.2009-2285
56. Fan MZ, Sauer WC. Additivity of apparent ileal and fecal phosphorus digestibility values measured in single feed ingredients for growing-finishing pigs. *Can J Anim Sci*. (2002) 82:183–91. doi: 10.4141/A01-072
57. Fan MZ, Archbold T, Sauer WC, Lackeyram D, Rideout T, Gao Y, et al. Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. *J Nutr*. (2001) 131:2388–96. doi: 10.1093/jn/131.9.2388
58. Petersen GI, Stein HH. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. *J Anim Sci*. (2006) 84:2126–32. doi: 10.2527/jas.2005-479
59. González-Vega JC, Walk CL, Stein HH. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. *J Anim Sci*. (2015) 93:4808. doi: 10.2527/jas.2015-8992
60. Liu JB, Yan HL, Cao SC, Liu J, Zhang HF. Effect of feed intake level on the determination of apparent and standardized total tract digestibility of phosphorus for growing pigs. Anim Feed Sci Technol. (2018) 246:137–43. doi: 10.1016/j.anifeedsci.2018.10.012
61. Anwar MN, Ravindran V, Morel PCH, Ravindran G, Cowieson AJ. Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens using the direct method. *Poultry Sci*. (2016) 95:70–6. doi: 10.3382/ps/pev319
62. Mutucumarana RK, Ravindran V. Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens using the direct method. Anim Feed Sci Technol. (2016) 219:249–56. doi: 10.1016/j.anifeedsci.2016.06.025
63. Merriman LA. *Factors Affecting the Digestibility of Calcium in Feed Ingredients By Pigs*. Univ. Illinois (2016).
64. Zhang F, Adeola O. Techniques for evaluating digestibility of energy, amino acids, phosphorus, and calcium in feed ingredients for pigs. *Anim Nutr*. (2017) 3:344–52. doi: 10.1016/j.aninu.2017.06.008
65. Bikker P, Blok MC. *Phosphorus and Calcium Requirements of Growing Pigs and Sows*. Technical Report (2017).
66. González-Vega JC, Walk CL, Liu Y, Stein HH. Determination of endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs. *J Anim Sci*. (2013) 91:4807–16. doi: 10.2527/jas.2013-6410
67. Liu JB, Cao SC, Chen L, Zhang HF. Effect of dietary phosphorus level on the determination of standardized and true total tract digestibility of phosphorus for growing pigs. *Anim Feed Sci Technol*. (2016) 215:117–23. doi: 10.1016/j.anifeedsci.2016.02.022
68. Stein HH, Adeola O, Cromwell GL, Kim SW, Mahan DC, Miller PS. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. *J Anim Sci*. (2011) 89:2139–44. doi: 10.2527/jas.2010-3522
69. Younes H, Demigné C, Rémésy C. Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat. *Br J Nutr*. (1996) 75:301–14. doi: 10.1079/BJN19960132
70. Rodehutscord M. Determination of phosphorus availability in poultry. *Worlds Poultry Sci J*. (2013) 69:687–98. doi: 10.1017/S0043933913000688
71. Létourneau-Montminy MP, Narcy A, Lescoat P, Magnin M, Bernier JF, Sauvant D, et al. Modeling the fate of dietary phosphorus in the digestive tract of growing pigs. *J Anim Sci*. (2011) 89:3596–611. doi: 10.2527/jas.2010-3397
72. Létourneau-Montminy MP, Jondreville C, Sauvant D, Narcy A. Meta-analysis of phosphorus utilization by growing pigs: effect of dietary phosphorus, calcium and exogenous phytase. *Animal*. (2012) 6:1590–600. doi: 10.1017/S1751731112000560
73. Sauvant D, Schmidely P, Daudin JJ, St-Pierre NR. Meta-analyses of experimental data in animal nutrition. *Animal*. (2008) 2:1203–14. doi: 10.1017/S1751731108002280
74. Sauvant D, Letourneau-Montminy MP, Schmidely P, Boval M, Loncke C, Daniel JB. Review: use and misuse of meta-analysis in animal science. *Animal*. (2020) 14:s207–22. doi: 10.1017/S1751731120001688
75. Jongbloed AW, Mroz Z, Kemme PA. The effect of supplementary Aspergillus niger phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. *J Anim Sci*. (1992) 70:1159–68. doi: 10.2527/1992.7041159x
76. Rapp C, Lantzsch HJ, Drochner W. Hydrolysis of phytic acid by intrinsic plant and supplemented microbial phytase (Aspergillus niger) in the stomach and small intestine of minipigs fitted with re-entrant cannulas. 3. Hydrolysis of phytic acid (IP6) occurrence of hydrolysis products (IP5, IP4, IP3 and IP2. *J Anim Physiol Anim Nutr*. (2001) 85:420–30. doi: 10.1046/j.1439-0396.2001.00337.x
77. Kemme PA, Schlemmer U, Mroz Z, Jongbloed AW. Monitoring the stepwise phytate degradation in the upper gastrointestinal tract of pigs. *J Sci Food Agric*. (2006) 86:612–22. doi: 10.1002/jsfa.2380
78. Adeola O, Olukosi OA, Jendza JA, Dilger RN, Bedford MR. Response of growing pigs to Peniophora lycii- and Escherichia coli-derived phytases or varying ratios of calcium to total phosphorus. *Anim Sci*. (2006) 82:637–44. doi: 10.1079/ASC200676
79. Létourneau-Montminy MP, Narcy A, Magnin M, Sauvant D, Bernier JF, Pomar C, et al. Effect of reduced dietary calcium concentration and phytase supplementation on calcium and phosphorus utilization in weanling pigs with modified mineral status. *J Anim Sci*. (2010) 88:1706–17. doi: 10.2527/jas.2008-1615
80. NRC. *Nutrient Requirements of Poultry: Ninth Revised Edition*. Washington, DC: National Academies Press (1994).
81. FAO, WHO. *Human Vitamin and Mineral Requirements*. Human Vitamin and Mineral Requirements (2001). p. 303.
82. Baker DH. Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. *J Nutr*. (1986) 116:2339–49. doi: 10.1093/jn/116.12.2339
83. Sauveur B, Perez JM. Alimentation minérale des animaux monogastriques. In: *L'alimentation des Animaux Monogastriques: porc, lapin, volailles*. 2e ed. Paris: INRA. (1989). p. 23–31.
84. Jondreville C, Dourmad JY. Phosphorus in pig nutrition. *Prod Anim*. (2006) 18:183–92. doi: 10.20870/productions-animales.2005.18.3.3523

85. Guéguen L, Perez JM. A re-evaluation of recommended dietary allowances of calcium and phosphorus for pigs. *Proc Nutr Soc*. (1981) 40:273–8. doi: 10.1079/PNS19810043
86. Jongbloed AW, Everts H, Kemme PA, Mroz Z. Quantification of absorbability and requirements of macroelements. In: Kyriazakis I, editor. Quantitative Biology of the pig. Wallingford, UK: CAB International (1999). p. 275–98.
87. Gauthier R, Largouët C, Gaillard C, Cloutier L, Guay F, Dourmad JY. Dynamic modeling of nutrient use and individual requirements of lactating sows. *J Anim Sci*. (2019) 97:2822–36. doi: 10.1093/jas/skz167
88. Gaillard C, Gauthier R, Cloutier L, Dourmad JY. Exploration of individual variability to better predict the nutrient requirements of gestating sows. *J Anim Sci*. (2019) 97:4934–45. doi: 10.1093/jas/skz320
89. Couture C, Chiasson R, Pomar C, Letourneau MP. Évolution de la teneur en protéine corporelle et du contenu minéral osseux des porcs charcutiers nourris avec différents niveaux de phosphore et calcium alimentaires. *J Rech Porcine*. (2018) 50:167–8.
90. Sauvant D, Grizard J. Bases d'un modèle décrivant la régulation du métabolisme du glucose de la chèvre en lactation. *Listing*. (1992) 41:115–6. doi: 10.1051/rnd:19920164
91. van Milgen J, Valancogne A, Dubois S, Dourmad JY, Sève B, Noblet J. InraPorc: a model and decision support tool for the nutrition of growing pigs. *Anim Feed Sci Technol*. (2008) 143:387–405. doi: 10.1016/j.anifeedsci.2007.05.020
92. Lautrou M, Létourneau-Montminy MP, Schmidely P, Pomar C. Anatomical and chemical composition of growing pigs at different bodyweights. *Banff Pork Seminar*. (2021).
93. Alexander LS, Qu A, Cutler SA, Mahajan A, Lonergan SM, Rothschild MF, et al. Response to dietary phosphorus deficiency is affected by genetic background in growing pigs. *J Anim Sci*. (2008) 86:2585–95. doi: 10.2527/jas.2007-0692
94. Cloutier L, Pomar C, Létourneau Montminy MP, Bernier JF, Pomar J. Evaluation of a method estimating real-time individual lysine requirements in two lines of growing-finishing pigs. *Animal*. (2015) 9:561–8. doi: 10.1017/S1751731114003073
95. Dersjant-Li Y, Awati A, Schulze H, Partridge G. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *J Sci Food Agric*. (2015) 95:878–96. doi: 10.1002/jsfa.6998
96. Greiner R, Konietzny U. Phytase for food application. *Food Technol Biotechnol*. (2006) 44:125–140.
97. Dersjant-Li Y, Hruby M, Evans C, Greiner R. A critical review of methods used to determine phosphorus and digestible amino acid matrices when using phytase in poultry and pig diets. *J Appl Anim Nutr*. (2019) 7:e2. doi: 10.1017/JAN.2019.1
98. Lei XG, Weaver JD, Mullaney E, Ullah AH, Azain MJ. Phytase, a new life for an "old" enzyme. *Ann Rev Anim Biosci*. (2013) 1:283–309. doi: 10.1146/annurev-animal-031412-103717
99. Augspurger NR, Webel DM, Lei XG, Baker DH. Efficacy of an *E*. coli phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. *J Anim Sci*. (2003) 81:474–83. doi: 10.2527/2003.812474x
100. Vasudevan UM, Krishna S, Jalaja V, Pandey A. Microbial phytase: impact of advances in genetic engineering in revolutionizing its properties and applications. *Bioresource Technol*. (2017) 245:1790–9. doi: 10.1016/j.biortech.2017.05.060
101. van Dijck PWM, Selten GCM, Hempenius RA. On the safety of a new generation of DSM Aspergillus niger enzyme production strains. *Regul Toxicol Pharmacol*. (2003) 38:27–35. doi: 10.1016/S0273-2300(03)00049-7
102. Maenz DD, Engele-Schaan CM, Newkirk RW, Classen HL. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal. *Anim Feed Sci Technol*. (1999) 81:177–92. doi: 10.1016/S0377-8401(99)00085-1
103. Adeola O, Cowieson AJ. BOARD-INVITED REVIEW: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *J Anim Sci*. (2011) 89:3189–218. doi: 10.2527/jas.2010-3715
104. Lei XG, Porres JM. Phytase enzymology, applications, and biotechnology. *Biotechnol Lett*. (2003) 25:1787–94. doi: 10.1023/A:1026224101580
105. Menezes-Blackburn D, Gabler S, Greiner R. Performance of seven commercial phytases in an in vitro simulation of poultry digestive tract. *J Agric Food Chem*. (2015) 63:6142–9. doi: 10.1021/acs.jafc.5b01996
106. Morales GA, Moyano FJ, Marquez L. In vitro assessment of the effects of phytate and phytase on nitrogen and phosphorus bioaccessibility within fish digestive tract. *Anim Feed Sci Technol*. (2011) 170:209–21. doi: 10.1016/j.anifeedsci.2011.08.011
107. Christensen T, Mejldal R, Dersjant-li Y, Debicki-Garnier AM, Messager B. Activité phytasique relative dans une gamme de pH allant résultats d ' une étude in vitro comparant trois phytases. *J Rech Porcine*. (2019) 51:1134.
108. De Jong JA, Woodworth JC, DeRouchey JM, Goodband RD, Tokach MD, Dritz SS, et al. Stability of four commercial phytase products under increasing thermal conditioning temperatures. *Transl Anim Sci*. (2017) 1:255–60. doi: 10.2527/tas2017.0030
109. Eeckhout W, De Paepe M. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Anim Feed Sci Technol*. (1994) 47:19–29. doi: 10.1016/0377-8401(94)90156-2
110. Kornegay ET. Digestion of phosphorus and other nutrients: the role of phytases and factors influencing their activity. In: Bedford MR, Partridge GG, editors. *Enzymes in Farm Animal Nutrition*. Wallingford, UK: CABI Publishing (2001).
111. Garrett JB, Kretz KA, O'Donoghue E, Kerovuo J, Kim W, Barton NR, et al. Enhancing the thermal tolerance and gastric performance of a microbial phytase for use as a phosphate-mobilizing monogastric-feed supplement. *Appl Environ Microbiol*. (2004) 70:3041–6. doi: 10.1128/AEM.70.5.3041-3046.2004
112. Outchkourov N, Petkov S. Phytases for feed applications. In: Vogel A, May O, editors. *Industrial Enzyme Applications*. Weinheim: Wiley-VCH (2013). p. 255–86.
113. Champagne ET, Fisher MS. Binding differences of Zn(II) and Cu(II) ions with phytate. *J Inorg Biochem*. (1990) 38:217–23. doi: 10.1016/0162-0134(90)84014-G
114. Blavi L, Sola-Oriol D, Perez JF, Stein HH. Effects of zinc oxide and microbial phytase on digestibility of calcium and phosphorus in maize-based diets fed to growing pigs. *J Anim Sci*. (2017) 95:847–54. doi: 10.2527/jas2016.1149
115. Augspurger NR, Spencer JD, Webel DM, Baker DH. Pharmacological zinc levels reduce the phosphorus-releasing efficacy of phytase in young pigs and chickens. *J Anim Sci*. (2004) 82:1732–9. doi: 10.2527/2004.8261732x
116. Pang Y, Applegate TJ. Effects of copper source and concentration on in vitro phytate phosphorus hydrolysis by phytase. *J Agric Food Chem*. (2006) 54:1792–6. doi: 10.1021/jf052053b
117. Ren P, Chen J, Wedekind K, Hancock D, Vázquez-A nón M. Interactive effects of zinc and copper sources and phytase on growth performance, mineral digestibility, bone mineral concentrations, oxidative status, and gut morphology in nursery pigs. *Transl Anim Sci*. (2020) 4:783–98. doi: 10.1093/tas/txaa083
118. Ren P, Chen J, Hancock D, Vazquez-A nón M. Interactive effects of copper sources and a high level of phytase in phosphorus-deficient diets on growth performance, nutrient digestibility, tissue mineral concentrations, and plasma parameters in nursery pigs. *Biol Trace Elem Res*. (2021) 199:4582–92. doi: 10.1007/s12011-021-02580-x
119. Richards JD, Zhao J, Harrell RJ, Atwell CA, Dibner JJ. Trace mineral nutrition in poultry and swine. *Asian Aust J Anim Sci*. (2010) 23:1527–34. doi: 10.5713/ajas.2010.r.07
120. Cheryan M, Rackis JJ. Phytic acid interactions in food systems. *Crit Rev Food Sci Nutr*. (1980) 13:297–335. doi: 10.1080/10408398009527293
121. Cowieson AJ, Wilcock P, Bedford MR. Super-dosing effects of phytase in poultry and other monogastrics. *Worlds Poultry Sci J*. (2011) 67:225–35. doi: 10.1017/S0043933911000250
122. Walk CL. The influence of calcium on phytase efficacy in non-ruminant animals. *Anim Prod Sci*. (2016) 56:1345. doi: 10.1071/AN15341
123. Lei XG, Ku PK, Miller ER, Yokoyama MT, Ullrey DE. Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean meal diets of weanling pigs1. *J Anim Sci*. (1994) 72:139–43. doi: 10.2527/1994.721139x
124. Qian H, Kornegay ET, Conner DEJ. Adverse effects of wide calcium:phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. *J Anim Sci*. (1996) 74:1288–97. doi: 10.2527/1996.7461288x

125. Liu J, Bollinger DW, Ledoux DR, Veum TL. Lowering the dietary calcium to total phosphorus ratio increases phosphorus utiliza- tion in low-phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. *J Anim Sci*. (1998) 76:808–13. doi: 10.2527/1998.763808x
126. Driver JP, Pesti GM, Bakalli RI, Edwards HM. Effects of calcium and nonphytate phosphorus concentrations on phytase efficacy in broiler chicks. *Poultry Sci.* (2005) 84:1406–17. doi: 10.1093/ps/84.9.1406
127. Wu F, Tokach MD, Dritz SS, Woodworth JC, Derouchey JM, Goodband RD, et al. Effects of dietary calcium to phosphorus ratio and addition of phytase on growth performance of nursery pigs. *J Anim Sci*. (2018) 96:1825–37. doi: 10.1093/jas/sky101
128. Underwood EJ, Mertz W. Introduction. In: *Trace Elements in Human and Animal Nutrition. 5th rev. ed*. New York, NY: Academic Press (1987). p. 1–19.
129. Stauffer M, Baylink D, Wergedal J, Rich C. Bone repletion in calcium deficient rats fed a high calcium diet. *Calcif Tissue Res*. (1972) 9:163–72. doi: 10.1007/BF02061954
130. Stauffer M, Baylink D, Wergedal J, Rich C. Decreased enhanced bone formation, mineralization, and enhanced resorption and in calcium-deficient rats. *Am J Physiol*. (1973) 225:163–72. doi: 10.1152/ajplegacy.1973.225.2.269
131. Drivdahl RH, Liu CC, Baylink DJ. Regulation of bone repletion in rats subjected to varying low-calcium stress. *Am J Physiol*. (1984) 246:R190–6. doi: 10.1152/ajpregu.1984.246.2.R190
132. Åkesson K, Lau KHW, Johnston P, Imperio E, Baylink DJ. Effects of short-term calcium depletion and repletion on biochemical markers of bone turnover in young adult women. *J Clin Endocrinol Metab*. (1998) 83:1921–7. doi: 10.1210/jc.83.6.1921
133. Goff JP. Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. *Anim Feed Sci Technol*. (2006) 126:237–57. doi: 10.1016/j.anifeedsci.2005.08.005
134. Friggens NC, Andersen JB, Larsen T, Aaes O, Dewhurst RJ. Priming the dairy cow for lactation: a review of dry cow feeding strategies. *Anim Res*. (2004) 53:453–73. doi: 10.1051/animres:2004037
135. Kasper C, Schlegel P, Ruiz-Ascacibar I, Stoll P, Bee G. Accuracy of predicting chemical body composition of growing pigs using dual-energy X-ray absorptiometry. *bioRxiv*. (2020). 1–24. doi: 10.1101/2020.09.15.286153
136. Gonzalo E, Létourneau-Montminy MP, Narcy A, Bernier JF, Pomar C. Consequences of dietary calcium and phosphorus depletion and repletion feeding sequences on growth performance and body composition of growing pigs. *Animal*. (2018) 12:1165–73. doi: 10.1017/S1751731117002567
137. Yan F, Angel R, Ashwell C, Mitchell A, Christman M. Evaluation of the broiler's ability to adapt to an early moderate deficiency of phosphorus and calcium. *Poultry Sci*. (2005) 84:1232–41. doi: 10.1093/ps/84.8.1232
138. Ashwell CM, Angel R. Nutritional genomics: a practical approach by early life conditioning with dietary phosphorus. *Rev Brasileira de Zootecnia*. (2010) 39(Suppl. spe):268–78. doi: 10.1590/S1516-35982010001300030
139. Rousseau X, Valable AS, L'Etourneau-Montminy MP, Meme N, Godet E, Magnin M, et al. Metabolism and nutrition: adaptive response of broilers to dietary phosphorus and calcium restrictions. *Poultry Sci*. (2016) 95:2849–60. doi: 10.3382/ps/pew172
140. Valable AS, Narcy A, Duclos MJ, Pomar C, Page G, Nasir Z, et al. Effects of dietary calcium and phosphorus deficiency and subsequent recovery on broiler chicken growth performance and bone characteristics. *Animal*. (2018) 12:1555–63. doi: 10.1017/S1751731117003093
141. Valable AS, Létourneau-Montminy MP, Klein S, Lardic L, Lecompte F, Metayer-Coustard S, et al. Early-life conditioning strategies to reduce dietary phosphorus in broilers: underlying mechanisms. *J Nutr Sci*. (2020) 9:e28. doi: 10.1017/jns.2020.17
142. Ryan WF, Lynch PB, O'Doherty JV. Compensatory effect of dietary phosphorus on performance of growing pigs and development of bone mineral density assessed using dual energy X-ray absorptiometry. *Livestock Sci*. (2011) 138:89–95. doi: 10.1016/j.livsci.2010.12.006
143. Lautrou M, Pomar C, Schmidely P, Laval U, Mosar UMR, Paris-saclay U, et al. Effets d'une déplétion-réplétion en phosphore et calcium sur les performances et la minéralisation osseuse des porcs en croissance. *J Rech Porcine*. (2021) 53:163–8.
144. Nussey SS, Whitehead SA. Endocrinology, An Integrated Approach. Oxford, U.K: BIOS Scientific (2001).
145. Gonzalo E. *Consequences of a dietary phosphorus and calcium depletion and repletion strategy in growing-finishing pigs* (Ph.D. thesis) (2017).
146. Schanler RJ, Abrams SA, Sheng HP. Calcium and phosphorus deficiencies affect mineral distribution in neonatal miniature piglets. *Am J Clin Nutr*. (1991) 54:420–4. doi: 10.1093/ajcn/54.2.420
147. Mitchell AD, Scholz AM, Pursel VG. Total body and regional measurements of bone mineral content and bone mineral density in pigs by dual energy X-ray absorptiometry. *J Anim Sci*. (2001) 79:2594–2604. doi: 10.2527/2001.79102594x
148. Schröder B, Schlumbohm C, Kaune R, Breves G. Role of calbindin-D9k in buffering cytosolic free Ca2+ ions in pig duodenal enterocytes. *J Physiol*. (1996) 492:715–22. doi: 10.1113/jphysiol.1996.sp021340
149. Allen MJ. Biochemical markers of bone metabolism in animals: uses and limitations. *Vet Clin Pathol*. (2003) 32:101–13. doi: 10.1111/j.1939-165X.2003.tb00323.x
150. Lagos LV, Lee SA, Bedford MR, Stein HH. Formulation of diets for pigs based on a ratio between digestible calcium and digestible phosphorus results in reduced excretion of calcium in urine without affecting retention of calcium and phosphorus compared with formulation based on values for total. *J Anim Sci*. (2021) 99:1–7. doi: 10.1093/jas/skab138
151. Pomar C, Hauschild L, Zhang GH, Pomar J, Lovatto PA. Applying precision feeding techniques in growing-finishing pig operations. *Rev Brasileira de Zoot*. (2009) 38:226–37. doi: 10.1590/S1516-35982009001300023
152. Andretta I, Pomar C, Rivest J, Pomar J, Lovatto PA, Radünz Neto J. The impact of feeding growing–finishing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition1. *J Anim Sci*. (2014) 92:3925–36. doi: 10.2527/jas.2014-7643

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Comparison of Gut Microbiota and Metabolic Status of Sows With Different Litter Sizes During Pregnancy

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The experiment was conducted to compare the differences of gut microbiota and metabolic status of sows with different litter sizes on days 30 and 110 of gestation, and uncover the relationship between the composition of maternal gut microbiota during gestation and sow reproductive performance. Twenty-six Large White × Landrace crossbred multiparous sows (2nd parity) with similar back fat thickness and body weight were assigned to two groups [high-reproductive performance group (HP group) and low-reproductive performance group (LP group)] according to their litter sizes and fed a common gestation diet. Results showed that compared with LP sows, HP sows had significantly lower plasma levels of triglyceride (TG) on gestation d 30 ($P < 0.05$), but had significantly higher plasma levels of TG, non-esterified fatty acid, tumor necrosis factor-α, and immunoglobulin M on gestation d 110 ($P < 0.05$). Consistently, HP sows revealed increased alpha diversity and butyrate-producing genera, as well as fecal butyrate concentration, on gestation d 30; HP sows showed significantly different microbiota community structure with LP sows ($P < 0.05$) and had markedly higher abundance of Firmicutes (genera *Christensenellaceae_R-7_group* and *Terrisporobacter*) which were positively related with litter size on gestation d 110 than LP sows ($P < 0.05$). In addition, plasma biochemical parameters, plasma cytokines, and fecal microbiota shifted dramatically from gestation d 30 to d 110. Therefore, our findings demonstrated that microbial abundances and community structures differed significantly between sows with different litter sizes and gestation stages, which was associated with changes in plasma biochemical parameters, inflammatory factors, and immunoglobulin. Moreover, these findings revealed that there was a significant correlation between litter size and gut microbiota of sows, and provided a microbial perspective to improve sow reproductive performance in pig production.

Keywords: gestation stage, gut microbiota, litter size, metabolic status, reproductive performance, sow

INTRODUCTION

Diverse microbial communities reside at various sites within a mammalian body (1, 2). Gut microbiota makes up the vast majority of body's microbes and with an estimated number of several trillion most probably outnumber human body cells (3). The gut microbiota is shaped by many environmental factors, such as host genetics (4), diet (5), and the immune system (6), and has been reported to play a vital role in inflammation, metabolic syndrome (7), energy metabolism (8), and immunity (9).

Previous study in humans showed that the body experiences extensive hormonal, metabolic, and immunological changes over the course of normal and healthy pregnancy (10), accompanied by dramatic changes in maternal gut microbiota (11). Koren et al. (10) showed normal pregnancy to be accompanied by a profound change of gut microbiota from the first to the third trimester with an increase in the Proteobacteria and Actinobacteria abundances which might be connected with the maternal metabolic profile. Uryu et al. (12) demonstrated that sow productivity on different farms was likely related to changes in fecal microbe composition. Besides, research showed that dietary probiotic supplementation in gestating sow diet could increase the number of piglets total born (13, 14). Further, Al-Asmakh et al. (15) found that maternal microbiota could regulate placental development and then might affect the development of the growing offspring in mice. This research suggests that maternal gut microbiota during gestation is affecting sow reproductive performance. However, there is little literature available about whether the composition of gut microbiota during gestation is associated with improved sow reproductive performance.

The early and late pregnancy are two critical stages for embryonic survival and development (16, 17). In the present study, we aimed to explore the relationship between reproductive performance and maternal gut microbiota during gestation through comparing the fecal microbiota characteristics and metabolic status of sows with high (>12 piglets per litter) and low litter size (≤12 piglets per litter) on day 30 of gestation (G30) and on day 110 of gestation (G110).

MATERIALS AND METHODS

Ethical Approval

This study was conducted at the pig breeding farm in Shandong Province. The animal use protocol for this research was approved by the Animal Care and Use Committee of Shandong Agricultural University (Approval Number: SDAUA-2019-019).

Animals and Experimental Design

Twenty-six Large White × Landrace crossbred multiparous sows (2nd parity) with similar back fat thickness (BF, 15.28 ± 0.45 mm) and body weight (174.34 ± 2.72 kg) were used in this study. The BF at the last rib was measured using a HG 9300 digital diagnostic ultrasound device (Caresono Technology Co. Ltd., Nanjing, China). After artificial insemination, the individual sow was housed individually in a gestation stall (2.37 × 0.65 × 1.13 m) kept at 21 ± 1°C. All the sows were mated within 3 days and fed a common fortified corn–soybean meal gestation diet (**Supplementary Table 1**) which was formulated to meet or exceed National Research Council (18) nutrient requirements. All sows received a daily meal at 0900 h and were fed the same amount of feed (days 1 to 89 of gestation 2.46 kg/d; days 90 of gestation to farrowing, 2.89 kg/d) during the entire gestation. On day 110 of gestation, sows were moved from gestation to farrowing rooms and kept in individual farrowing crates measuring 2.40 × 1.80 × 0.90 m thereafter. Backfat thickness and body weight of individual sow were measured at breeding and within 24 h of farrowing. At farrowing, the numbers of total born piglets, live born piglets, and dead born piglets per litter, as well as litter weight, were recorded, and the averages were calculated. Thus, two groups were generated (**Table 1**): 13 sows with litter size lower than the average in this trial (12.7 piglets) were classified as the low-reproductive performance group (LP group), while 13 sows with litter size higher than the average in this trial (12.7 piglets) labeled as the high-reproductive performance group (HP group). Sows had free access to water throughout the experiment and did not receive vaccine, antibiotics, or other medication in the feed or for any therapeutic purposes after insemination.

TABLE 1 | Litter size and litter weight in low- and high-reproductive performance groups.

Items	Group[a]		*P*-value
	LP	HP	
No. of sows	13	13	-
Backfat thickness, mm			
Breeding	15.33 ± 0.74	14.84 ± 0.53	0.599
Farrowing	19.16 ± 0.72	17.82 ± 0.77	0.218
Body weight, kg			
Breeding	179.32 ± 4.34	175.31 ± 4.18	0.631
Farrowing	238.77 ± 3.50	231.84 ± 3.91	0.199
Litter size			
Total born	9.77 ± 0.53	15.54 ± 0.66	<0.001
Born alive	9.46 ± 0.57	14.31 ± 0.61	<0.001
Dead	0.31 ± 0.13	1.23 ± 0.30	0.013
Litter weight, kg			
Total born	16.55 ± 0.64	22.16 ± 0.84	<0.001
Born alive	16.03 ± 0.66	21.21 ± 0.83	<0.001

[a]LP, Sows in low-Reproductive Performance Group; HP, Sows in High-Reproductive Performance Group.
Values are mean ± standard error (n = 13).

Sample Collection

Fasting blood samples (12 h overnight) and fresh fecal samples from all healthy sows were collected on day 30 and day 110 of gestation before feeding in the morning. Samples were grouped as follows: LP30 and LP110: sows with low-reproductive performance on day 30 and day 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on day 30 and day 110 of gestation, respectively. Blood samples (5 mL) from the ear veins were collected into a tube containing heparin sodium and centrifuged at 3,000 × g for 15 min. Plasma samples was transferred to 200 μL centrifuge tubes and stored at −20°C until analysis. Fecal samples (about 5 g) were collected from the rectum by a sterilized fecal collection tube and then stored at −80°C immediately for the detection of short-chain fatty acids (SCFAs) and analysis of microbiota.

Plasma Biochemical Parameters Analysis

Plasma biochemical parameters, including glucose (GLU), cholesterol (CHOL), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and non-esterified fatty acid (NEFA), were determined with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) using standard spectrophotometric methods on an Autolab-PM4000 Automatic Analyzer (AMS Co., Rome, Italy) as previously described (19).

Analysis of Inflammatory Factors, Immunoglobulins, and Reproductive Hormones

Concentrations of interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), progesterone, estrogen, lutropin, and prolactin in the plasma of sows were determined with commercial ELISA kits (Feiya Biotechnology Co. Ltd., Yancheng, China) as described in **Supplementary Methods**.

Determination of Fecal SCFAs

The fecal SCFAs of sows were measured by a Varian CP-3800 gas chromatograph (Palo Alto, CA, USA) equipped with a micro-injector, a flame ionization detector, and a capillary chromatographic column as described in **Supplementary Methods**.

Microbial Analysis

Microbial composition and diversity were analyzed as previously described in Li et al. (20). Briefly, bacterial genomic DNA was extracted from frozen fecal samples with an E.Z.N.A.^{TM} Stool DNA kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocol. After DNA concentration and purity monitoring, DNA was diluted to 1 ng/μL using sterile water, and the V4 hypervariable region of 16S rDNA was amplified with 515F and 806R primer (5′-GTGCCAGCMGCCGCGGTAA-3′ and 5′-GGACTACHVGGGTWTCTAAT-3′, respectively), on the Illumina HiSeq PE2500 platform by Novogene (Beijing, China). Filtered, non-chimeric high-quality sequences (tags) sharing over 97% sequence similarity were clustered into the same operational taxonomic units (OTUs) by Uparse software (21), and then classified to different taxonomic levels with SILVA database (22) based on Mothur algorithm to annotate taxonomic information. Operational taxonomic units abundance information were normalized using a standard of sequence number corresponding to the sample with the least sequences for subsequent analysis of alpha diversity and beta diversity. Shannon, Simpson, Chao 1, and ACE indexes were chosen to ascertain differences in alpha diversity based on different groups (23, 24), and Bray-Curtis distances were calculated and visualized using Principal Coordinate Analysis (PCoA) (25). The statistical differences in alpha and beta diversity of bacterial communities between the two groups were examined using the Wilcoxon rank-sum test. Significant difference among the microbial communities was accessed with the analysis of similarity (ANOSIM) test.

Statistical Analysis

The individual sow was regarded as the experimental unit for all variables. Differences in the data including plasma biochemical parameters, inflammatory factors, and fecal SCFAs were evaluated using the independent *t*-test (LP vs. HP) or paired *t*-test (G30 vs. G110) procedure of SAS 9.0 (Institute Inc., Cary, NC, USA) following normal distribution assessment using a Shapiro-Wilk's statistic ($W > 0.05$). Multiple testing was corrected by using the Benjamini-Hochberg false discovery rate. Spearman's correlations were used to assess the associations between bacterial abundance and litter size, as well as plasma biochemical indices. Treatment differences were considered statistically significant at $P < 0.05$, and $0.05 \leq P < 0.10$ was considered a statistical trend. Values are expressed as mean ± standard error in tables and figures.

RESULTS

Changes of Fecal Microbial Diversity

A total of 4,392,562 total tags, 4,118,486 taxon tags, and 274,447 unique tags were obtained from 52 sow fecal samples, with an average of 84,472 ± 730, 79,202 ± 720 and 5,278 ± 179 per sample, respectively (**Figures 1A–C**). Based on 97% sequence similarity, a total of 21,114 OTUs were found in the HP group on day 30 of gestation, with an significantly higher average of 1,624 ± 10 OUT per sample compared to an average of 1,589 ± 12 OUT per sample in the LP group, where 20,657 OTUs were found in total (**Figure 1D**); the HP group tended had lower unique tags than the LP group on day 110 of gestation (5,500 ± 248 vs. 6,397 ± 389; $P = 0.064$; **Figure 1C**). In addition, from gestation d 30 to d 110, the unique tags number was significantly increased on average ($P < 0.05$), but OTUs number was decreased ($P = 0.047$).

To determine whether the sample size was sufficient for OUT testing, the species accumulation curves (SAC) was used in the present study. The SAC (**Supplementary Figure 1**) tended to flatten as the sample number of analyzed sequences increased up to 52, suggesting that the sample size was enough for OTUs testing and could estimate the species richness of the habitat.

The results of fecal microbial community structures assessment are shown in **Figure 2**. On d 30 of gestation, the HP group had significantly higher Shannon index ($P < 0.05$) and tended to have a higher Chao 1 index compared with LP group ($P = 0.069$); on d 110 of gestation, no significant differences were observed in alpha diversity between the two groups ($P > 0.05$).

In addition, to measure the evolutionary distance between microbiotas (beta diversity), the PCoA profile for sow fecal samples based on the Bray-Curtis distance was used in the present study, and ANOSIM test was used to assess significant differences among the microbial communities (**Figures 3A,B**). The results suggested that LP and HP groups had close distance

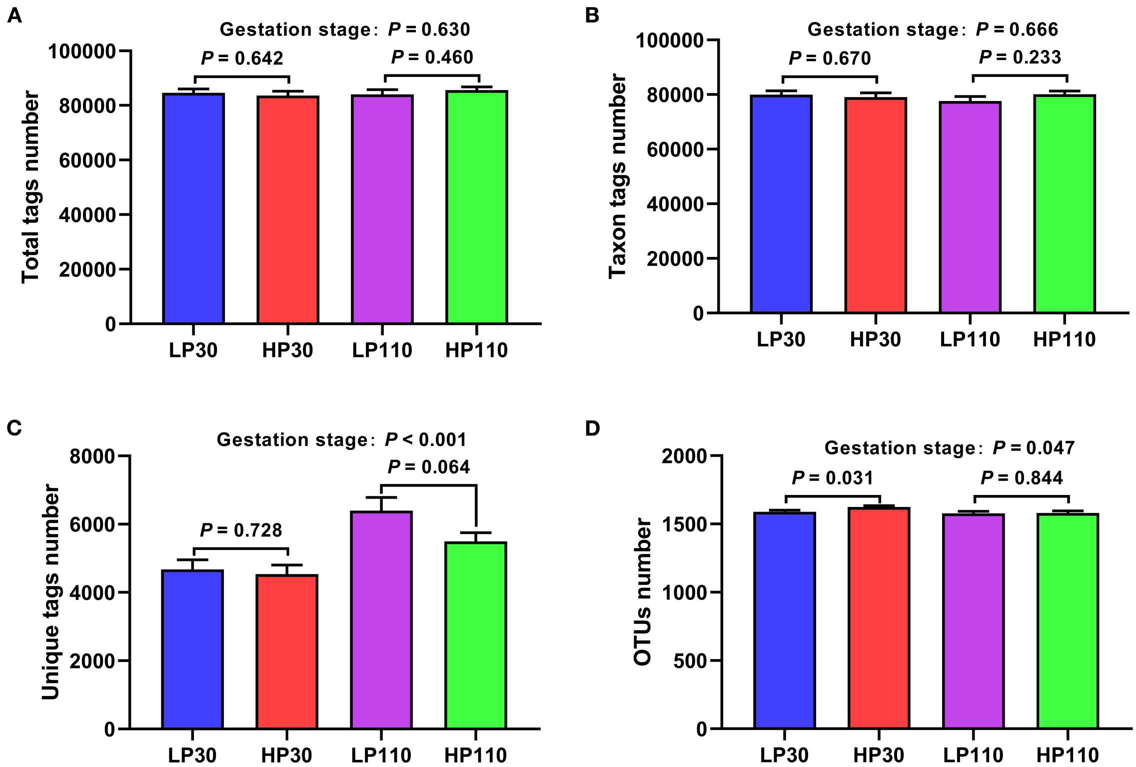

FIGURE 1 | Operational taxonomic unit (OUT) clustering and annotation of sow fecal samples on d 30 and d 110 of gestation. **(A)** Total tags number; **(B)** taxon tags number; **(C)** unique tags number; **(D)** OTUs number. LP30 and LP110: sows with low-reproductive performance on d 30 and d 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on d 30 and d 110 of gestation, respectively. Gestation stage: difference in the variations between gestation d 30 and d 110. Values are mean ± standard error (n = 13).

on gestation d 30 which showed that the two groups had no significant difference in the microbial community ($P = 0.189$, **Figure 3C**); an obvious separation was observed in PCoA between samples from HP group and LP group on d 110 of gestation. The ANOSIM test also indicated that the two groups had notably different microbiota structures on gestation d 110 ($P = 0.006$, **Figure 3D**), and sows in HP group had greater beta diversity compared to sows in LP group at 110 days of gestation ($P < 0.05$). Besides, the Bray-Curtis distance analysis showed a global shift in microbial community composition from gestation d 30 to d 110 ($P = 0.001$, **Figures 3B,E**).

Changes in Relative Abundance of Phyla and Genera

As shown in **Figure 4**, Firmicutes and Bacteroidetes were the most predominant phyla which accounted for more than 75%, followed by Spirochaetes and Tenericutes, in both groups during gestation (**Figure 4A**). No significant differences were observed in the top 10 phyla which accounted for more than 99.5% of the total bacteria population between the LP and HP groups on d 30 of gestation ($P > 0.05$, **Figure 5A**). However, the relative abundance of Firmicutes in the HP groups was significantly higher ($P < 0.05$) than that in the LP group, while the relative abundances of Bacteroidetes, Spirochaetes, and Fibrobacteres were significantly lower ($P < 0.05$) than that in the LP group on d 110 of gestation. In addition, the relative abundances of Firmicutes and Actinobacteria were significantly decreased ($P < 0.05$), while Bacteroidetes and Verrucomicrobia were significantly increased from gestation d 30 to d 110 ($P < 0.05$).

The species phylogenetic tree evolution was constructed by multiple sequences alignments to obtain the representative sequence of the top 35 genera. As shown in **Figure 4B**, the relative abundance of Firmicutes was contributed by *Clostridium_sensu_stricto_1*, *Streptococcus*, *Lactobacillus*, *Christensenellaceae_R-7_group*, *Ruminococcaceae_UCG-002*, *Ruminococcaceae_NK4A214_group*, *Ruminococcaceae_UCG-005*, *Ruminococcaceae_UCG-014*, *Ruminococcus_1*, and *Lachnospiraceae*_XPB1014_group; Bacteroidetes mainly distributed with *Rikenellaceae_RC9_gut_group*, *Prevotellaceae_UCG-001,* and *Prevotellaceae_NK3B31_group*; Spirochaetes was dominated by *Treponema_2*. In the LP and HP groups, *Treponema_2* and *Clostridium_sensu_stricto_1* were the top two genera on d 30 of gestation, and *Treponema_2* and *Streptococcus* were the most dominant on d 110 of gestation. Of the top 35 genera, compared with sows in the LP group, sows in the HP group had significantly higher ($P < 0.05$) relative abundances of *Eubacterium_coprostanoligenes_group*,

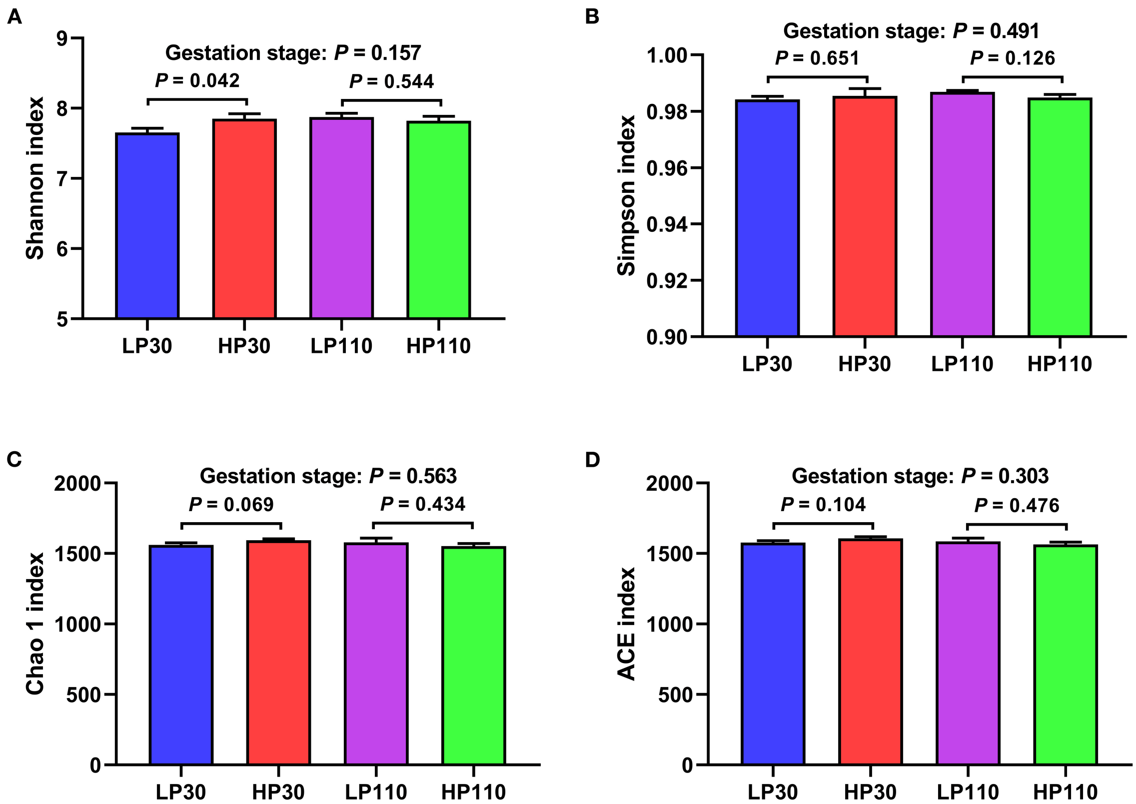

FIGURE 2 | Difference on bacteria community diversity and richness among different groups on d 30 and 110 of gestation. **(A)** Shannon index; **(B)** Simpson index; **(C)** Chao 1 index; **(D)** ACE index. LP30 and LP110: sows with low-reproductive performance on d 30 and 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on d 30 and 110 of gestation, respectively. Gestation stage: difference in the variations between gestation d 30 and 110. Values are mean ± standard error ($n = 13$).

Lachnospiraceae_XPB1014_group, *Sphaerochaeta*, *Ruminococcaceae_UCG-010*, *Roseburia*, *Ruminococcaceae_UCG-002,* and *Family_XIII_AD3011_group*, and significantly lower ($P < 0.05$) *Succinvibrio* on d 30 of gestation; sows in the HP group had significantly lower ($P < 0.05$) *Treponema_2*, *Prevotellaceae_UCG-001*, *Prevotella_1* and *dgA-11_gut_group*, as well as significantly higher ($P < 0.05$) *Lactobacillus*, *Christensenellaceae_R-7_group*, *Terrisporobacter,* and *Escherichia-Shigella* on d 110 of gestation (**Figure 5B**). Besides, sow fecal samples from gestation d 110 had significantly higher abundances of *Streptococcus*, *Prevotellaceae_NNK3B31_group*, *Oscillospira*, *Eubacterium_coprostanoligenes_group,* and *Ruminococcaceae_UCG-010*, but significantly lower *Prevotellaceae_UCG-001*, *Terrisporobacter*, *Escherichia-Shigella*, *Ruminococcaceae_NK4A214_group*, *Christensenellaceae_R-7_group,* and *Romboutsia* than those of sow fecal samples from gestation d 30 ($P < 0.05$).

Correlation Analysis Between Sow Reproductive Performance and Fecal Microbiota

On day 30 of gestation, at the phylum level, the relative abundances of Firmicutes and Actinobacteria tended to be positively correlated with litter size ($P < 0.10$), while the relative abundance of Proteobacteria tended to be negatively correlated with litter size ($P < 0.10$); at the genus level, the relative abundances of *Turicibacter* and *Ruminococcaceae_UCG-014* had significant positive correlations with litter size ($P < 0.05$), and the relative abundances of *Clostridium_sensu_stricto_1* and *Romboutsia* tended to be positively correlated with litter size ($P < 0.10$, **Table 2**).

On d 110 of gestation, at the phylum level, significant positive correlation between the relative abundance of Firmicutes and litter size was observed ($P < 0.05$), and the relative abundances of Bacteroidetes, Spirochaetes, and Actinobacteria were all significantly negatively correlated with litter size ($P < 0.05$); at the genus level, *Clostridium_sensu_stricto_1*, *Turicibacter*, *Terrisporobacter*, *Christensenellaceae_R-7_group,* and *Escherchia-Shigella* exhibited the significantly positive correlations with litter size ($P < 0.05$), while *Rikenellaceae_RC9_gut_group*, *Treponema_2*, and *Sphaerochaeta* had significant negative correlations with litter size ($P < 0.05$). In addition, the phylum Proteobacteria and genus *Lactobacillus* displayed a tendency to be positively correlated with litter size ($P < 0.10$), and the genus *Sphaerochaeta* tended to be negatively correlated with litter size ($P < 0.10$).

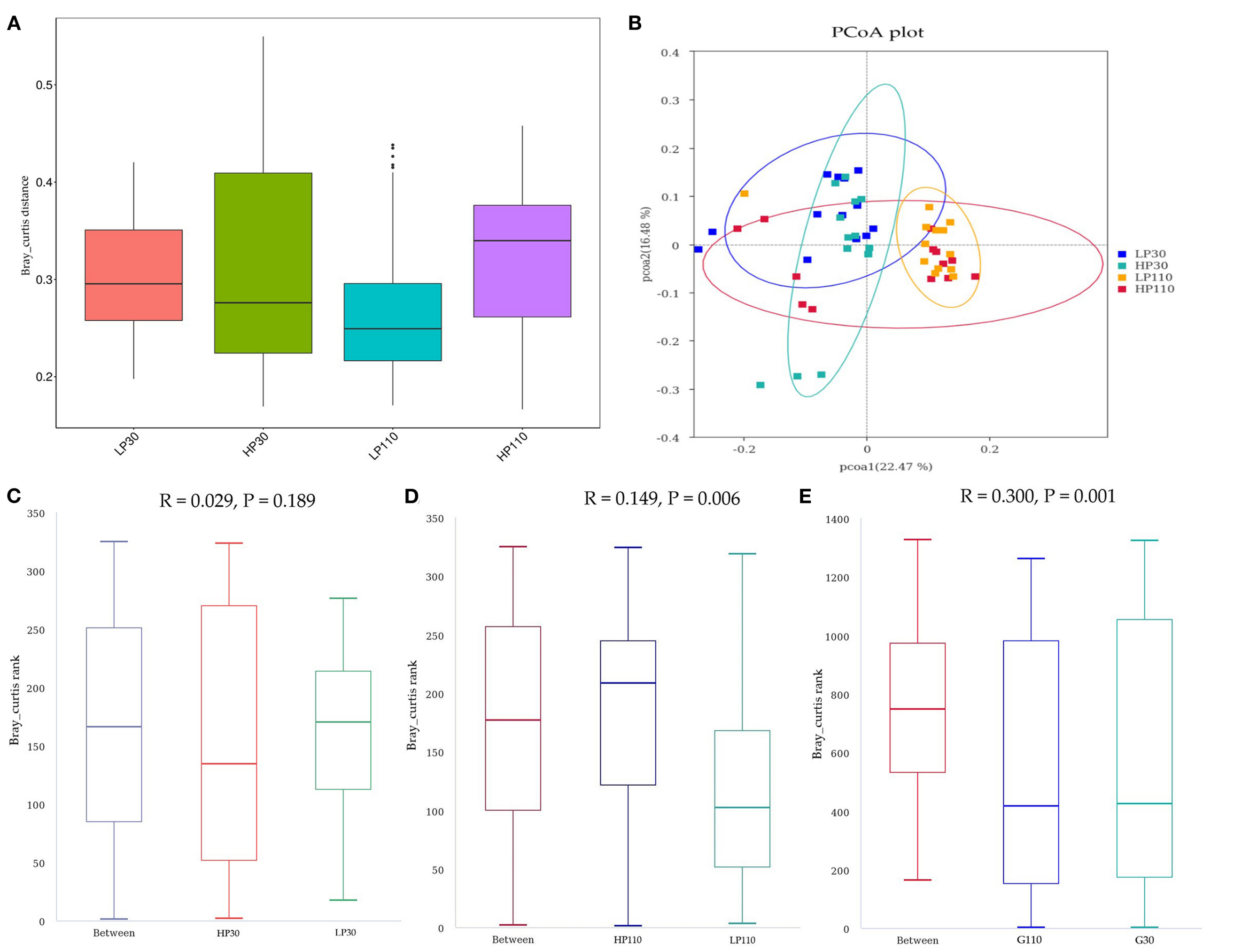

FIGURE 3 | The beta diversity of microbial communities in the groups on d 30 and d 110 of gestation. **(A)** The Bray-Curtis distance within each group. **(B)** The principal coordinate analysis (PCoA) profile of the two groups displayed with the Bray-Curtis distance. Each dot represents one sample from each group. The percent variation explained by each principal coordinate is indicated on the X and Y axis. **(C–E)** Analysis of similarity (ANOSIM). R value is scaled to lie between −1 and +l. Generally, $0 < R < 1$ and $P < 0.05$ represents that there were significant differences between the groups. LP30 and LP110: sows with low-reproductive performance on d 30 and d 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on d 30 and d 110 of gestation, respectively. $n = 13$ for each group.

Changes of Fecal SCFAs Concentrations During Gestation

The concentrations of fecal short-chain fatty acids on d 30 and d 110 of gestation in the two groups are listed in **Figure 6**. On d 30 of gestation, there were no significant differences in fecal acetate, propionate, and total SCFAs concentrations between the LP group and HP group ($P > 0.05$), but sows from HP group showed significantly higher butyrate concentration than those of sows from LP group ($P < 0.05$). On d 110 of gestation, sows in the HP group had significantly lower acetate and total SCFAs concentrations than sows in the LP group ($P < 0.05$), and the propionate concentration in the HP group tended to be lower than that in the LP group ($P = 0.053$). The fecal SCFAs concentrations of sows on d 110 of gestation did not differ with that of sows on d 30 of gestation ($P > 0.05$).

Changes in Plasma Metabolites During Gestation

As shown in **Figure 7**, on day 30 of gestation, significantly lower plasma TG levels were observed in the HP group compared with those in the LP group ($P < 0.05$); sows in the HP group tended to have a lower plasma GLU concentration than sows in the LP group ($P = 0.070$). On d 110 of gestation, sows in the HP group had significantly higher plasma TG and NEFA levels ($P < 0.05$) and tended to have lower plasma HDL-C concentration compared with those of sows in the LP group ($P = 0.055$). Besides, the concentrations of CHOL ($P = 0.018$), HDL-C ($P = 0.085$), and LDL-C ($P = 0.061$) were decreased, and the levels of TG ($P = 0.020$) were increased from gestation d 30 to d 110.

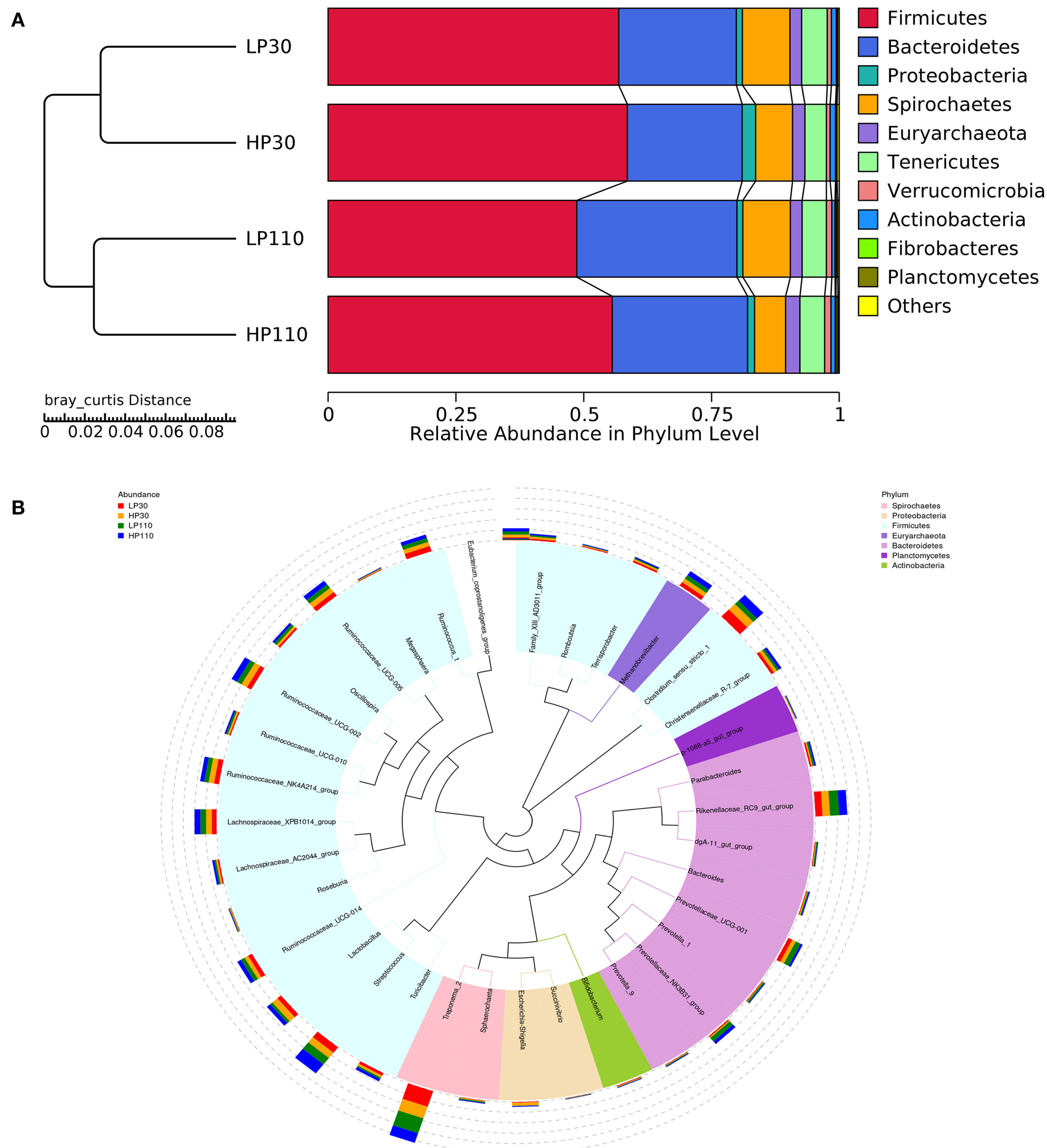

FIGURE 4 | Changes of the relative abundance at phylum and genus levels. **(A)** Unweighted pair-group method with arithmetic mean (UPGMA) clustering analysis with the Bray-Curtis distance. The left panel shows the phylogenic tree, and the right panel displays the relative abundance of each group at the phylum level. **(B)** The phylogenetic tree constructed based on the sequence of the top 35 genera. The branches with different colors in the inner circle represent their corresponding phylum, and the stacked column chart in the outer circle indicates the relative abundance of each genus in different treatments. LP30 and LP110: sows with low-reproductive performance on d 30 and 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on d 30 and 110 of gestation, respectively. $n = 13$ for each group.

Changes of Plasma Inflammatory Factors and Immunoglobulins During Gestation

The levels of plasma inflammatory factors and immunoglobulins are shown in **Figure 8**. There were no significant differences in the plasma concentrations of inflammatory factors and immunoglobulins on d 30 of gestation between the LP and HP groups ($P > 0.05$). However, significantly higher TNF-α and IgM concentrations were observed in the HP group compared with those in the LP group on d 110 of gestation ($P < 0.05$). In addition, the plasma IL-6 and IL-10 levels were significantly increased ($P < 0.05$) from gestation d 30 to d 110.

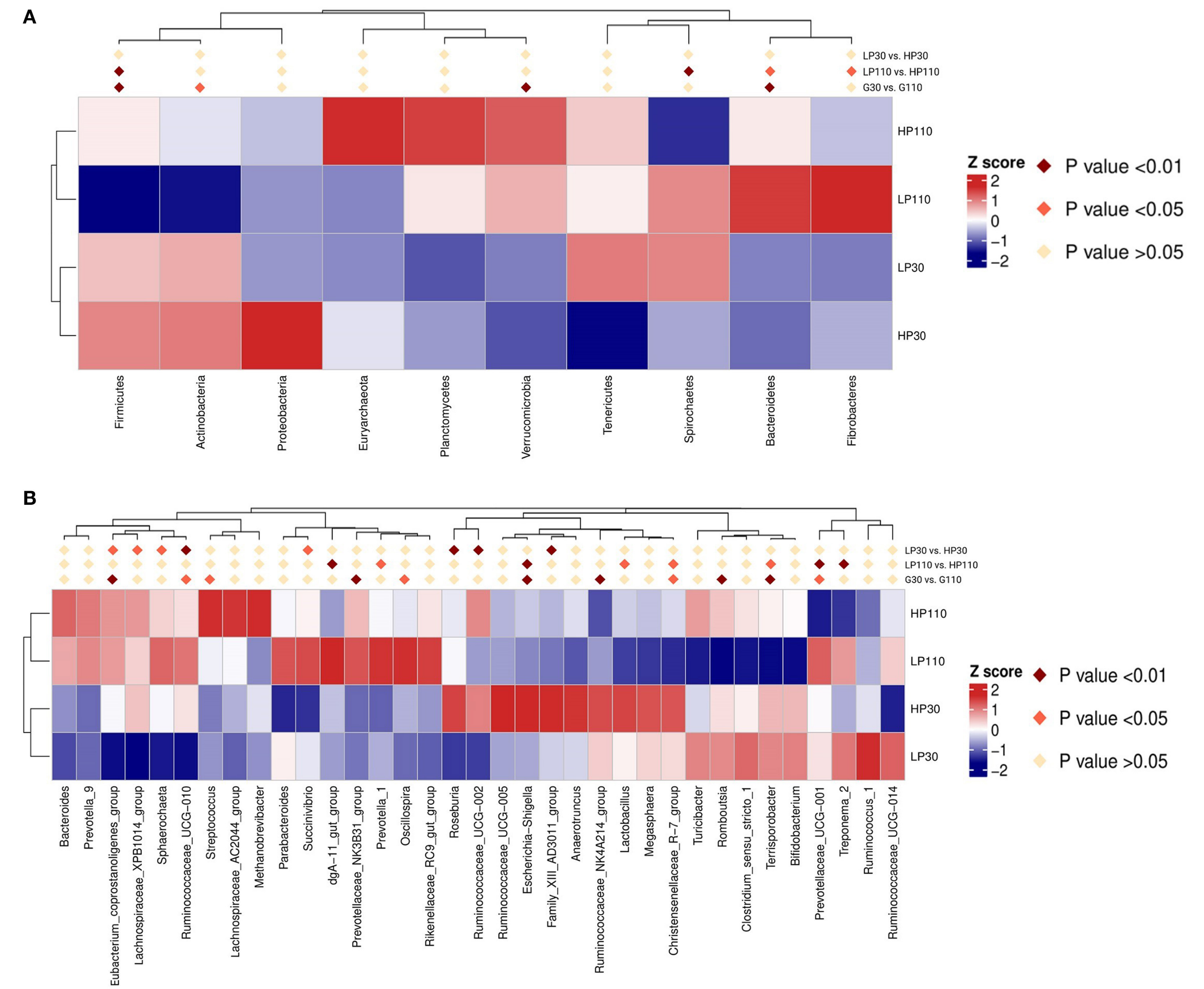

FIGURE 5 | Heatmap distribution of OTUs in sow feces for all groups. **(A)** Comparison of the relative abundances of sow fecal microbiota in the top 10 at the phylum level. **(B)** Comparison of the relative abundances of sow fecal microbiota in the top 35 at the genus level. Different colors show the relative abundance of taxa. Fecal bacterial abundances were standardized with Z-score prior to the analyses. Positive z-scores reflect abundances above the average, whereas a negative z-score indicates abundance below the average. LP30 and LP110: sows with low-reproductive performance on d 30 and d 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on d 30 and d 110 of gestation, respectively. $n = 13$ for each group.

Changes of Plasma Hormone Contents During Gestation

The plasma hormone contents are shown in **Figure 9**. There were no significant differences in the plasma hormone concentrations on d 30 and d 110 of gestation between the LP and HP groups ($P > 0.05$). The plasma hormone contents of sows on gestation d 110 did not differ from those of sows on gestation d 30 ($P > 0.05$).

Correlation Analysis Between Fecal Microbial Abundance and Plasma Biochemical Indices

At the phylum level (**Figure 10A**), the plasma levels of CHOL, HDL-C, and LDL-C showed positive correlations with the abundances of Firmicutes and Actinobacteria ($P < 0.05$) and negative correlations with the abundances of Bacteroidetes and Fibrobacteres ($P < 0.05$); the plasma TG level had significant positive correlation with Euryarchaeota abundance ($P < 0.05$); the plasma IL-2 concentration was significantly positively correlated with Proteobacteria abundance ($P < 0.05$); the plasma IL-6 concentration was significantly positively correlated with the abundance of Bacteroidetes ($P < 0.05$); the plasma IgA concentration had significant negative correlation with Verrucomicrobia ($P < 0.05$). In addition, the IL-10 concentration tended to be negatively correlated with the abundance of Tenericutes ($P < 0.10$), and the IgM concentration tended to be negatively correlated with the abundance of Euryarchaeota ($P < 0.10$).

TABLE 2 | The Spearman's correlation test between the sow fecal microbiota and litter size.

Phase of gestation	Phylum	Genus
D 30 of gestation	Firmicutes (0.351+)	*Clostridium_sensu_stricto_1* (0.376+)
		Turicibacter (0.479*)
		Ruminococcaceae_UCG-014 (0.533**)
		Romboutsia (0.346+)
	Proteobacteria (−0.331+)	-
	Actinobacteria (0.369+)	-
D110 of gestation	Firmicutes (0.492*)	*Lactobacillus* (0.365+)
		Clostridium_sensu_stricto_1 (0.434*)
		Turicibacter (0.445*)
		Terrisporobacter (0.466*)
		Christensenellaceae_R-7_group (0.406*)
	Bacteroidetes (−0.402*)	*Rikenellaceae*_RC9_gut_group (−0.495*)
	Spirochaetes (−0.526**)	*Treponema_2* (−0.490*)
		Sphaerochaeta (−0.347+)
	Proteobacteria (0.365+)	*Escherichia-Shigella* (0.578**)
	Actinobacteria (−0.627**)	-

+*The correlation tends to be significant at a level of 0.10; *the correlation is significant at a level of 0.05; **the correlation is significant at a level of 0.01.*

At the genus level (**Figure 10B**), the plasma level of CHOL showed positive correlations with the abundances of *Clostridium_sensu_stricto_1*, *Christensenellaceae_R-7_group,* and *Terrisporobacter* ($P < 0.05$) and negative correlations with the abundances of *Eubacterium_coprostanoligenes_group*, *dgA-11_gut_group*, *Ruminococcaceae_UCG-010,* and *Sphaerochaeta* ($P < 0.05$). The plasma HDL-C concentration was significantly positively correlated with the abundance of *Christensenellaceae_R-7_group* ($P < 0.05$) and was significantly negatively correlated with the abundances of *Eubacterium_coprostanoligenes_group*, *dgA-11_gut_group,* and *Sphaerochaeta* ($P < 0.05$). The plasma LDL-C level had significant positive correlations with the abundances of *Clostridium_sensu_stricto_1* and *Christensenellaceae_R-7_group* ($P < 0.05$) and had significant negative correlations with the abundances of *dgA-11_gut_group*, *Ruminococcaceae_UCG-010,* and *Sphaerochaeta* ($P < 0.05$). The plasma NEFA concentration displayed positive correlation with the abundance of *Eubacterium_coprostanoligenes_group* and negative correlations with the abundances of *Turicibacter*, *Terrisporobacter,* and *Romboutsia* ($P < 0.05$). The plasma IL-2 concentration was significantly positively correlated with the abundances of *Clostridium_sensu_stricto_1* and *Succinivibrio*, and significantly negatively correlated with the abundance of *Ruminococcaceae_UCG-010* ($P < 0.05$). The plasma IL-6 level showed positive correlation with the *Prevotellaceae_NNK3B31_group* abundance ($P < 0.05$). The plasma IL-10 concentration had significant positive correlations with the abundances of *Prevotellaceae_NNK3B31_group* and *Sphaerochaeta* ($P < 0.05$). The plasma TNF-α concentration was significantly positively correlated with the abundances of *Anaerotruncus* ($P < 0.05$). The plasma level of IgA showed positive correlation with the *Lachnospiraceae_XPB1014_group* abundance ($P < 0.05$). The plasma level of IgG indicated negative correlation with the *Christensenellaceae_R-7_group* abundance ($P < 0.05$). The plasma IgM concentration was significantly positively correlated with the abundances of *Ruminococcaceae_UCG-014* and *Lachnospiraceae_AC2044_group* ($P < 0.05$), and was significantly negatively correlated with the abundances of *Methanobrevibacter* and *dgA-11_gut_group* ($P < 0.05$).

DISCUSSION

Maternal metabolism changes dramatically during the gestation period. Especially, maternal glucose and lipid metabolism plays a vital role in the initiation and development of gestation (26). The early stage of gestation can be regarded as an anabolic state to meet the fetal-placental and maternal demands of late gestation and lactation, with an increase in maternal fat stores and small increases in insulin sensitivity (27). The present study showed that the sows in the HP group had lower plasma levels of GLU and TG than those in LP group on d 30 of gestation. Plasma levels of GLU and TG are important indicators of glycolipid metabolism. Plasma lipid profiles at early pregnancy may predict the incidence and severity of pre-eclampsia in humans (28). The previous study in humans showed that higher plasma GLU concentration in the first trimester of pregnancy was a risk factor for adverse perinatal and neonatal outcomes, such as diabetes-related complications, gestational hypertension, and obesity (29). Similarly, a study in dairy cows demonstrated that high glucose levels at early gestation had an adverse impact on early embryonic development (30). The reason might be related to high nutritional level that increased the metabolic clearance rate of progesterone (31). The results might suggest that higher glucose level was not conducive to the development of embryos. Besides, higher plasma TG concentration is usually associated with abnormal lipid metabolism and causally related to an increased risk of cardiovascular disease in the clinic (32). Previous research indicated that higher plasma TG concentration demonstrated a poor health status of a gestating sow (33). Therefore, the sows in HP group is in a better physical state than those in LP group on d 30 of gestation.

In contrast, the sows in HP group showed higher plasma levels of NEFA and TG on d 110 of gestation. Late pregnancy is characterized as a catabolic state with increased insulin resistance which leads to increases in concentrations of maternal glucose and NEFA in plasma, allowing for greater substrate availability for rapid fetal development (27, 34). Serum NEFA, one of the most important biomarkers of energy balance status, is the product of lipolysis of storage fat, such as TG. Elevated

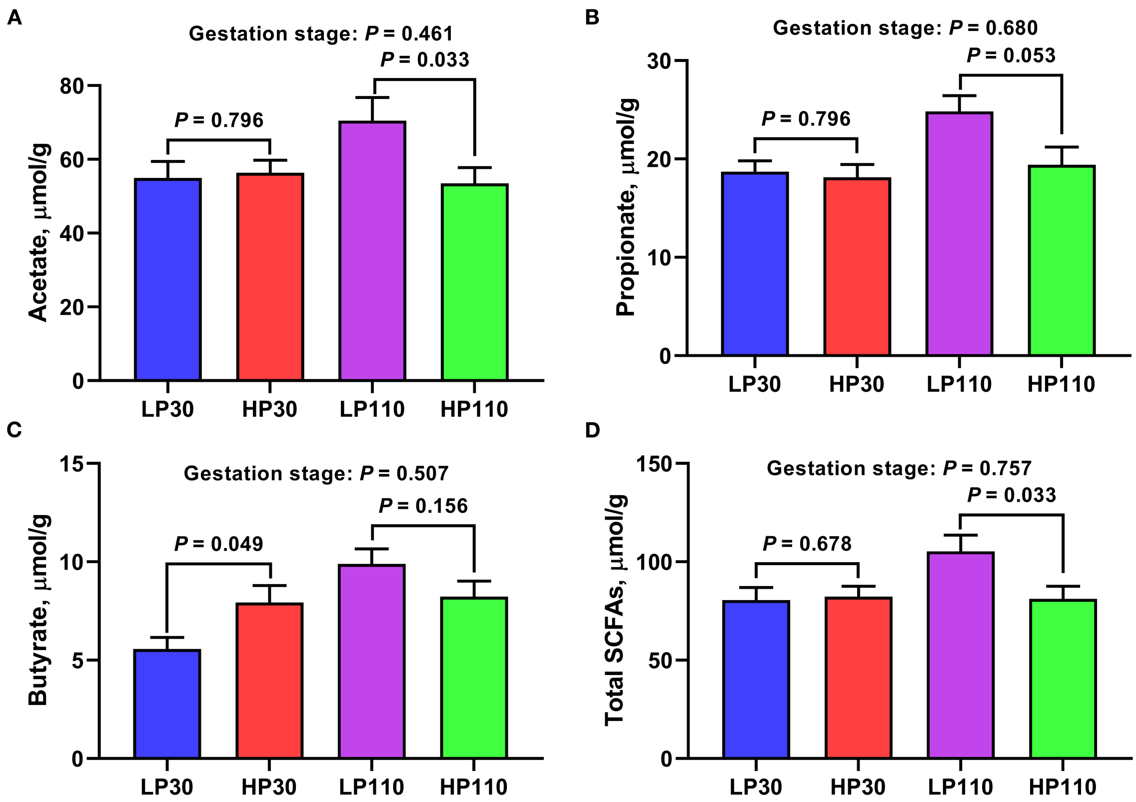

FIGURE 6 | Fecal short-chain fatty acids (SCFAs) concentrations in low- and high-reproductive performance groups on d 30 and 110 of gestation. **(A)** Acetate. **(B)** Propionate. **(C)** Butyrate. **(D)** Total SCFAs. Total SCFAs, the sum of acetate, propionate, and butyrate. LP30 and LP110: sows with low-reproductive performance on d 30 and 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on d 30 and 110 of gestation, respectively. Gestation stage: difference in the variations between gestation d 30 and d 110. Values are mean ± standard error ($n = 13$).

plasma NEFA level mediates many adverse metabolic effects, including obesity, insulin resistance, hypertension, and chronic inflammation (35–38). Consistently, increased plasma TNF-α concentration was found in the sows of HP group on d 110 of gestation. Tumor necrosis factor-alpha is a highly pleiotropic cytokine and is thought of as a vital mediator of inflammatory responses, metabolic activation, and cell death (39). The results of the present study demonstrated that HP sows might be in a more dramatic catabolic status to ensure the normal growth and development of the fetus during late gestation, leading to greater inflammation than LP sows, which was in accord with previous results in Shao et al. (40).

It is well-known that the dramatic changes of the microbial community can usually affect the health status of the host. In the present study, higher observed species, Shannon index, and Chao 1 index, as well as OTUs number, which was used to assess fecal microbial community richness and diversity, were observed in the HP group compared with the LP group on d 30 of gestation. Gut microbial diversity has been regarded as a new biomarker of health and metabolic capacity and low microbial diversity was often associated with poor health status such as inflammatory response, oxidative stress, and obesity (41, 42). In addition, sows in the HP group had the higher abundances of *Eubacterium_coprostanoligenes_group*, *Lachnospiraceae_XPB1014_group*, *Ruminococcaceae_UCG-010*, *Roseburia*, and *Ruminococcaceae_UCG-002* on d 30 of gestation. *Eubacterium coprostanoligenes* is a cholesterol-reducing bacterium and inversely correlated with the inflammatory response (43, 44). Li et al. (45) found that feeding *Eubacterium coprostanoligenes* to germ-free mice decreased blood CHOL concentration. Consistently, the correlation analysis in the present study also demonstrated that the relative abundance of *Eubacterium_coprostanoligenes_group* was negatively correlated with plasma CHOL concentration. Lachnospiraceae family are abundant in healthy humans (46) and can impact their hosts by producing SCFAs, converting primary to secondary bile acids, and competitively inhibiting colonization of intestinal pathogens (47, 48). *Ruminococcaceae*, which has carbohydrate-active enzymes, sugar transport mechanisms, and metabolic pathways for the degradation of complex plant materials (49), is a common digestive tract microbe. Fomenky et al. (50) showed that *Ruminococcaceae* might enhance mucus production and benefit to improve inflammatory responses in calves. In the present study, *Ruminococcaceae_UCG-010* was shown to be negatively associated with the plasma concentration of proinflammatory factor IL-2. *Roseburia* is a prominent

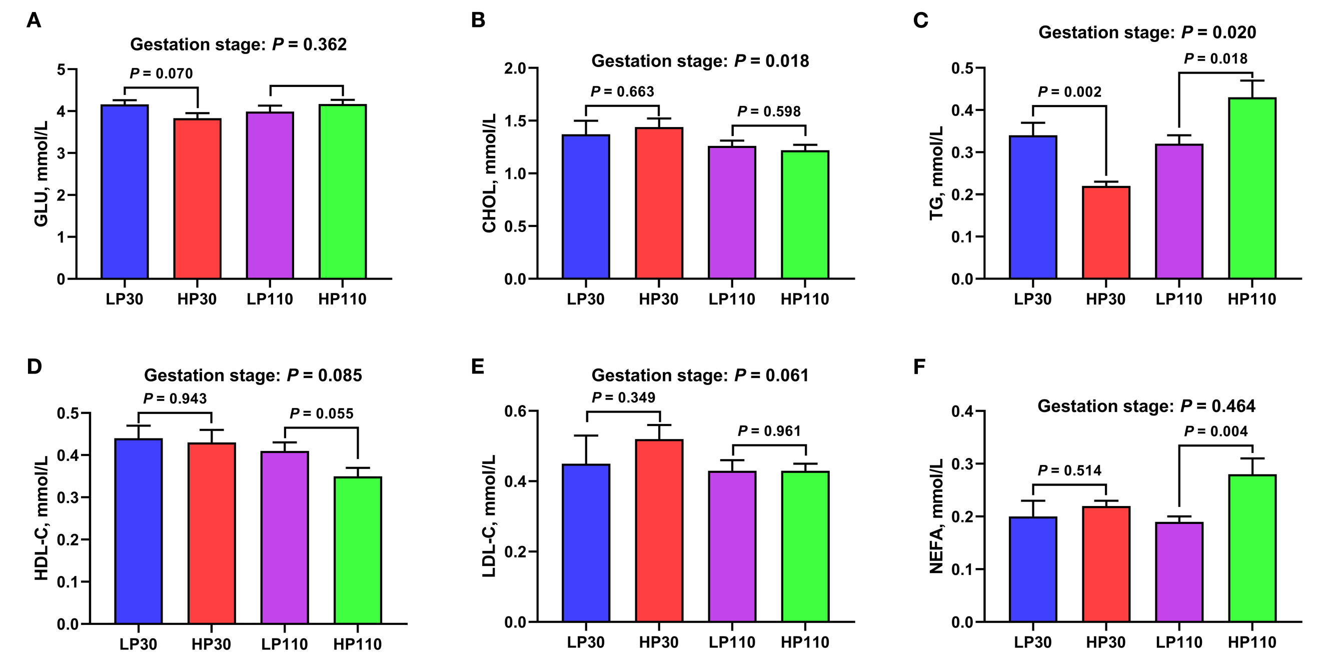

FIGURE 7 | The levels of plasma metabolites in low- and high-reproductive performance groups on d 30 and 110 of gestation. **(A)** Glucose (GLU); **(B)** Cholesterol (CHOL); **(C)** Triglyceride (TG); **(D)** High density lipoprotein cholesterol (HDL-C); **(E)** Low density lipoprotein cholesterol (LDL-C); **(F)** Non-esterified fatty acid (NEFA). LP30 and LP110: sows with low-reproductive performance on d 30 and 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on d 30 and 110 of gestation, respectively. Gestation stage: difference in the variations between gestation d 30 and d 110. Values are mean ± standard error ($n = 13$).

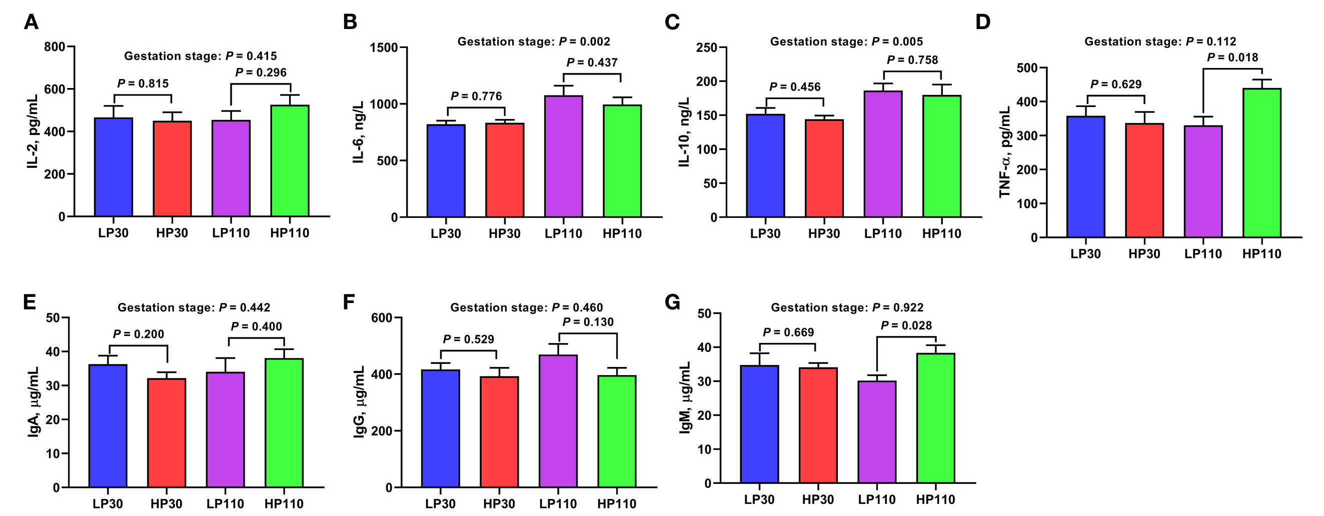

FIGURE 8 | The levels of inflammatory factors and immunoglobulins in low- and high-reproductive performance groups on d 30 and 110 of gestation. **(A)** Interleukin-2 (IL-2); **(B)** Interleukin-6 (IL-6); **(C)** Interleukin-10 (IL-10); **(D)** Tumor necrosis factor-α (TNF-α); **(E)** Immunoglobulin A (IgA); **(F)** Immunoglobulin G (IgG); **(G)** Immunoglobulin M (IgM). LP30 and LP110: sows with low-reproductive performance on d 30 and 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on d 30 and 110 of gestation, respectively. Gestation stage: difference in the variations between gestation d 30 and d 110. Values are mean ± standard error ($n = 13$).

gut-associated butyrate-producing genus (51) and inversely correlated with many diseases, such as inflammatory bowel disease (52) and atherosclerotic lesion (53). Consistently, increased fecal butyrate concentration was found in sows in the HP group. Microbial-driven butyrate has been shown to exhibit protective effects toward inflammatory diseases (54). Previous study has shown that butyrate oxidation can make up around 70 and 60% of the oxygen consumption in human descending colon and ascending colon, and inhibit the proliferation of aerobic pathogens (55). These findings

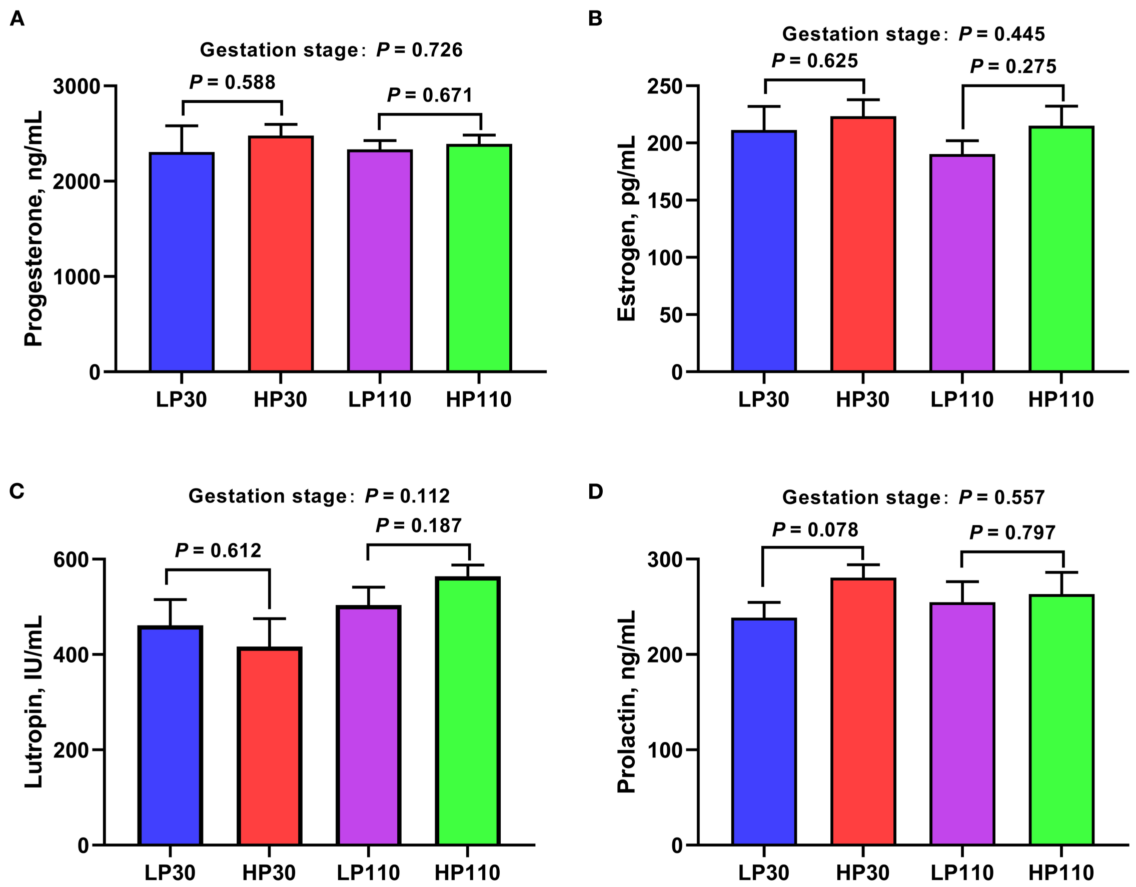

FIGURE 9 | The plasma concentrations of reproductive hormones in low- and high-reproductive performance groups on d 30 and 110 of gestation. **(A)** Progesterone; **(B)** Estrogen; **(C)** Lutropin; **(D)** Prolactin. LP30 and LP110: sows with low-reproductive performance on d 30 and 110 of gestation, respectively; HP30 and HP110: sows with high-reproductive performance on d 30 and 110 of gestation, respectively. Gestation stage: difference in the variations between gestation d 30 and d 110. Values are mean ± standard error ($n = 13$).

might partly explain the better health status of HP sows at early gestation.

Interestingly, the significant difference in alpha diversity disappeared, but significant difference was observed in beta diversity between HP and LP groups on d 110 of gestation. This was in keeping with the results in Uryu et al. (12) who explored the relationship between sow productive capacities and the fecal microbiota in different farms. However, Shao et al. (40) reported that alpha diversity and beta diversity both differed between sows with high- and low-reproductive performance during late gestation. It might suggest that the beta diversity, not alpha diversity, was a critical factor to evaluate the effect of gut microbiota on sow reproductive performance (12).

In addition, compared with sows in the LP group, sows in the HP group had the lower abundances of Bacteroidetes (including *Prevotellaceae_UCG-001*, *Prevotella_1*, and *dgA-11_gut_group*) and Spirochaetes (*Treponema_2*) which were negatively correlated with litter size, but the higher abundance of Firmicutes (containing *Lactobacillus*, *Christensenellaceae_R-7_group*, and *Terrisporobacter*) and genus *Escherichia-Shigella* exhibited positive correlations with litter size on day 110 of gestation. In the present study, Firmicutes and Bacteroidetes were the most predominant phyla, regardless of the stage of gestation, which were in accordance with previous studies on sows (40, 56, 57). A previous study in obese children showed that the abundance of Firmicutes had the positive association with plasma TNF-α level (58). *Bacteroidetes*, as well as *Treponema_2*, includes a large number of cellulases, glycoside hydrolases, glycosyl transferases, and have the capacity to degrade polymers such as cellulose, hemicellulose, and lignin (59, 60), which might be the reason for the decreases in fecal concentrations of acetate, propionate, and total SCFAs. Previous studies indicated that a changed gut microbiota characterized by increased levels of Firmicutes and depleted Bacteroidetes was associated with chronic or low-grade inflammation (11). *Escherichia-Shigella,* belonging to phylum *Proteobacteria*, is generally taken as non-pathogenic bacteria and can become pathogenic bacteria when stimulated by stress (61). Shao et al. (40) also reported that predicted metabolic functions related to lipopolysaccharide biosynthesis significantly higher in HP sows than in LP sows during late gestation. The greater production of total SCFAs and propionate on d 110 of gestation in the

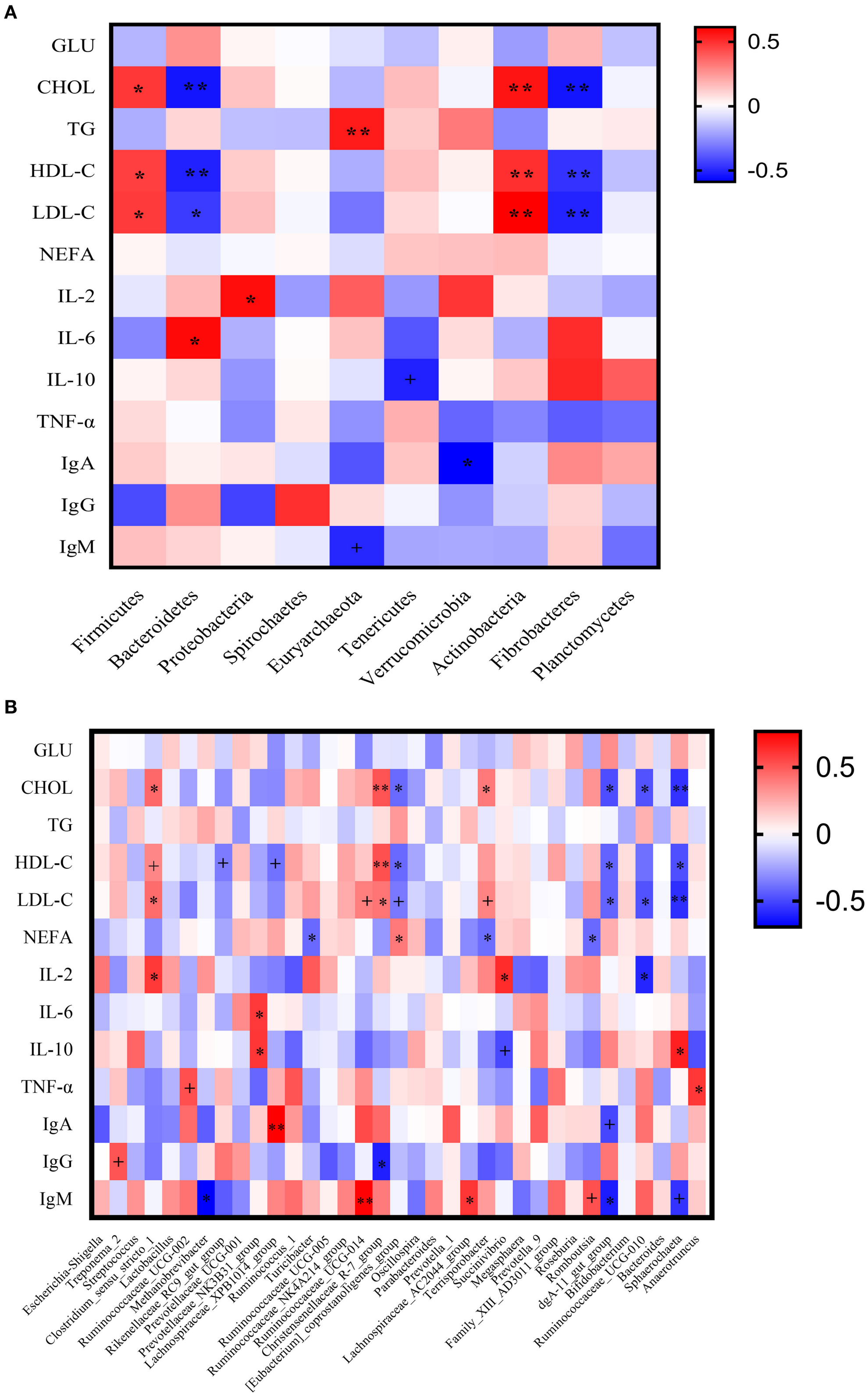

FIGURE 10 | Correlation analysis between the plasma biochemical indices and sow fecal microbiota. **(A)** At phylum level; **(B)** At genus level. GLU, Glucose; CHOL, cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol, LDL-C, low density lipoprotein cholesterol; NEFA, non-esterified fatty acid; IL-2, interleukin-2; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor-α; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M. $^{+}$The correlation tends to be significant at a level of 0.10; *the correlation is significant at a level of 0.05; ** the correlation is significant at a level of 0.01.

LP group may be a compensatory mechanism in order to ensure the survival of fetuses and try to reduce pathogenic microorganisms, which need to be further studied. Moreover, Koren et al. (10) showed that dramatical alterations of species and abundance of gut microbiota contributed to the metabolic changes during gestation which was characterized by greater adiposity and insulin resistance to meet the needs of the rapid growth of fetuses during late gestation in human. Therefore, it might suggest that the alteration in gut microbiota during late gestation, associated with the increases in plasma TG and NEFA, in sows with high-reproductive performance might be more conducive to the growth and development of the fetus.

Interestingly, we also found increased abundance of *Terrisporobacter* that had significant negative correlations with the plasma NEFA concentration, which might be helpful to decrease the plasma NEFA from the HP sow and resist inflammatory response during late gestation. We also found increased plasma IgM concentration in HP sows on d 110 of gestation, which might be related to the increased abundance of *Lactobacillus* and the decreased abundance of *dgA-11_gut_group*. Wang et al. (62) reported that *Lactobacillus* supplementation in weanling piglets could increase plasma level of IgM. Immunoglobulin M, serving as the first line of host defense against infections, is the first antibody isotype to appear during immune responses and plays a vital role in immune regulation and immunological tolerance (63). The abundance of *dgA-11_gut_group* was negatively correlated with the plasma IgM concentration in the present study. This might be an important reason that the microecological balance of the intestinal tract of HP sows could restore during lactation (40).

In addition, we explored the shifts in plasma parameters, fecal metabolites, and microbiota from gestation d 30 to d 110 in the present study. The results indicated that plasma level of TG was increased, but levels of CHOL, HDL-C, and LDL-C were reduced on d 110 of gestation. Ji et al. (56) also showed that plasma concentrations of total CHOL and HDL-C were reduced from gestation d 60 to d 110. It suggested that lipid metabolism in the hepatic and adipose tissues of sows were activated to maintain the nutritional needs of the fetus in late gestation. Dramatic switches in lipid catabolism were often associated with inflammatory responses (64). Consistently, plasma concentrations of IL-6 and IL-10 were both elevated on d 110 of pregnancy. Interleukin-6 is a pleiotropic pro-inflammatory cytokine and involved in chronic inflammation and immune regulatory cascades (65). Interleukin-10, a prototypical anti-inflammatory cytokine produced by CD4 (+) cells, plays an important role in inhibiting inflammatory reaction by suppressing the upstream activities of antigen presenting cells and T cell functions (66). Increased serum concentration of IL-6 frequently accompanied an increased level of IL-10 in serum under inflammatory conditions (67). The alteration of abundances of phyla Firmicutes, Bacteroidetes, and Verrucomicrobia was in keeping with the results in Zhou et al. (11) that Firmicutes was significantly decreased while Bacteroidetes and Verrucomicrobia increased from d 30 to d 110 of gestation. However, Zhou et al. (11) observed an increase in Actinobacteria at late gestation, which was in line with Liu et al. (68). It suggested that the changes of abundance of Actinobacteria might be not associated with the progress of gestation. In terms of the genus level, the relative abundances of fecal *Streptococcus*, *Oscillospira,* and *Ruminococcaceae_UCG-010* were increased, and that of fecal *Terrisporobacter* was decreased with progression of pregnancy. Zhou et al. (11) also showed increased *Oscillospira* and decreased *Terrisporobacter* in sow feces from gestation d 30 to d 110. The changes of abundances of *Terrisporobacter* and *Ruminococcaceae_UCG-010* were in accord with alteration of plasma CHOL concentration. *Streptococcus*, including Gram-positive organisms shaped in cocci and organized in chains, are commensals, pathogens, and opportunistic pathogens for humans and animals (69). Previous study in humans also reported that *Streptococcus* was enriched in late gestation compared to in early gestation (10). However, Zhou et al. (11) found a reduction in fecal *Streptococcus* from gestation d 30 to d 110. Therefore, further study is required to indentify which microbiome is involved in the progress of pregnancy. Interestingly, the SCFAs were not significantly altered during gestation although significant microbiota compositions occurred, which was consistent with Liu et al. (68) and Zhou et al. (11). Above all, the sows underwent dramatic metabolic changes over the course of a normal pregnancy, which was associated with the profound alteration of the gut microbiota.

CONCLUSION

In summary, our findings demonstrated that microbial abundances and community structures differed significantly between sows with different litter sizes during gestation, which was associated with changes in plasma biochemical parameters, inflammatory factors, and immunoglobulin, as well as fecal metabolites. Besides, plasma biochemical parameters and cytokines shifted dramatically from gestation d 30 to d 110, which were associated with the alterations in microbial composition and diversity. These findings revealed that sow reproductive performance might be associated with the changes of maternal gut microbiota during gestation and provided a microbial perspective to improve sow reproductive performance in pig production.

DATA AVAILABILITY STATEMENT

The assembled HiSeq sequences obtained in the present study were submitted to National Center of Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession PRJNA721963 (Illumina sequences).

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Care and Use Committee of Shandong Agricultural University.

AUTHOR CONTRIBUTIONS

YL: conceptualization, investigation, supervision, and writing—review and editing. JC: data curation, project administration, and writing—original draft. JC and FL: formal analysis. WY: funding acquisition. SJ and YL: methodology. JC and YL: software. FL, WY, and YL: validation. JC and SJ: visualization. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.793174/full#supplementary-material

REFERENCES

1. Camp JG, Kanther M, Semova I, Rawls JF. Patterns and scales in gastrointestinal microbial ecology. *Gastroenterology.* (2009) 136:1989–2002. doi: 10.1053/j.gastro.2009.02.075
2. Letarov A, Kulikov E. The bacteriophages in human- and animal body-associated microbial communities. *J Appl Microbiol.* (2009) 107:1–13. doi: 10.1111/j.1365-2672.2009.04143.x
3. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.* (2016) 14:e1002533. doi: 10.1371/journal.pbio.1002533
4. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat Rev Microbiol.* (2011) 9:279–90. doi: 10.1038/nrmicro2540
5. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science.* (2011) 334:105–8. doi: 10.1126/science.1208344
6. Slack E, Hapfelmeier S, Stecher B, Velykoredko Y, Stoel M, Lawson MA, et al. Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science.* (2009) 325:617–20. doi: 10.1126/science.1172747
7. Chassaing B, Ley RE, Gewirtz AT. Intestinal epithelial cell toll-like receptor 5 regulates the intestinal microbiota to prevent low-grade inflammation and metabolic syndrome in mice. *Gastroenterology.* (2014) 147:1363–77.e1317. doi: 10.1053/j.gastro.2014.08.033
8. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab.* (2011) 13:517–26. doi: 10.1016/j.cmet.2011.02.018
9. Peng J, Tang Y, Huang Y. Gut health: The results of microbial and mucosal immune interactions in pigs. *Anim Nutr.* (2021) 7:282–94. doi: 10.1016/j.aninu.2021.01.001
10. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell.* (2012) 150:470–80. doi: 10.1016/j.cell.2012.07.008
11. Zhou P, Zhao Y, Zhang P, Li Y, Gui T, Wang J, et al. Microbial mechanistic insight into the role of inulin in improving maternal health in a pregnant sow model. *Front Microbiol.* (2017) 8:2242. doi: 10.3389/fmicb.2017.02242
12. Uryu H, Tsukahara T, Ishikawa H, Oi M, Otake S, Yamane I, et al. Comparison of productivity and fecal microbiotas of sows in commercial farms. *Microorganisms.* (2020) 8:1469. doi: 10.3390/microorganisms8101469
13. Böhmer BM, Kramer W, Roth-Maier DA. Dietary probiotic supplementation and resulting effects on performance, health status, and microbial characteristics of primiparous sows. *J Anim Physiol Anim Nutr.* (2006) 90:309–15. doi: 10.1111/j.1439-0396.2005.00601.x
14. Zhang Q, Li J, Cao M, Li Y, Zhuo Y, Fang Z, et al. Dietary supplementation of Bacillus subtilis PB6 improves sow reproductive performance and reduces piglet birth intervals. *Anim Nutr.* (2020) 6:278–87. doi: 10.1016/j.aninu.2020.04.002
15. Al-Asmakh M, Hedin L, Pettersson S. Maternal microbiota regulate glucocorticoids levels and placental development in mice. *Endocr Abstr.* (2015) 37:1. doi: 10.1530/endoabs.37.OC5.1
16. Che L, Yang Z, Xu M, Zhang Z, Liu P, Xu S, et al. Dietary energy intake affects fetal survival and development during early and middle pregnancy in Large White and Meishan gilts. *Anim Nutr.* (2015) 1:152–9. doi: 10.1016/j.aninu.2015.08.009
17. Che L, Xu M, Yang Z, Xu S, Che L, Lin Y, et al. Detection of placental proteomes at different uterine positions in Large White and Meishan Gilts on gestational day 90. *PLoS ONE.* (2016) 11:e0167799. doi: 10.1371/journal.pone.0167799
18. National Research Council. *Nutrient Requirements of Swine, 11th rev. ed.* Washington, DC: National Academy Press (2012).
19. Li Y, He J, Zhang L, Liu H, Cao M, Lin Y, et al. Effects of dietary fiber supplementation in gestation diets on sow performance, physiology and milk composition for successive three parities. *Anim Feed Sci Tech.* (2021) 276:114945. doi: 10.1016/j.anifeedsci.2021.114945
20. Li Y, Zhao X, Zhang L, Zhan X, Liu Z, Zhuo Y, et al. Effects of a diet supplemented with exogenous catalase from penicillium notatum on intestinal development and microbiota in weaned piglets. *Microorganisms.* (2020) 8:30391. doi: 10.3390/microorganisms8030391
21. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods.* (2013) 10:996–8. doi: 10.1038/nmeth.2604
22. Glöckner FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, et al. 25 years of serving the community with ribosomal RNA gene reference databases and tools. *J Biotechnol.* (2017) 261:169–76. doi: 10.1016/j.jbiotec.2017.06.1198
23. Chen L, Li S, Zheng J, Li W, Jiang X, Zhao X, et al. Effects of dietary Clostridium butyricum supplementation on growth performance, intestinal development, and immune response of weaned piglets challenged with lipopolysaccharide. *J Anim Sci Biotechnol.* (2018) 9:62. doi: 10.1186/s40104-018-0275-8
24. Gallego S, Esbr,í JM, Campos JA, Peco JD, Martin-Laurent F, Higueras P. Microbial diversity and activity assessment in a 100-year-old lead mine. *J Hazard Mater.* (2021) 410:124618. doi: 10.1016/j.jhazmat.2020.124618
25. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol.* (2005) 71:8228–35. doi: 10.1128/AEM.71.12.8228-8235.2005
26. Pinto J, Barros AS, Domingues MR, Goodfellow BJ, Galhano E, Pita C, et al. Following healthy pregnancy by NMR metabolomics of plasma and correlation to urine. *J Proteome Res.* (2015) 14:1263–74. doi: 10.1021/pr5011982
27. Lain KY, Catalano PM. Metabolic changes in pregnancy. *Clin Obstet Gynecol.* (2007) 50:938–48. doi: 10.1097/GRF.0b013e31815a5494
28. Ekhator CN, Ebomoyi MI. Blood glucose and serum lipid profiles during pregnancy. *Afr J Diabetes Med.* (2012) 20:e15320. doi: 10.1097/MD.0000000000015320
29. Salman L, Arbib N, Borovich A, Shmueli A, Chen R, Wiznitzer A, et al. The impact of first trimester fasting glucose level on adverse perinatal outcome. *J Perinatol.* (2018) 38:451–5. doi: 10.1038/s41372-018-0045-7
30. Leane S, Herlihy MM, Curran F, Kenneally J, Forde N, Simintiras CA, et al. The effect of exogenous glucose infusion on early embryonic development in lactating dairy cows. *J Dairy Sci.* (2018) 101:11285–96. doi: 10.3168/jds.2018-14894
31. Jindal R, Cosgrove JR, Foxcroft GR. Progesterone mediates nutritionally induced effects on embryonic survival in gilts. *J Anim Sci.* (1997) 75:1063–70. doi: 10.2527/1997.7541063x
32. Kockx M, Kritharides L. Triglyceride-rich lipoproteins. *Cardiol Clin.* (2018) 36:265–75. doi: 10.1016/j.ccl.2017.12.008
33. Bin P, Azad MAK, Liu G, Zhu D, Kim SW, Yin Y. Effects of different levels of methionine on sow health and plasma metabolomics during late gestation. *Food Funct.* (2018) 9:4979–88. doi: 10.1039/C8FO01477A

34. Père MC, Etienne M. Insulin sensitivity during pregnancy, lactation, and postweaning in primiparous gilts. *J Anim Sci.* (2007) 85:101–10. doi: 10.2527/jas.2006-130
35. Shi X, Li D, Deng Q, Li Y, Sun G, Yuan X, et al. NEFAs activate the oxidative stress-mediated NF-κB signaling pathway to induce inflammatory response in calf hepatocytes. *J Steroid Biochem Mol Biol.* (2015) 145:103–12. doi: 10.1016/j.jsbmb.2014.10.014
36. Spiller S, Blüher M, Hoffmann R. Plasma levels of free fatty acids correlate with type 2 diabetes mellitus. *Diabetes Obes Metab.* (2018) 20:2661–9. doi: 10.1111/dom.13449
37. Wang Y, Li C, Ali I, Li L, Wang G. N-acetylcysteine modulates non-esterified fatty acid-induced pyroptosis and inflammation in granulosa cells. *Mol Immunol.* (2020) 127:157–63. doi: 10.1016/j.molimm.2020.09.011
38. Yang WS, Li R, Shen YQ, Wang XC, Liu QJ, Wang HY, et al. Importance of lipid ratios for predicting intracranial atherosclerotic stenosis. *Lipids Health Dis.* (2020) 19:160. doi: 10.1186/s12944-020-01336-1
39. Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM. Suppression of TNF-α-induced apoptosis by NF-κB. *Science.* (1996) 274:787–9. doi: 10.1126/science.274.5288.787
40. Shao Y, Zhou J, Xiong X, Zou L, Kong X, Tan B, et al. Differences in gut microbial and serum biochemical indices between sows with different productive capacities during perinatal period. *Front Microbiol.* (2020) 10:3047. doi: 10.3389/fmicb.2019.03047
41. Qiao Y, Sun J, Ding Y, Le G, Shi Y. Alterations of the gut microbiota in high-fat diet mice is strongly linked to oxidative stress. *Appl Microbiol Biotechnol.* (2013) 97:1689–97. doi: 10.1007/s00253-012-4323-6
42. Gong D, Gong X, Wang L, Yu X, Dong Q. Involvement of reduced microbial diversity in inflammatory bowel disease. *Gastroent Res Pract.* (2016) 2016:1–7. doi: 10.1155/2016/6951091
43. Ye JZ, Li YT, Wu WR, Shi D, Fang DQ, Yang LY, et al. Dynamic alterations in the gut microbiota and metabolome during the development of methionine-choline-deficient diet-induced nonalcoholic steatohepatitis. *World J Gastroenterol.* (2018) 24:2468–81. doi: 10.3748/wjg.v24.i23.2468
44. Vlacil AK, Schuett J, Ruppert V, Soufi M, Oberoi R, Shahin K, et al. Deficiency of Nucleotide-binding oligomerization domain-containing proteins (NOD) 1 and 2 reduces atherosclerosis. *Basic Res Cardiol.* (2020) 115:47. doi: 10.1007/s00395-020-0806-2
45. Li L, Batt SM, Wannemuehler M, Dispirito A, Beitz DC. Effect of feeding of a cholesterol-reducing bacterium, Eubacterium coprostanoligenes, to germ-free mice. *Lab Anim Sci.* (1998) 48:253–5.
46. Hold GL, Pryde SE, Russell VJ, Furrie E, Flint HJ. Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. *FEMS Microbiol Ecol.* (2002) 9:33–9. doi: 10.1111/j.1574-6941.2002.tb00904.x
47. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, et al. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature.* (2015) 517:205–8. doi: 10.1038/nature13828
48. Rivera-Chávez F, Zhang LF, Faber F, Lopez CA, Byndloss MX, Olsan EE, et al. Depletion of butyrate-producing clostridia from the gut microbiota drives an aerobic luminal expansion of Salmonella. *Cell Host Microbe.* (2016) 19:443–54. doi: 10.1016/j.chom.2016.03.004
49. Biddle A, Stewart L, Blanchard J, Leschine S. Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. *Diversity.* (2013) 5:627–40. doi: 10.3390/d5030627
50. Fomenky BE, Do DN, Talbot G, Chiquette J, Bissonnette N, Chouinard YP, et al. Direct-fed microbial supplementation influences the bacteria community composition of the gastrointestinal tract of pre- and post-weaned calves. *Sci Rep.* (2018) 8:14147. doi: 10.1038/s41598-018-32375-5
51. Robinson H, Barrett H, Gomez-Arango L, McIntyre HD, Callaway L, Dekker Nitert M. Ketonuria is associated with changes to the abundance of Roseburia in the gut microbiota of overweight and obese women at 16 weeks gestation: a cross-sectional observational study. *Nutrients.* (2019) 11:1836. doi: 10.3390/nu11081836
52. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine.* (2020) 51:102590. doi: 10.1016/j.ebiom.2019.11.051
53. Kasahara K, Krautkramer KA, Org E, Romano KA, Kerby RL, Vivas EI, et al. Interactions between Roseburia intestinalis and diet modulate atherogenesis in a murine model. *Nat Microbiol.* (2018) 3:1461–71. doi: 10.1038/s41564-018-0272-x
54. Zhong X, Zhang Z, Wang S, Cao L, Zhou L, Sun A, et al. Microbial-driven butyrate regulates jejunal homeostasis in piglets during the weaning stage. *Front Microbiol.* (2019) 9:3335. doi: 10.3389/fmicb.2018.03335
55. Hu J, Lin S, Zheng B, Cheung PC. Short-chain fatty acids in control of energy metabolism. *Crit Rev Food Sci Nutr.* (2018) 58:1243–49. doi: 10.1080/10408398.2016.1245650
56. Ji YJ, Li H, Xie PF, Li ZH, Li HW, Yin YL, et al. Stages of pregnancy and weaning influence the gut microbiota diversity and function in sows. *J Appl Microbiol.* (2019) 127:867–79. doi: 10.1111/jam.14344
57. Li Y, Liu H, Zhang L, Yang Y, Lin Y, Zhuo Y, et al. Maternal dietary fiber composition during gestation induces changes in offspring antioxidative capacity, inflammatory response, and gut microbiota in a sow model. *Int J Mol Sci.* (2020) 21:31. doi: 10.3390/ijms21010031
58. Orbe-Orihuela YC, Lagunas-Martínez A, Bahena-Román M, Madrid-Marina V, Torres-Poveda K, Flores-Alfaro E, et al. High relative abundance of firmicutes and increased TNF-α levels correlate with obesity in children. *Salud Publica Mex.* (2018) 60:5–11. doi: 10.21149/8133
59. Rosewarne CP, Cheung JL, Smith WJ, Evans PN, Tomkins NW, Denman SE, et al. Draft genome sequence of Treponema sp. strain JC4, a novel spirochete isolated from the bovine rumen. *J Bacteriol.* (2012) 194:4130. doi: 10.1128/JB.00754-12
60. Fernández-Gómez B, Richter M, Schüler M, Pinhassi J, Acinas SG, González JM, et al. Ecology of marine Bacteroidetes: a comparative genomics approach. *ISME J.* (2013) 7:1026–37. doi: 10.1038/ismej.2012.169
61. Lutful Kabir SM. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *Int J Environ Res Public Health.* (2010) 7:89–114. doi: 10.3390/ijerph7010089
62. Wang T, Teng K, Liu Y, Shi W, Zhang J, Dong E, et al. Lactobacillus plantarum PFM 105 promotes intestinal development through modulation of gut microbiota in weaning piglets. *Front Microbiol.* (2019) 10:90. doi: 10.3389/fmicb.2019.00090
63. Liu J, Wang Y, Xiong E, Hong R, Lu Q, Ohno H, et al. Role of the IgM Fc receptor in immunity and tolerance. *Front Immunol.* (2019) 10:529. doi: 10.3389/fimmu.2019.00529
64. Kim SW, Weaver AC, Shen YB, Zhao Y. Improving efficiency of sow productivity: nutrition and health. *J Anim Sci Biotechnol.* (2013) 4:26. doi: 10.1186/2049-1891-4-26
65. Jordan SC, Choi J, Kim I, Wu G, Toyoda M, Shin B, et al. Interleukin-6, a cytokine critical to mediation of inflammation, autoimmunity and allograft rejection: therapeutic implications of il-6 receptor blockade. *Transplantation.* (2017) 101:32–44. doi: 10.1097/TP.0000000000001452
66. Kumar R, Ng S, Engwerda C. The role of IL-10 in malaria: a double edged sword. *Front Immunol.* (2019) 10:229. doi: 10.3389/fimmu.2019.00229
67. Jndoyan ZT, Bablumyan AY, Ginosyan KV, Shekoyan SV. Correlations between indicators of interleukin-10 and interleukin-6 in patients with periodic disease. *Ter Arkh.* (2018) 90:38–41. doi: 10.26442/terarkh201890338-41
68. Liu H, Hou C, Li N, Zhang X, Zhang G, Yang F, et al. Microbial and metabolic alterations in gut microbiota of sows during pregnancy and lactation. *FASEB J.* 33:4490–501. doi: 10.1096/fj.201801221RR
69. Haenni M, Lupo A, Madec JY. Antimicrobial resistance in *Streptococcus* spp. *Microbiol Spectr.* (2018) 6:9804. doi: 10.1128/9781555819804.ch8

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