frontiers | Research Topics

Alternative feed ingredients for sustainable livestock production

Edited by
Juana Catarina Cariri Chagas, Gabriel Da Silva Viana and Marcos Inacio Marcondes

Published in
Frontiers in Animal Science

ISSN 1664-8714
ISBN 978-2-8325-6530-8
DOI 10.3389/978-2-8325-6530-8

Generative AI statement
Any alternative text (Alt text) provided alongside figures in the articles in this ebook has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Alternative feed ingredients for sustainable livestock production

Topic editors

Juana Catarina Cariri Chagas — Swedish University of Agricultural Sciences, Sweden

Gabriel Da Silva Viana — Natural Resources Institute Finland (Luke), Finland

Marcos Inacio Marcondes — William H. Miner Agricultural Research Institute, United States

Citation

Chagas, J. C. C., Viana, G. D. S., Marcondes, M. I., eds. (2025). *Alternative feed ingredients for sustainable livestock production*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6530-8

Table of contents

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 25 October 2023
DOI 10.3389/fanim.2023.1260787

OPEN ACCESS

EDITED BY
Juana Catarina Cariri Chagas,
Swedish University of Agricultural Sciences, Sweden

REVIEWED BY
Gibson Maswayi Alugongo,
China Agricultural University, China
Halima Sultana,
University of Florida, United States

*CORRESPONDENCE
Sameer J. Mabjeesh
sameer.mabjeesh@mail.huji.ac.il

RECEIVED 18 July 2023
ACCEPTED 04 October 2023
PUBLISHED 25 October 2023

CITATION
Wagali P, Ngomuo G, Kilama J, Sabastian C, Ben-Zeev S, Ben-Meir YA, Argov-Argaman N, Saranga Y and Mabjeesh SJ (2023) The effect of teff (*Eragrostis tef*) hay inclusion on feed intake, digestibility, and milk production in dairy cows. *Front. Anim. Sci.* 4:1260787. doi: 10.3389/fanim.2023.1260787

The effect of teff (*Eragrostis tef*) hay inclusion on feed intake, digestibility, and milk production in dairy cows

Philip Wagali[1], Godliver Ngomuo[1], Justine Kilama[1], Chris Sabastian[1], Shiran Ben-Zeev[2], Yehoshav A. Ben-Meir[3], Nurit Argov-Argaman[1], Yehoshua Saranga[2] and Sameer J. Mabjeesh[1]*

[1]Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Rehovot, Israel, [2]The Robert H. Smith Institute of Plant Sciences & Genetics in Agriculture, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel, [3]Institute of Animal Science, Agricultural Research Organization, Rishon LeTsiyon, Israel

Teff (*Eragrostis tef*) is a drought-tolerant, multi-harvest, high-quality summer forage crop. We conducted a study aiming at assessing the effect of replacing wheat hay with teff hay in diets on the feed intake, digestibility, and lactational performance of dairy cows. Thirty-four multiparous (≥3rd parity) Israeli Holstein Friesian dairy cows averaging (± SD) 182 days in milk ± 8 days in milk, 45 kg/d ± 4.8 kg/d of milk yield, and a body weight of 647.1 kg ± 51 kg at the beginning of the study were recruited to a 6-week feeding trial. Cows were randomly divided into two balanced groups based on parity, days in milk, and milk yield. Cows were subjected to two low-roughage dietary treatments (~30% roughage): a control wheat hay-based diet and a teff hay-based diet. Production performances, dry matter intake, and nutrient digestibility were measured. Milk samples were analyzed for their composition and fatty acids profile. Blood samples were used to measure metabolite concentrations. The statistical model included fixed effects of dietary treatments, time, and random effects of cows nested in treatment. Production data and feed intake were analyzed as repeated measures using a covariance structure. Dietary treatments did not affect dry matter intake (26 kg/d). However, the teff-fed cows demonstrated higher crude protein digestibility than control cows (61.9% vs. 59.2%). Dietary teff inclusion increased milk yield by 1.5 kg/d. Polyunsaturated fatty acids and omega-3 fatty acids profiles in milk were greater in the teff cows than in the control cows (4.77 g/100 g vs. 4.36 g/100 g and 3.71 g/100 g vs. 3.43 g/100 g, respectively). Non-esterified fatty acids, beta-hydroxybutyrate, and blood urea nitrogen concentrations in circulation were higher in the control group than in the teff group. The acetic-to-propionic-acid ratio in the rumen fluid was higher in control cows than in teff cows (2.90 vs. 2.43). However, the ruminal ammonia-N concentration was higher in the teff cows than in the control cows (18.5 mg/dL vs. 15.8 mg/dL). In conclusion, teff hay inclusion in the rations of high-producing dairy cows increased milk yield, which could be attributed to improved crude protein digestibility and energy partition to production.

KEYWORDS

teff hay, dairy cows, milk production, digestibility, productivity

Introduction

Global milk production increased to 887 million tons per annum in 2021, with 81.0% of the total milk produced coming from cows, and this figure is projected to reach 1,060 million tons per annum by 2031 (OECD-FAO, 2022). The increase in milk production is mainly due to a continuous increase in the number of herds, rain-fed fodder availability, and the intensification of modern production systems. However, dairy production is dependent on traditional forages that are often scarce, expensive, and seasonal, which highlights the importance of improving forage production and feeding efficiencies to meet the milk demand.

In dairy production, the cost of feeding constitutes 60.0% of the total production costs (Lawrence et al., 2008). To address this issue and offset the higher prices of feeds in the dairy industry and their limited availability, there is a need to identify cost-effective and well-adapted forages. Teff (*Eragrostis tef*), a C4 warm-season annual crop from the Chloridoideae subfamily of grasses, is one such alternative, with over 4,000 varieties identified (Miller, 2011; Assefa et al., 2013). Moreover, it is tolerant to a wider range of biotic and abiotic stresses and rarely requires irrigation during the growing period (Davison and Creech, 2011; Roseberg et al., 2018). Teff is a multipurpose crop used for both human and livestock consumption (NRC, 1996; Miller, 2011; Habte et al., 2019).

The crop has been used mainly by humans in the horn of Africa for over 6,000 years to make the traditional Ethiopian bread "*Injera*" and other meals (Roseberg et al., 2018; Chanyalew et al., 2019; Girija et al., 2022). Teff grain is gluten-free and contains higher levels of minerals such as calcium, iron, and magnesium than wheat, barley, and sorghum grains (Mengesha, 1966). Teff can also be used to feed livestock as it is a multi-harvest forage that performs better during summer. It can produce high-quality hay in a short growing period, unlike most common forages such as Timothy grass (Miller, 2011; Roseberg et al., 2018; Habte et al., 2019). Teff straw, after grain harvest, is also used to feed farm animals. Teff can be ensiled and preserved as a high-quality forage.

Teff silage has been proven to have high-quality *in vitro* DM digestibility (IVDMD) (57.0% to 66.1%) (Wagali et al., 2023) and teff straw is also considered to have high digestibility values compared with barley and wheat straw (Assefa et al., 2013). Several studies have investigated the nutritional value of teff hay and silages. For example, CP concentration was shown to range from 7.5%–18.7%, whereas NDF concentration ranged from 58.6%–70.8% in teff hay (Vinyard et al., 2018; Ream et al., 2020; Wagali et al., 2023). The IVDMD of teff silage and *in vivo* DMD of teff hay ranged from 58.0% to 67.0% (Ream et al., 2020; Wagali et al., 2023). The *in vitro* NDF digestibility of teff silage was up to 55.0% (Wagali et al., 2023), and the *in vivo* NDF digestibility of teff hay in beef cattle was up to 69.0% (Ream et al., 2020). However, the nutritional composition of teff, similar to that of other forages, may vary greatly depending on its variety, growth conditions, and maturity stage at harvest, as elaborated by Buxton (1996) and Wagali et al. (2023).

Although previous studies have investigated the effect of teff forage digestibility in horses, beef cattle, and sheep (Hagos and Melaku, 2009; Staniar et al., 2010; Vinyard et al., 2018), the effect of feeding teff-based rations to high-producing dairy cows has scarcely been studied. Our goal is to establish teff as a multi-harvest summer fodder crop in Israeli dairy cows' rations. We hypothesized that a teff-based diet fed to high-producing dairy cows might be an alternative feeding strategy that could replace the wheat hay-based diet that is common in Israel. Therefore, the objective of this study was to evaluate the effect of a teff-based diet compared with a wheat-based diet on the feed intake, digestibility, and lactational performance of high-yielding dairy cows.

Materials and methods

Experimental animals, study design, and treatments

The experiment was conducted for 42 consecutive days, and all procedures followed the ethical guidelines of the Volcani Center Animal Committee (approval number B17051), and all procedures involving the use of animals for *in situ* experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Hebrew University of Jerusalem (ethical approval number AG-14102). The study recruited 34 multiparous high-yielding Israeli Holstein dairy cows for a feeding experiment, and they were housed in covered loose pens at the Volcani Center experimental farm in Rishon LeZion, Israel. The pens were equipped with real-time electronic individual feeders that could record the individual feed intake of cows via sensors that opened specific feeders for each cow (ID tag, S.A.E Kibbutz Afikim, Israel).

Cows were randomly divided into two balanced groups, each containing 17 cows, on the basis of their DIM, BW, parity, and milk yield (MY). After a covariate period of 14 days, to allow the cows to adapt to the feeders and diets, we began to take measurements. The cows were fed basal diets formulated to meet the net energy of lactation (NE_L) and metabolizable protein (MP) requirements (NRC, 2001) based on real production data collected.

The control group, with an average (± SD) 50.0 kg/d ± 6.0 kg/d MY, 178.0 ± 88.5 DIM, 3.0 ± 1.1 parity, and 647.4 kg ± 47.0 kg BW, was fed a wheat-based TMR. The teff group, with 49.9 kg/d ± 3.5 kg/d MY, 185.3 ± 73.0 DIM, 3.1 ± 1.3 parity, and 646.8 kg ± 55.0 kg BW, was fed a TMR containing 11.9% teff hay as a replacement for wheat hay. Both diets were prepared and delivered by Yavne feed center, Kibbutz Yavne, Israel (Table 1). Rations were designed to have similar nutrient concentrations and NE_L.

Feed intake and chemical analysis

The cows were fed daily at 11:00 by a feeder wagon (RMH Mixell 14, Lachish Industries Ltd, Israel) at 105% of the expected intake, which was then adjusted based on each cow's intake data recorded on the previous day. The actual feeding trial was conducted for 28 consecutive days, after a 14-day adaptation period to feeders and diets, during which samples were collected. Feed samples for evaluation of digestibility, intake, and chemical composition were collected twice per week. Ort samples were collected from each feeder at 07:00, before the feeders were

cleaned and fresh feed was added for 3 consecutive days per week and sorted by cow and week for digestibility and intake analysis. Particle size fractions of the two diets (teff and control) and ort samples were determined once every week using the Penn State Particle Size Separator (PSPS) as summarized in Table 2. The PSPS is designed to separate particles by repeated 40 shaking motions through screens of 19.0 mm, 8.0 mm, and 4.0 mm and the bottom pan (Kononoff et al., 2003). Before analysis, feed and ort samples were dried at 60°C for 48 hours and ground using a hammer mill with a 4.0-mm screen, and later to finer particles in a knife mill (Thomas–Willey Laboratory Mill, model 4, Arthur H. Thomas Company, Philadelphia, PA, USA) to pass through a 2-mm screen. Samples of the two diets and ort samples were then analyzed for CP, DM, EE, and OM content in accordance with AOAC (1990) methods. Fiber analyses, i.e., α-amylase heat-stable-treated aNDF, ADF, hemicellulose, cellulose, and ADL were conducted in accordance with the method described by Van Soest et al. (1991), utilizing an Ankom^{200} Fiber Analyzer, model 200l (ANKOM Technology Corp., Macedon, NY, USA).

The gross energy contents of the two diets, ort, and fecal samples were determined using a 6100 Compensated Calorimeter (Parr Instrument Company, Moline, IL, USA). Dry matter intake, CP intake (CPI), NDF intake (NDFI), OM intake (OMI), and ADF intake (ADFI) were computed from pooled weekly samples. The intake of each nutrient was computed as the difference between the content of a specific nutrient found in the feed offered and that found in the orts. The nutrient composition of the two diets was calculated from the chemical composition as presented in Table 3. The balances of rumen-degradable protein (RDP), rumen-undegradable protein (RUP), NE_L, and MP were estimated based on the method described (NRC, 2001) using the average DMI, MY, milk composition, and BW of the cows during the experimental period.

Digestibility measurements

To determine the apparent total tract digestibility of treatment diets, fecal samples were collected by rectal grab sampling from each cow during week 6 of the experiment. Samples were taken for 2 consecutive days at 07:00, 10:30, 15:00, and 22:00. Eight samples were collected from each cow, pooled, then dried in an air-forced oven at 60°C for 48 hours and ground to pass through a 2-mm screen using a knife mill (Thomas–Willey Laboratory Mill, model 4, Arthur H. Thomas Company, Philadelphia, PA, USA). Ground fecal samples were subjected to chemical fiber-based analysis if DM, OM, CP, AND EE, and gross energy was calculated in accordance with the method described above. Approximately 500 mg of each of the sorted fecal, orts, and diet samples were weighed into F57 Dacron filter bags with 25-micron porosity and incubated for 240 hours using a Daisy^{II220} incubator (ANKOM Corp, Fairport, NY, USA) to determine the indigestible NDF (iNDF). The iNDF was used as an internal marker to estimate the fecal amount and calculate nutrient digestibility (Huhtanen et al., 1994). The digestibility of CP, NDF, ADF, OM, and DM were calculated based on the relationship between the feed intake and fecal output of each cow (Adin et al., 2009).

TABLE 1 Ingredients and chemical composition of the experimental diets.

	Treatment	
Item, % of DM	Control	Teff
Ingredients		
Corn, ground	19.7	20.0
Soybean meal	1.9	1.9
Wheat grain	2.9	3.5
Soap stock oil	0.1	0.1
Teff hay	0	11.9
Wheat hay	12.6	0
Wheat silage	23.3	22.7
Rapeseed meal	11.0	11.0
Dried distillers grains	6.6	6.6
Wheat bran	4.2	4.2
Gluten feed	12.4	12.4
Calcium salts	3.1	3.3
Palmitic acid	0.4	0.4
Sodium bicarbonates	0.7	0.7
Calcium carbonate	0.2	0.3
Lactose water	0.8	0.8
[1]Vitamin mix	0.05	0.05
Urea	0.04	0.04
Chemical composition		
CP %	16.5	17.0
NDF %	31.8	32.4
Forage, NDF %	17.5	17.9
Starch %	24	23.2
Ether extract %	5.2	5.3
Calcium %	0.9	0.9
Phosphorus %	0.5	0.7
[2]RDP %	11.4	14.5
[2]RUP %	5.2	4.9
[3]NEL, Mcal/kg DM	1.78	1.78
[4]Gross energy, Mcal/kg	4.05	4.10

[1] Contained 20,000,000 IU/kg of vitamin A; 2,000,000 IU/kg of vitamin D; 15,000 IU/kg of vitamin E; 1,500 mg/kg of Cu; 6,000 mg/kg of Mn; 6,000 mg/kg of Zn; 2,000 mg/kg of Fe; 120 mg/kg of I; 50 mg/kg of Se; and 20 mg/kg of Co.
[2] RDP and RUP are calculated based on the data from NASEM (2021).
[3] Net energy captured in milk calculated according to the data from NASEM (2021).
[4] Gross energy based on bomb calorimeter results.

To determine *in situ* degradability of teff and wheat hay, approximately 4.0 g of teff sample were weighed into Ankom R510, a 5 cm×10 cm Dacron bags with 50-micron porosity and incubated in duplicates inside rumen utilizing two cannulated

TABLE 2 Particle size distribution (DM-Basis) in feed and refusal samples.

	Diet			*p*-value		
Item[1]	Control	Teff	SEM	Diet	Week	Diet X week
Feed						
>19mm	28.8	25.2	1.459	0.108	0.002	0.176
8 mm–19 mm	10.7	8.36	0.662	0.082	0.4262	0.5686
4 mm–8 mm	10.5[a]	8.96[b]	0.303	<0.001	<0.001	<0.239
<4mm	50.0[b]	57.5[a]	1.428	<0.001	0.001	0.004
Refusals						
>19 mm	42.1[b]	62.2[a]	1.762	<0.001	0.003	0.001
8 mm–19 mm	13.9[a]	7.99[b]	0.409	<0.001	0.002	0.0014
4 mm–8 mm	8.78[a]	4.93[b]	0.502	<0.001	<0.001	0.022
<4mm	35.2[a]	24.9[b]	1.373	0.008	0.004	0.001

Different superscripts on LS mean values indicate significant differences at p≤ 0.05. SEM, standard error of the mean. Item[1]-Distribution of particles was conducted using the Penn State Particle size separator tool—calculated as TMR proportions in feed and refusals.

wether Assaf sheep fed a standard diet containing 2.42 Mcal ME, 120.0 g of CP per kg DM basis. Sheep diets comprised 73.0% roughage feeds including wheat silage, wheat hay, clover hay, concentrates, minerals, and vitamins to meet the maintenance requirements (NRC, 2007). Bags were incubated in duplicates at 0 hours, 3 hours, 6 hours, 9 hours, 12 hours, 24 hours, 48 hours, and 96 hours. It is worth noting that incubation at time 0, bags were placed in a hot water bath at 39°C for 1 hour and agitated intermittently to determine the solubility of the samples. After the incubation, the bags were hand-washed until the water was clear

TABLE 3 Effects of diets on the intake and digestibility of nutrients in dairy cows.

	Treatments			
Variable	Control	Teff	SEM	*p*-value
[1]Intake, kg/d				
DM	25.3	25.7	0.560	0.754
OM	24.5	23.6	0.580	0.455
CP	4.87	4.83	0.119	0.870
aNDF	10.7	10.3	0.259	0.430
ADF	4.69	4.46	0.113	0.313
Energy	116.4	110.3	2.784	0.279
Digestibility, %				
DM	62.5	61.7	0.401	0.287
OM	63.9	63.8	0.389	0.928
CP	59.2[b]	61.9[a]	0.682	0.042
[2]aNDF	49.3	49.7	0.454	0.697
ADF	47.2	46.0	0.636	0.353
Energy	61.0	61.0	0.709	0.719
Energy balance				
[3]DEI, Mcal/d	74.2[a]	67.1[b]	1.585	0.022
[4]DE, Mcal/kg DM	2.76[a]	2.65[b]	0.024	0.024

(Continued)

TABLE 3 Continued

	Treatments			
Variable	Control	Teff	SEM	*p*-value
[5]NE_M, Mcal/d	10.3	10.3	0.102	0.978
[6]NE_L, Mcal/d	34.2	33.5	0.563	0.529
[7]EB, Mcal/d	29.6[a]	23.3[b]	1.318	0.014
BW	645.9	644.2	4.310	0.551

LS means with different superscripts are statistically different at $p \leq 0.05$. SEM=standard error of the mean.
[1]Intake=intake during the period of digestibility data collection.
[2]aNDF=amylase-treated NDF.
[3]DEI= DE × DMI.
[4]DE= digestible energy.
[5]NE_M= $BW^{0.75}$ × 0.08.
[6]NE_L= ECM × 0.715.
[7]EB= calculated energy balance.

and dried at 60°C for 48 hours to determine the degradability of nutrients in accordance with the method described by Ørskov and McDonald (1979). Degradability parameters: a (solubility, %), b (potential degradability by time, %), and kd (rate of degradability, %/hours). DM degradability (DMD), crude protein degradability (CPD), NDF degradability (NDFD), and OM degradability (OMD) were computed using a passage rate (kp) of 8.0%.

Milk yield and milk composition analysis

Cows were milked three times a day at 06:00, 14:00, and 21:00, and milk yield was recorded automatically during each milking using the Afifarm software, v. 5.2.1 (Afimilk Ltd, Kibbutz Afikim, 1514800, Israel), installed at the milking parlor. Cows were weighed automatically after each milking by a walk-on scale in the system when exiting the milking parlor. Milk samples were collected from three consecutive milking occasions once every 7 days, four different tests using these samples were conducted. Milk samples were collected into 50.0-mL tubes containing bromo-2-nitropropane-1,3-diol tablets (Advanced Instruments Inc, Two-technology way, Norwood, MA 02062) as a preservative and sent to the Central Laboratory in Caesarea, Israel, for compositional analysis. Milk percentages of fat, lactose, true protein, urea, and somatic cell count were analyzed. Prior to sending the milk samples, 5.0 mL from each milking occasion were pooled from cows to make a total of 15 mL for further analysis. A 2-mL volume of milk was transferred from 15.0-mL tubes to Eppendorf tubes for fatty acid profile analysis as described by Mesilati-Stahy and Argov-Argaman (2014) using an Agilent 6890N network gas chromatograph (Agilent Technologies, Santa Clara, CA, USA).

Calculations of production efficiency and energy balance

Energy-corrected milk (ECM) was calculated using the formula: ECM, kg/d = milk yield (kg/d) × {[0.3887 × milk fat (%)] + [0.2356 × milk protein (%)] + [0.1653 × milk lactose (%)]}/3.1338 kg/d of standard milk containing 3.5% fat, 5% lactose, and 3.5% protein, and an energy value of 0.715 Mcal/d (NRC, 2001). The efficiency of milk production was calculated as ECM/DMI. The energy content in milk was computed following (NRC, 2001) equations. The energy balance (EB) was also calculated on a daily basis using values from NRC (1989) as shown in the equations below.

$$EB = DEI—(NE_M + NE_L)$$

$$DEI\ (Mcal/d) = digestible\ energy\ (Mcal/kg)\ ñ\ DMI\ (kg/d)$$

$$NE_m(Mcal/d) = BW^{0.75} ñ\ 0.08$$

$$NEL\ (Mcal/d) = ECM\ (kg/d)\ ñ\ (0.715)$$

In which, EB = energy balance, NE_L = net energy of lactation, NE_m = net energy required for maintenance, DE = digestible energy, and DEI = digestible energy intake.

Blood metabolites analyses

During week 6 of the experiment, blood samples were collected twice from each cow. Blood was withdrawn from the coccygeal blood vessel during the last day of the experiment using a 20-gauge×2.54 mm needle into 9-mL vacutainer tubes containing Lithium Heparin (BD Vacutainer, Plymouth, UK). The first sampling was carried out in the morning (07:00) before feeding (i.e., rations were removed from feeders) and the second collection was carried out 3 hours after feeding (10:00). Blood glucose and β-hydroxybutyrate (βHB) were measured immediately after sampling (Pichler et al., 2014) using the Freestyle Optimum β-ketone test strips and Freestyle Optimum glucose test strips (Abbott Diabetes Care Ltd, Berkshire, UK). The rest of the blood samples were kept on ice for 4 hours before being centrifuged at 1,500x*g* at 4°C for 20 minutes to obtain plasma. Plasma samples were stored at −20°C until the further analysis of non-esterified fatty acids (NEFA) was carried out in accordance with the method described by Johnson and Peters (1993) using the Wako NEFA kit (Wako chemicals, GmbH, Neuss, Germany). Blood urea nitrogen (BUN) was also analyzed using a calorimetric method read in 96-well plates (96

MaxisorpNunc- Immuno plates, Roskilde, Denmark) in a spectrophotometer (Biotek ELx808, Lumitron Ltd) in accordance with the method described by Coulombe and Favreau (1963).

Rumen fluid analyses

Rumen fluid samples were collected from each cow twice during the last day of the experiment in 50-mL tubes to determine the changes in the concentration of volatile fatty acids (VFA) and ammonia nitrogen ($N–NH_3$) with time. The first collection was conducted at 07:00, after milking and before feeding. The second collection was conducted 3 hours after feeding. All collections were conducted with great care by trained personnel in accordance with the method described in the study by Ben Meir et al. (2021). Rumen fluid (400 mL) was collected from each cow using a self-made esophageal metal-coated rubber pipe (2 m length, 15 mm internal diameter, with 24 pores of 4 mm diameter each around the edge of a metal head) connected to a vacuum pump. To avoid saliva contamination and to ensure an adequate volume of fiber-containing rumen fluid, the vacuum pump was turned on only after the sampler pipe was inserted and the first 200 mL of rumen fluid was discarded to prevent contamination with saliva. The pH of each rumen fluid sample was measured immediately using a pH meter (Sartorius Company, Göttingen, Germany). After centrifugation at 2,000×*g* at 4°C for 10 minutes, samples were stored at −20°C for further analysis. The VFA concentrations were analyzed as described in the study by Erwin et al. (1961) using a Hewlett Packard HP 5890 gas chromatograph (Conquer Scientific Company, Poway, CA, USA). Ammonia concentrations were measured as explained by Bower and Holm-Hansen (1980).

Statistical analysis

Statistical analyses of the data collected were conducted using JMP Pro (version 16.0.0, SAS Institute Inc., Cary, NC, USA). The production data collected during the second week of the experiment were treated as a covariate when relevant. Continuous data, including DMI, OMI, milk yield, milk composition (lactose, fat, protein, urea, and somatic cell count), production efficiency, and ECM were averaged per experimental week and analyzed using a MIXED procedure and a suitable covariance structure with data from the covariate period. The autoregressive order 1 (AR1) structure was found to be the most suitable to be used. Blood metabolites (NEFA, βHB, and blood glucose), milk FA profile, and rumen metabolites ($N–NH_3$, VFA, and rumen pH) were also analyzed using a two-way ANOVA, with the treatment and time of sampling as fixed factors. The statistical model included the fixed effects of treatment (diet), week, and their interaction, and cow as a random effect nested within treatment.

$$Y = \mu + T_i + C(T)_{ij} + L_k + L_k \tilde{n}\ T_i + E_{ijk}$$

In which μ = overall mean, T_i = fixed effect of treatment *i*, $C(T)_{ij}$ = random effect of cow *j* = nested within treatment *i*, L_k = fixed effect of week *k*, $L_k \times T_i$ = the fixed effect of interaction between treatment and week, E_{ijk} = residual error.

Nutrient intake (CP, ADF, and NDF), nutrient digestibility, energy balance, and whole-chemical composition (i.e., CP, OM, DM, ADL, EE, NDF, ADF, hemicellulose, cellulose, and starch) were analyzed using MIXED procedures as previously described, but without the repeated measurements and week effect. The data are presented as LS means with adjusted standard error of the mean (SEM). Statistical differences were considered significant at $p \leq 0.05$ and tendencies at $0.05 < p \leq 0.10$.

Results

Effects of diet and refusals on particle size

The feed particle size distribution (Table 2) was similar for both diets, except for minor differences in the 4 mm–8 mm fraction, in which the particle size was greater in the control group than in the teff group ($p < 0.001$), and the< 4 mm fraction had an interaction between diet and week ($p = 0.004$), with these particles being fewer in the control group than in the teff group in week 2 and week 4 (≈ 17 and ≈8% units, respectively), but slightly more numerous in the control group than in the teff group during week 5 of the trial. On average the 4-mm fraction in teff was greater than the control. Refusals differed between diets, with an interaction between diet and week observed. The greatest differences were noted in week 5 of the trial, with the control group having more than twice the particle distribution than the teff group. In terms of refusal particles > 19 mm in size, we observed that there was a larger number of these in the teff-fed group than in the control group, whereas for<19-mm refusal particles, the teff-fed group had fewer of these than the control group (Table 2).

Feed intake, nutrient digestibility, and energy balance

Feed intake and digestibility did not differ among diets, except in terms of crude protein digestibility, which was greater in teff cows than in control cows by 2.7% (Table 3; $p = 0.042$, 61.9% vs. 59.2%, respectively). Digestible energy intake was higher in control cows than in teff-fed cows ($p = 0.022$; 74.2 Mcal/d vs. 67.1 Mcal/d, respectively). A similar trend was noted with regard to energy balance ($p = 0.014$; 29.6 Mcal/d vs. 23.3 Mcal/d; Table 3).

The *in situ* degradability of teff and wheat hay used for formulating the diets showed that NDF was almost insoluble, with a lower solubility for OM in teff than wheat hay (Figure S1) as elaborated in Table S1. However, CP solubility in teff hay was higher than it was in wheat hay by 18% (55.1% vs. 37.5%, respectively). Despite the potential CPD of teff hay with time being lower than that of wheat hay, its potential OMD with time was higher. Interestingly, despite being almost insoluble, teff hay had a very high potential degradability with time, and its NDFD was up to 80.0%, similar to that of wheat hay. Teff hay had lower rates of degradability per hour than wheat hay for all the parameters (DMD, OMD, CPD, and NDFD) *in situ*. A comparison between the effective degradabilities of teff hay and wheat hay showed that teff

hay had higher CPD values than wheat hay Figure S1, and as detailed in Table S1.

Milk yield and composition

Despite the lack of difference in intake between the diets, teff increased the daily milk yield by 1.5 kg/d ($p< 0.001$; 43.1 kg/d vs. 44.6 kg/d, respectively). Similarly, a higher trend in milk protein ($p = 0.009$; 1.42 kg/d vs. 1.51 kg/d, respectively) and milk lactose yields ($p = 0.003$; 2.10 kg/d vs. 2.26 kg/d, respectively; Table 4) was observed in the teff group than in the control group. There was an interaction between diet and week, which was nearly statistically different for daily milk fat yield ($p = 0.094$), with a higher milk fat yield in teff cows than in control cows in the last week of the experiment (1.73 kg/d vs. 1.60 kg/d, respectively). Milk lactose showed a nearly significant effect of time ($p = 0.078$) as it decreased from 4.90% in week 5 to 4.73% in week 6. Milk yield efficiency expressed per DMI was also nearly significant ($p = 0.063$), with that in the teff-fed group at week 5 being higher than that in the control group (1.75 kg/d vs. 1.64 kg/d, respectively). The ECM yield efficiency expressed per DMI tended to have an interaction effect between diet and week ($p = 0.086$) and tended to be higher in the teff group than in the control group during week 5 of the trial (1.77 kg/d vs. 1.54 kg/d, respectively; Table 4).

Fatty acids profile

A comparison between the two diets showed that teff led to an increase in the levels of some fatty acids, such as C18:1, trans 9, C18:2n6cis, polyunsaturated fatty acids (PUFA), omega-3, and total trans fatty acids concentration, and that the increase associated with C20:0 and this tended to be statistically significant (Table 5). However, levels of C16:0 and saturated fatty acids were reduced in the teff treatment compared with the control, and this tended to be statistically significant. The levels of the rest of the fatty acids were similar among the diets. The analysis of the TMR FA profiles of the control and teff diets showed that the teff diet had higher levels of PUFA than the control diet (Table S2).

Rumen parameters

The pH tended to be statistically different and was higher in the control treatment than in the teff treatment ($p = 0.075$), whereas the concentration of N–NH_3 was higher in the teff treatment than in the control treatment ($p = 0.004$, 18.50 mg/dL vs. 15.83 mg/dL, respectively; Table 6). A comparison of the volatile fatty acids concentration between the teff and control diets showed that the concentration of acetic acid (C2) was higher in the control treatment than in the teff treatment ($p = 0.040$, 61.7 mmol/dL vs. 58.8 mmol/dL, respectively), propionic acid (C3) was higher in the teff treatment than in the control treatment ($p = 0.001$, 26.2 vs. 22.5 mmol/100 mmol, respectively), and the ratio of C2 to C3 was higher in the control treatment than in the teff treatment ($p = 0.014$). The rumen pH decreased between 0 hours and 3 hours in a similar pattern in both the teff and control groups (Δ 0.40, teff vs. Δ 0.22, control). A similar trend was observed for C2 and isovaleric acid [I-C5 (Δ 12.7, teff vs. Δ 14.7, control, and Δ 0.14, teff vs. Δ 0.12, control, respectively]. The rumen N–NH_3 increased between 0 hours and 3 hours (Δ 16.6, teff vs. Δ 14.9, control). In addition, C3, butyric acid (C4), valeric acid (C5), and total VFAs increased between 0 hours and 3 hours (C3; Δ 6.42 vs. Δ 6.86, C4; Δ 5.09 vs. Δ 6.47, C5; Δ 0.99 vs. Δ 1.07 and total VFAs concentration; Δ 39.67 vs. Δ 32.49, for teff vs. control, respectively). It is worth noting that the ratio of C2 to C3

TABLE 4 Effects of diets on dry matter intake, milk yield, and components in dairy cows.

	Diet			*p*-value		
Item	Control	Teff	SEM	Diet	Week	Diet X week
Milk yield (kg/d)	43.1[b]	44.6[a]	0.432	0.045	<0.001	0.941
Milk fat, %	3.96	3.76	0.052	0.172	0.414	0.664
Fat yield, kg/d	1.71	1.72	0.024	0.911	<0.001	0.094
Milk true protein, %	3.32	3.27	0.030	0.565	0.348	0.760
Protein yield, kg/d	1.42[b]	1.51[a]	0.016	0.009	<0.001	0.133
Milk lactose, %	4.86	4.88	0.034	0.723	0.078	0.625
Lactose yield, kg/d	2.10[b]	2.26[a]	0.029	0.003	<0.001	0.235
ECM, kg/d	42.9	44.1	0.538	0.304	<0.001	0.107
MUN, mg/dL	0.22	0.21	0.030	0.664	<0.001	0.992
SCC, × 10^3 cells/mL	145.4	103.9	26.017	0.574	0.047	0.453
Milk yield/DMI, kg/d	1.74	1.74	0.024	0.991	0.067	0.003
ECM/DMI, kg/d	1.71	1.76	0.030	0.427	0.145	0.086

LS means with different superscripts are statistically different at p ≤ 0.05. SEM, standard error of the mean; MUN, milk urea nitrogen; SCC, somatic cell count.

TABLE 5 Effects of diets on the fatty acid composition of milk fat (g/100 g of total fatty acids) in dairy cows.

	Treatment			
Item	Control	Teff	SEM	*p*-value
C8:0	0.42	0.48	0.074	0.703
C10:0	2.87	2.90	0.165	0.914
C12:0	5.18	4.97	0.135	0.456
C14:0	14.8	14.9	0.155	0.870
C16:0	40.1	39.2	0.261	0.073
Sat<C16:0	23.3	23.2	0.380	0.940
Sat >C16:0	8.76	8.40	0.177	0.320
C16:1n7	1.75	1.78	0.064	0.830
C16:1/C16:0	0.04	0.05	0.002	0.586
C18:0	8.42	8.04	0.180	0.296
C18:1n7	0.75	0.79	0.027	0.426
C18:1, trans 9	1.73	1.93	0.053	0.051
C18:2n6 trans	0.53	0.60	0.022	0.135
C18:2n6 cis	3.34[b]	3.62[a]	0.063	0.019
C18:3n6	0.03	0.05	0.010	0.256
C18:3n3	0.28	0.27	0.007	0.803
C18:1, cis 9	19.1	19.8	0.242	0.169
C18:1/C18:0	2.31	2.48	0.055	0.113
C18:0/C16:0	0.21	0.21	0.005	0.661
C20:0	0.34	0.37	0.007	0.078
C20:1n9	0.10	0.09	0.010	0.760
C20:4n6	0.16	0.16	0.003	0.813
C20:5n3	0.02	0.05	0.012	0.224
C20:1n9/C20:0	2.89	3.22	0.138	0.238
C22:6n3	0.01	0.01	0.004	0.766
Saturated	72.2	70.8	0.370	0.067
MUFA	23.4	24.3	0.319	0.138
PUFA	4.36[b]	4.77[a]	0.084	0.013
Omega-3	3.43[b]	3.71[a]	0.062	0.022
Omega-6	0.31	0.34	0.016	0.377
∑ trans fatty acids	2.26[b]	2.53[a]	0.065	0.033

LS means with different superscripts are statistically different at $p \leq 0.05$. MUFA, monounsaturated fatty acids. PUFA, polyunsaturated fatty acids; ∑ trans fatty acids, total trans fatty acids concentration.

decreased between 0 hours and 3 hours (Δ 1.24, teff vs. Δ 1.60, control).

Blood metabolites

The glucose level was similar between the treatments and averaged 46.2 mg/dL. Beta-hydroxybutyrate tended to be statistically different with higher concentrations in the control treatment than in the teff treatment (p = 0.072, 1.02 mmol vs. 0.83 mmol, respectively). A similar trend was noted in NEFA (p = 0.015, 201.8 μEq/L vs. 166.1 μEq/L, respectively), whereas the BUN was lower in the control treatment than in the teff treatment (p = 0.0460, 24.4 mg/dL vs. 27.8 mg/dL, respectively; Table 7). Blood glucose and BUN decreased in both diets between 0 hours and 3 hours (glucose; Δ 4.35 vs. Δ 1.71, BUN; Δ 2.78 vs. Δ 2.99 for teff vs.

10.3389/fanim.2023.1260787

TABLE 6 Effects of diet on N–NH3, rumen pH and VFA in dairy cows.

	Diet			*p*-value		
Item	Control	Teff	SEM	Diet	Time	Diet x Time
$N–NH_3$, mg/dL	15.8[b]	18.5[a]	1.106	0.040	<0.001	0.314
Rumen pH	6.89	6.75	0.039	0.075	<0.001	0.124
Volatile fatty acids, mmol/100 mmols						
C2	61.7[a]	58.8[b]	0.997	0.040	<0.001	0.133
C3	22.5[b]	26.2[a]	0.610	0.001	<0.001	0.667
C2:C3	2.90[a]	2.43[b]	0.119	0.014	<0.001	0.147
C4	13.3	12.2	0.458	0.105	<0.001	0.189
I-C5	1.06	1.18	0.039	0.141	0.080	0.882
C5	1.29	1.40	0.070	0.161	<0.001	0.451
Total VFAs	91.8	97.4	3.345	0.326	<0.001	0.429

LS means with different superscripts are statistically different at p≤ 0.05. SEM, standard error of the mean.

control, respectively) whereas NEFA and βHB increased between 0 hours and 3 hours (NEFA; Δ 17.5 vs. Δ 51.3, βHB; Δ 0.70 vs. Δ 0.83 for teff vs. control, respectively).

Discussion

In this study, we aimed to assess the effect of teff hay inclusion in rations on dairy cows' production and performance. We compared feed intake and digestibility, milk yield and composition, fatty acids composition, rumen fluid parameters (pH and $N–NH_3$ and VFA concentrations), and blood metabolites (glucose, βHB, NEFA, and BUN) between teff- and wheat-based rations. The inclusion of teff led to increased milk production, milk protein, and lactose yields.

Feed intake, nutrient digestibility, and energy efficiency

Feed intake was similar for both diets, with an average DMI of 25.5 kg/d and DM and OM digestibilities having similar averages (62.1% and 63.9%, respectively). Factors such as particle size, particle fragility, NDF content, forage-to-concentrate ratio, the passage rate of feed in the reticulorumen, and the ruminal distension of the reticulorumen resulting from gut fill may influence intake in ruminants (Allen, 1996; Allen, 2000; Zebeli et al., 2007). The NDF content tended to be higher in teff hay than in wheat hay (70% vs. 62%; respectively) as reported by Beck et al. (2009) and Staniar et al. (2010). Hence, we expected to observe differences in the intake of nutrients between the two diets. However, there were no actual differences observed, probably because of the different inclusion levels of dietary forage (11.9% teff vs. 12.6% wheat in DM). In addition, cows in the control group consumed more of the long particles (>19 mm), whereas those in the teff group consumed more of the short particles (<19 mm) which could partially explain the similarities in intake. Longer particle size leads to greater fill as a result of a slow passage rate, whereas a reduction in particle size increases the passage rate (Allen, 2000; Yansari et al., 2004). Longer particles contain more NDF and ADF content than finely chopped particles (Sudweeks et al., 1979; Bal et al., 2000). Similar DMI results (ranging from 25 kg/d–27 kg/d) were reported by Rezac et al. (2012) between the cows fed 20% tall prairie hay (68.0% NDF) and cows fed corn silage (43.0% NDF), alfalfa hay (44.0% NDF), and wet corn gluten feed (38.0% NDF). Saylor et al. (2018) also reported a similar intake averaging 28 kg/d in cows fed different forages (teff vs. corn silage, alfalfa, and prairie hay).

The higher CPD in the teff diet (61.9%) might be attributable to the higher level of RDP in and solubility of teff hay than in wheat hay. However, digestibility in ruminants can be influenced by other

TABLE 7 Effects of diets on blood concentration of glucose, βHB, NEFA, and BUN in dairy cows.

	Diets			*p*-value		
Variable	Control	Teff	SEM	Diet	Time	Diet x Time
Glucose (mg/dL)	46.4	46.1	1.054	0.896	0.085	0.444
βHB(mmol)	1.02	0.83	0.072	0.072	<0.001	0.414
NEFA (μEq/L)	201.8[a]	166.1[b]	7.643	0.015	0.024	0.252
BUN (mg/dL)	24.4[b]	27.8[a]	0.712	0.046	0.011	0.921

LS means with different superscript letters are statistically different at p≤ 0.05. SEM, standard error mean.

factors such as their eating patterns, the retention time of feed in the rumen, fiber composition, level of intake, passage rate, and particle size of feed (Van Soest, 1994). In our case, similarities in DMD, OMD, NDFD, and ADFD could be attributed to similar intakes of nutrients, as discussed earlier, and differences in the inclusion levels of forage, but most importantly, the similarities in their retention time in the rumen. The selective consumption of particles between the two groups could also explain the similar digestibilities observed, which was in agreement with a study by Yansari et al. (2004), who reported a similar digestibility of nutrients in TMR diets containing different particle sizes in dairy cows. A study by Saylor et al. (2018) also found no differences in DMD and NDFD when teff hay was used solely to replace alfalfa hay, corn silage, and prairie hay in dairy cows. It might be concluded that cows consuming the teff-based TMR diet selected and consumed smaller particles, which in turn might support the similar retention time in the rumen and apparent total tract digestibility of nutrients in both the teff-based and wheat-based diets.

The DEI, DE, and EB were lower in the teff treatment than in the control treatment, whereas the BW, NE_M, and NE_L were similar between the teff and control treatments. Despite the lower energy balance values in the teff than in the control diets, the cows fed the teff diet had higher milk yields, which would imply that they had more efficient energy utilization. We postulated that the energy in the teff-fed cows was retained for production purposes rather than losses in heat production.

Milk yield, composition, and efficiency of production

An increment of 1.5 kg/d milk yield was recorded for cows fed the teff diet compared with cows fed the control diet, with a similar trend in milk lactose and true protein yields. The concentrations of milk fat, milk true proteins, milk lactose, and ECM were similar in both diets. The milk parameters assessed in our study were within acceptable ranges in high-yielding dairy cows, as reported by Ben Meir et al. (2021) and Habel et al. (2022). Our results are also similar to the findings of Saylor et al. (2018), who reported a tendency to increase milk protein yield and milk urea nitrogen (MUN) associated with a teff-based diet compared with a control diet. The ECM and ECM/DMI results were similar to earlier findings reported in dairy cows by Saylor et al. (2018) and Ben Meir et al. (2021). However, our results pertaining to milk protein concentration and lactose yield were contradictory to the earlier findings of Saylor et al. (2018). The differences between our findings and those of Saylor et al. (2018) could be attributed to the use of teff hay as the sole feed in their study; in our study, teff was mixed in TMR. The milk produced by cows consuming a teff-based diet had numerically higher total VFAs concentrations than that produced by those consuming a control diet with predominantly C2, C3, and C4, constituting approximately 70% of ME in ruminants (Dijkstra, 1994; Reynolds et al., 2003; Seymour et al., 2005). Our results showed that the fermentation of the teff-based diet increased propionate, lowering the ratio of C2 to C3 in the rumen. The increased C3 is a major glucogenic precursor (Larsen and Kristensen, 2009; Aschenbach et al., 2010; Dijkstra et al., 2012) supplying over 65% net glucose in lactating cows (Reynolds et al., 2003; Seymour et al., 2005) partitioned for the higher milk, lactose, and protein yields in the teff-based diet than in the control diet. The propionate and total VFA concentrations were positively correlated with milk yield, protein, and acetate, which was negatively correlated with milk yield (Seymour et al., 2005). Hence, feeding teff to high-yielding dairy cows could be beneficial as it increased propionate associated with increased milk yield and proteins.

Rumen ammonia nitrogen, VFA, and pH

In both treatments, $N–NH_3$ concentration was within normal ranges as reported by (Schaefer et al., 1980; Pengpeng and Tan, 2013) which is usually sufficient for rumen microbial growth and below toxicity levels (Patra, 2015). The teff-based diet had higher concentrations of ruminal $N–NH_3$. It increased 3 hours post-feeding and was still higher in the teff diet than in the control diet. The ruminal $N–NH_3$ concentrations in the teff diet in our study were higher than previously reported values in the diets of beef cattle (Vinyard et al., 2018; Ream et al., 2020). The differences between our findings and the earlier findings of Vinyard et al. (2018) and Ream et al. (2020) could be attributed to the level of feed intake (beef heifers and steers vs. dairy cows). In addition, the teff variety might affect its composition and nutritional value (Wagali et al., 2023). Nonetheless, the teff hay used in the earlier studies by Vinyard et al. (2018) and Ream et al. (2020) was harvested at boot, early heading, or late heading, whereas our teff hay was harvested at early heading. It is probable that the elevated concentrations of $N–NH_3$ observed in the teff-based diet are attributable to the high solubility of CP in teff hay compared with that in wheat hay and the salivary supply of urea that is converted to $N–NH_3$ (Bailey and Balch, 1961). However, the accumulation of $N–NH_3$ implies that there were inadequate levels of the ruminally degradable carbohydrates which initiate the fermentation of amino acids as an energy source, causing nitrogen loss (Nocek and Russell, 1988; Hristov and Jouany, 2005). The utilization of rumen $N–NH_3$ is dependent on the carbohydrate and nitrogen supply balance, synchronization, and availability for microbes (Hristov and Jouany, 2005). Carbohydrate availability influences the microbial growth rate and the efficient utilization of $N–NH_3$ in the rumen (Russell et al., 1983). The higher $N–NH_3$ concentration in the teff-based diet implies asynchrony between protein and available energy, in turn hindering the efficient conversion of nitrogen into MCP and amino acids. Thus, it is necessary to ensure the synchronization of available nitrogen and energy in the diet to improve ruminal fermentation, feed utilization, and MPS (Cabrita et al., 2006).

The pH of both diets was within the optimal range (6–7) for rumen physiology (Patra, 2015). Ruminal pH tended to have a lower value in the teff-based diet than in the control diet. A decrease (Δ 0.31) in pH was observed 3 hours post-feeding (6.98, control, vs. 6.67, teff). Similar findings were reported on dairy cows fed TMR containing mixed hay and haylage in the study by Duffield et al. (2004). The total VFA concentration had a greater influence on the

rumen pH than the concentrations of individual VFAs (Dijkstra, 1994). The slightly lower ruminal pH in the teff-based diet was attributed to the total VFA. A slight decline in the ruminal pH post-feeding (3 hours) could be due to the production of VFA during the fermentation process, as postulated by Allen (1997). That being said, it should be kept in mind that both TMRs in the current study contained a buffering agent (sodium bicarbonates) that might have compromised the real effect of fermentation on the pH values.

Our VFA results are within the acceptable ranges (≤ 130 mM) previously reported by Bannink and Tamminga (2005). Although slightly lower, the results were also similar to the VFA ranges summarized in the study by Seymour et al. (2005). A teff-based diet decreased the molar proportion of acetic acid, which is often correlated with decreased milk yield, but favored the production of more total VFA concentration and increased the molar proportion of propionic acid. This is desirable in increasing milk yield and proteins as propionic acid is glucogenic, constituting over 65% of the net glucose supply that could be partitioned for milk production in lactating cows, as reported by Reynolds et al. (2003) and Seymour et al. (2005).

Blood metabolites

The glucose levels were similar in both diets (averaged 46.3 mg/dL). However, during the pre-feeding period, the glucose level was higher than average 3 hours after feeding. Our results agree with the findings of Piantoni et al. (2015), who reported a decrease in plasma glucose levels 4 hours post-feeding in early and late lactating dairy cows. The increased production of propionate in the rumen, followed by higher absorption into the circulation, prompts insulin secretion and gluconeogenesis, resulting in lower levels of glucose 3 hours post-feeding compared with pre-feeding (Aschenbach et al., 2010). It was also postulated that immediately after feeding, glucose was converted into glycogen and stored in the liver during the absorptive phase of digestion to be used during the post-absorptive phase (Aschenbach et al., 2010).

Blood urea N concentrations were significantly higher in cows consuming the teff-based diet than those consuming the control diet. Despite the levels of BUN concentration dropping over time, teff steadily demonstrated elevated concentrations compared with the control. Teff proteins are highly soluble and rapidly degraded to N–NH_3; consequently, detoxification of N–NH_3 to urea-N in the liver triggers the accumulation of BUN in the blood (Satter, 1986; Nocek and Russell, 1988; Hristov et al., 2011). It is proposed that the increase in BUN could be related to the higher ruminal N–NH_3 concentrations. In this study, we showed that teff hay had a higher soluble CP fraction than wheat hay (this was demonstrated *in situ*). Apart from the dietary source, the continuous secretion of saliva by the parotid and sublingual glands enhances the urea supply to the rumen (Bailey and Balch, 1961). This could perhaps contribute to the higher concentration of BUN in the cows fed the teff diets. The decreased ruminal concentrations of BUN observed 3 hours post-feeding could be related to recycling it back to the rumen N–NH_3 pool, saliva, and excretion in urine and feces (Lapierre and Lobley, 2001; Marini and Van Amburgh, 2003), causing a decline in BUN concentration over time.

Cows fed the teff-based diet had lower plasma NEFA concentrations, with a slight decrease in βHB levels compared with the control group. The plasma NEFA concentrations and βHB levels declined with time, with those in the cows on the teff-based diet being consistently lower than in those cows on the control diet. Comparable findings on βHB were reported in dairy cows 4 hours post-feeding by Piantoni et al. (2015). High levels of plasma NEFA indicate negative energy balance (NEB) in ruminants, which is typically associated with low blood glucose concentrations and increased levels of plasma βHB (Bell, 1995). Although plasma NEFA and βHB were significantly lower in teff-fed cows, the blood glucose concentration was similar between the two dietary treatments. Ospina et al. (2010) benchmarked ≥ 600 μEq/L of NEFA as a higher concentration in dairy cows. Thus, the plasma concentrations of NEFA and βHB observed were within the tolerance threshold of high-yielding dairy cows in mid-lactation. The plasma concentrations of NEFA and βHB reduced as the cows' EB decreased, which contrasts with the findings of Gross et al. (2011), who reported elevated plasma NEFA and βHB levels along with a decrease in EB in dairy cows. The differences between our results and those of Gross et al. (2011) could be due to the fact that the cows studied in their experiment were in early lactation and ours were in late lactation. Furthermore, in the cows in their study, in contrast to ours, NEB was induced by physiological feed intake restriction. According to Craninx et al. (2008), reductions in the concentrations of short- and medium-chain fatty acids (SMCFA; C4:0-C14:0) in milk fat indicate elevated plasma NEFA concentrations. However, the SMCFA concentrations were similar in both dietary groups, suggesting that the plasma NEFA was within normal limits. Bjerre-Harpøth et al. (2012) also found no discernible effect of partial feed restriction on plasma βHB levels in mid- and late-lactating dairy cows. This suggests that the increase in plasma NEFA and βHB concentrations of mid-lactating dairy cows was not solely related to the NEB. It is worth noting that energy losses in heat production were not calculated in the current study. It is therefore likely that the EB in teff-fed cows might have been retained for production purposes, sparing fat mobilization and hence lowering NEFA and βHB concentrations.

Milk fatty acids profile

The FA profile was within the normal ranges reported in previous studies (Jensen et al., 1991; Palmquist et al., 1993; Fant et al., 2023). The higher concentration of C16:0 in the control diet is similar to that found previously by (Gross et al., 2011), who demonstrated that the increase in C16:0 was correlated with the increased EB of animals. However, in our study, we found that the teff diet was associated with an increase in the total trans FA concentration with decreased EB (relative to the control). This finding contradicts the report by Stoop et al. (2009), who found an increase in trans FA with improved EB. The differences in our results and the aforementioned study could be ascribed to differing stages of lactation. In Stoop et al. (2009), samples were collected from early-lactation cows, which could have had a higher NEB, whereas, we studied mid-to late-lactation cows, which could have

had a lower NEB. In addition, in the study by Stoop et al. (2009), primiparous Holstein–Friesian cows were used, whereas we used multiparous Holstein–Friesian cows. Triglycerides constitute approximately 98% of total milk fat (Jensen et al., 1991; Moate et al., 2007). Milk FA synthesis occurs through biohydrogenation or bacterial degradation in the rumen, *de novo* synthesis in the mammary gland, mobilization of body fats, and direct incorporation from the diet (Stoop et al., 2009; Mesilati-Stahy and Argov-Argaman, 2018). Alteration in these processes influences the FA composition of milk fat during lactation (van Knegsel et al., 2005; Stoop et al., 2009). The FA profile of the teff diet had numerically higher concentrations of PUFA than that of the control diet. In line with this, El-Alfy et al. (2012) showed that higher levels of oleic acids, linolenic acids, and PUFA existed in teff. This may explain the higher concentration of long-chain fatty acids (LCFA) in milk fat observed in the present study due to the fact that LCFA is sourced mostly from diet rather than fat immobilization (Craninx et al., 2008). In addition, calcium soap supplements that were added to rations in the current study to support NE_L requirements, might protect C18:2 against rumen biohydrogenation (Palmquist et al., 1993). Thus, the slightly higher calcium soaps incorporated in the teff diet to balance the NE_L could be a possible reason for higher levels of C18:2 in the milk fat observed. These findings agree with a study by (Palmquist et al., 1993) which demonstrated a higher concentration of C18:2 in the calcium soaps diet than in other dietary treatments. The higher level of dietary C18:2 may have been the source of the trans fatty acids that were found at higher concentrations in the milk of cows in the teff group. Trans fatty acids originate from the rumen biohydrogenation of PUFA (Lock and Bauman, 2004; Lock et al., 2005; Lock et al., 2006) In addition, LCFA transfer from plasma to milk FA inhibits the *de novo* synthesis of short-chain fatty acids (Garnsworthy and Huggett, 1992; Palmquist et al., 1993). It is likely that the trivial concentration of SCFA in teff could be due to the repressive effect of LCFA in cows. The decrease in the EB of dairy cows can impact their FA profile by reducing the supply of acetate and glucose for the *de novo* synthesis of SCFA (Palmquist et al., 1993; Gross et al., 2011), thus triggering an increase in LCFA (C18:0-C22:0). On the other hand, the high concentration of C16:0 in the control diet could be linked to a higher level of acetate production, similar to that in a study by Ørskov et al. (1969).

Conclusions

We assessed the effect of teff hay inclusion in rations on dairy cows' production and performances. We compared the feed intake, digestibility, energy balance, milk yield and composition, fatty acids profile, rumen parameters ($N–NH_3$, rumen pH, and VFA), and blood metabolites (glucose, NEFA, and βHB) of the teff and control diets. Our results showed that teff inclusion in dairy cow rations did not affect intake, but increased crude protein digestibility. It also increased milk, milk protein, and lactose yields, and levels of polyunsaturated fatty acids and omega-3 fatty acids (which would improve human health). Teff inclusion also increased propionic acid, ruminal ammonia nitrogen, and blood urea nitrogen in circulation, but decreased non-esterified fatty acids and beta-hydroxybutyrate in the blood circulation of the cows. These results suggest that teff can be used to improve the production and performance of dairy cows.

Data availability statement

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

Ethics statement

The animal study was approved by the Volcani Center Animal Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

PW: Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft. GN: Data curation, Formal Analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. JK: Formal Analysis, Methodology, Writing – review & editing. CS: Formal Analysis, Methodology, Writing – review & editing. SB-Z: Methodology, Writing – review & editing, Validation, Visualization. YB-M: Methodology, Writing – review & editing. NA-A: Methodology, Writing – review & editing, Validation. YS: Validation, Writing – review & editing, Conceptualization, Funding acquisition, Project administration, Supervision. SM: Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing, Data curation, Formal Analysis, Investigation, Methodology, Visualization.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded by The Chief Scientist of the Ministry of Agriculture, grant number 12-01-0032, and The Israeli Dairy Board, grant number 820-0328-17.

Acknowledgments

The authors thank The Chief Scientist of the Ministry of Agriculture and The Israeli Dairy Board for partially funding this research.

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2023.1260787/full#supplementary-material

References

Adin, G., Solomon, R., Nikbachat, M., Zenou, A., Yosef, E., Brosh, A., et al. (2009). Effect of feeding cows in early lactation with diets differing in roughage-neutral detergent fiber content on intake behavior, rumination, and milk production. *J. Dairy Sci.* 92, 3364–3373. doi: 10.3168/jds.2009-2078

Allen, M. S. (1996). Physical constraints on voluntary intake of forages by ruminants. *J. Anim. Sci.* 74, 3063. doi: 10.2527/1996.74123063x

Allen, M. S. (1997). Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80, 1447–1462. doi: 10.3168/jds.S0022-0302(97)76074-0

Allen, M. S. (2000). Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83, 1598–1624. doi: 10.3168/jds.S0022-0302(00)75030-2

AOAC (1990). ""Plants and animals,"," in *AOAC: official methods of analysis*. Ed. K. Helrich (Arligton: The Association of Agricultural Chemists), 40–82.

Aschenbach, J. R., Kristensen, N. B., Donkin, S. S., Hammon, H. M., and Penner, G. B. (2010). Gluconeogenesis in dairy cows: The secret of making sweet milk from sour dough. *IUBMB Life* 62, 869–877. doi: 10.1002/iub.400

Assefa, K., Chanyalew, S., and Tedele, Z. (2013). "Achievements and prospects of tef improvement," in *Proceedings of the second international workshop, november 7-9, 2013, debre zeit, Ethiopia*. Eds. K. Solomon, S. Chanyalew and Z. Tadele (Debre Zeit, Ethiopia: Universität Bern), 33–51.

Bailey, C. B., and Balch, C. C. (1961). Saliva secretion and its relation to feeding in cattle. *Br. J. Nutr.* 15, 383–402. doi: 10.1079/BJN19610048

Bal, M. A., Shaver, R. D., Jirovec, A. G., Shinners, K. J., and Coors, J. G. (2000). Crop processing and chop length of corn silage: effects on intake, digestion, and milk production by dairy cows. *J. Dairy Sci.* 83, 1264–1273. doi: 10.3168/jds.S0022-0302(00)74993-9

Bannink, A., and Tamminga, S. (2005). ""Rumen function.,"," in *Quantitative aspects of ruminant digestion and metabolism* (UK: CABI Publishing), 263–288. doi: 10.1079/9780851998145.0263

Beck, P. A., Stewart, C. B., Gray, H. C., Smith, J. L., and Gunter, S. A. (2009). Effect of wheat forage maturity and preservation method on forage chemical composition and performance of growing calves fed mixed diets12. *J. Anim. Sci.* 87, 4133–4142. doi: 10.2527/jas.2009-2184

Bell, A. W. (1995). Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.* 73, 2804. doi: 10.2527/1995.7392804x

Ben Meir, Y. A., Nikbachat, M., Portnik, Y., Jacoby, S., Adin, G., Moallem, U., et al. (2021). Effect of forage-to-concentrate ratio on production efficiency of low-efficient high-yielding lactating cows. *Animal* 15, 100012. doi: 10.1016/j.animal.2020.100012

Bjerre-Harpøth, V., Friggens, N. C., Thorup, V. M., Larsen, T., Damgaard, B. M., Ingvartsen, K. L., et al. (2012). Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. *J. Dairy Sci.* 95, 2362–2380. doi: 10.3168/jds.2011-4419

Bower, C. E., and Holm-Hansen, T. (1980). A salicylate–hypochlorite method for determining ammonia in seawater. *Can. J. Fisheries Aquat. Sci.* 37, 794–798. doi: 10.1139/f80-106

Buxton, D. R. (1996). Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Anim. Feed Sci. Technol.* 59, 37–49. doi: 10.1016/0377-8401(95)00885-3

Cabrita, A. R. J., Dewhurst, R. J., Abreu, J. M. F., and Fonseca, A. J. M. (2006). Evaluation of the effects of synchronising the availability of N and energy on rumen function and production responses of dairy cows – a review. *Anim. Res.* 55, 1–24. doi: 10.1051/animres:2005045

Chanyalew, S., Ferede, S., Damte, T., Fikre, T., Genet, Y., Kebede, W., et al. (2019). Significance and prospects of an orphan crop tef. *Planta* 250, 753–767. doi: 10.1007/s00425-019-03209-z

Coulombe, J. J., and Favreau, L. (1963). A new simple semimicro method for colorimetric determination of urea. *Clin. Chem.* 9, 102–108. doi: 10.1093/clinchem/9.1.102

Craninx, M., Steen, A., Van Laar, H., Van Nespen, T., Martín-Tereso, J., de Baets, B., et al. (2008). Effect of lactation stage on the odd- and branched-chain milk fatty acids of dairy cattle under grazing and indoor conditions. *J. Dairy Sci.* 91, 2662–2677. doi: 10.3168/jds.2007-0656

Davison, M. L., and Creech, E. (2011)The potential for teff as an alternative forage crop for irrigated regions. In: *2011 western alfalfa & Forage conference* (Las Vegas, Nevada: UC Cooperative Extension, Plant Sciences Department, University of California, Davis, CA 95616). Available at: https://alfalfa.ucdavis.edu/+symposium/2011/index.aspx (Accessed May 15, 2023).

Dijkstra, J. (1994). Production and absorption of volatile fatty acids in the rumen. *Livest Prod Sci.* 39, 61–69. doi: 10.1016/0301-6226(94)90154-6

Dijkstra, J., Ellis, J. L., Kebreab, E., Strathe, A. B., López, S., France, J., et al. (2012). Ruminal pH regulation and nutritional consequences of low pH. *Anim. Feed Sci. Technol.* 172, 22–33. doi: 10.1016/j.anifeedsci.2011.12.005

Duffield, T., Plaizier, J. C., Fairfield, A., Bagg, R., Vessie, G., Dick, P., et al. (2004). Comparison of techniques for measurement of rumen pH in lactating dairy cows. *J. Dairy Sci.* 87, 59–66. doi: 10.3168/jds.S0022-0302(04)73142-2

El-Alfy, T. S., Ezzat, S. M., and Sleem, A. A. (2012). Chemical and biological study of the seeds of Eragrostis tef (Zucc.) Trotter. *Nat. Prod Res.* 26, 619–629. doi: 10.1080/14786419.2010.538924

Erwin, E. S., Marco, G. J., and Emery, E. M. (1961). Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44, 1768–1771. doi: 10.3168/jds.S0022-0302(61)89956-6

Fant, P., Leskinen, H., Ramin, M., and Huhtanen, P. (2023). Effects of replacement of barley with oats on milk fatty acid composition in dairy cows fed grass silage-based diets. *J. Dairy Sci.* 106, 2347–2360. doi: 10.3168/jds.2022-22327

Garnsworthy, P. C., and Huggett, C. D. (1992). The influence of the fat concentration of the diet on the response by dairy cows to body condition at calving. *Anim. Sci.* 54, 7–13. doi: 10.1017/S000335610002050X

Girija, A., Jifar, H., Jones, C., Yadav, R., Doonan, J., and Mur, L. A. J. (2022). Tef: a tiny grain with enormous potential. *Trends Plant Sci.* 27, 220–223. doi: 10.1016/j.tplants.2021.11.011

Gross, J., van Dorland, H. A., Bruckmaier, R. M., and Schwarz, F. J. (2011). Milk fatty acid profile related to energy balance in dairy cows. *J. Dairy Res.* 78, 479–488. doi: 10.1017/S0022029911000550

Habel, J., Chapoutot, P., Koch, C., and Sundrum, A. (2022). Estimation of individual glucose reserves in high-yielding dairy cows. *Dairy* 3, 438–464. doi: 10.3390/dairy3030033

Habte, E., Muktar, M. S., Negawo, A. T., Lee, S.-H., Lee, K.-W., and Jones, C. S. (2019). An overview of teff (Eragrostis teff zuccagni) trotter) as a potential summer forage crop in temperate systems. *J. Korean Soc. Grassland Forage Sci.* 39, 185–188. doi: 10.5333/KGFS.2019.39.3.185

Hagos, T., and Melaku, S. (2009). Feed intake, digestibility, body weight and carcass parameters of Afar rams fed tef (Eragrostis tef) straw supplemented with graded levels of concentrate mix. *Trop. Anim. Health Prod* 41, 599–606. doi: 10.1007/s11250-008-9230-6

Hristov, A. N., Hanigan, M., Cole, A., Todd, R., McAllister, T. A., Ndegwa, P. M., et al. (2011). Review: Ammonia emissions from dairy farms and beef feedlots. *Can. J. Anim. Sci.* 91, 1–35. doi: 10.4141/CJAS10034

Hristov, A. N., and Jouany, J.-P. (2005). ""Factors affecting the efficiency of nitrogen utilization in the rumen. In Nitrogen and phosphorus nutrition of cattle: reducing the environmental impact of cattle operations,"," in *Nitrogen and phosphorus nutrition of cattle*. Eds. A. N. Haristov and J. P. Jouarny (Wallingford UK: CABI Publishing), 117–166.

Huhtanen, P., Kaustell, K., and Jaakkola, S. (1994). The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Anim. Feed Sci. Technol.* 48, 211–227. doi: 10.1016/0377-8401(94)90173-2

Jensen, R. G., Ferris, A. M., and Lammi-Keefe, C. J. (1991). The composition of milk fat. *J. Dairy Sci.* 74, 3228–3243. doi: 10.3168/jds.S0022-0302(91)78509-3

Johnson, M. M., and Peters, J. P. (1993). Technical note: an improved method to quantify nonesterified fatty acids in bovine plasma. *J. Anim. Sci.* 71, 753–756. doi: 10.2527/1993.713753x

Kononoff, P. J., Heinrichs, A. J., and Buckmaster, D. R. (2003). Modification of the penn state forage and total mixed ration particle separator and the effects of moisture content on its measurements. *J. Dairy Sci.* 86, 1858–1863. doi: 10.3168/jds.S0022-0302(03)73773-4

Lapierre, H., and Lobley, G. E. (2001). Nitrogen recycling in the ruminant: A review. *J. Dairy Sci.* 84, E223–E236. doi: 10.3168/jds.S0022-0302(01)70222-6

Larsen, M., and Kristensen, N. B. (2009). Effect of abomasal glucose infusion on splanchnic and whole-body glucose metabolism in periparturient dairy cows. *J. Dairy Sci.* 92, 1071–1083. doi: 10.3168/jds.2008-1453

Lawrence, J. D., Mintert, J., Anderson, J. D., and Anderson, D. P. (2008). Feed grains and livestock: impacts on meat supplies and prices. *CHOICES* 23, 11–15.

Lock, A. L., and Bauman, D. E. (2004). Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids* 39, 1197–1206. doi: 10.1007/s11745-004-1348-6

Lock, A. L., Overton, T. R., Harvatine, K. J., Giesy, J., and Bauman, D. E. (2006). Milk fat depression: impact of dietary components and their interaction during rumen fermentation, in *Proc. Cornell nutr. Conf.* Eds. A. L. Lock, T. R. Overton, K. J. Harvatine, J. Giesy and D. E. Bauman (Ithaca, New York: Cornell University), 75–85.

Lock, A. L., Parodi, P. W., and Bauman, D. E. (2005). The biology of trans fatty acids: implications for human health and the dairy industry. *Aust. J. Dairy Technol.* 60, 134–142.

Marini, J. C., and Van Amburgh, M. E. (2003). Nitrogen metabolism and recycling in Holstein heifers12. *J. Anim. Sci.* 81, 545–552. doi: 10.2527/2003.812545x

Mengesha, M. H. (1966). Chemical composition of teff (Eragrostis tef) compared with that of wheat, barley and grain sorghum. *Econ Bot.* 20, 268–273. doi: 10.1007/BF02904277

Mesilati-Stahy, R., and Argov-Argaman, N. (2014). The relationship between size and lipid composition of the bovine milk fat globule is modulated by lactation stage. *Food Chem.* 145, 562–570. doi: 10.1016/j.foodchem.2013.08.077

Mesilati-Stahy, R., and Argov-Argaman, N. (2018). Changes in lipid droplets morphometric features in mammary epithelial cells upon exposure to non-esterified free fatty acids compared with VLDL. *PloS One* 13, e0209565. doi: 10.1371/journal.pone.0209565

Miller, D. (2011) *Teff grass: crop overview and forage production guide* (California). Available at: http://www.calwestseeds.com/products/teff/ (Accessed May 15, 2023).

Moate, P. J., Chalupa, W., Boston, R. C., and Lean, I. J. (2007). Milk fatty acids. I. Variation in the concentration of individual fatty acids in bovine milk. *J. Dairy Sci.* 90, 4730–4739. doi: 10.3168/jds.2007-0225

NASEM (2021). *Nutrient requirements of dairy cattle. 8th ed* (NASEM Washington, D.C: National Academies Press). doi: 10.17226/25806

Nocek, J. E., and Russell, J. B. (1988). Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *J. Dairy Sci.* 71, 2070–2107. doi: 10.3168/jds.S0022-0302(88)79782-9

National Research Council (NRC) (1989). *Food and Nutrition Board Recommended Dietary Allowances*. *10*th Edition (National Academies Press), 82. doi: 10.17226/1349

NRC (1996). *Lost crops of africa.* (Washington, D.C: National Academies Press). doi: 10.17226/2305

NRC (2001). *Nutrient requirements of dairy cattle.* (Washington, D.C: National Academies Press). doi: 10.17226/9825

NRC (2007). *Nutrient requirements of small ruminants.* (Washington, D.C: National Academies Press). doi: 10.17226/11654

OECD/FAO (2022). *OECD-FAO Agricultural Outlook 2022-2031* (Paris: OECD Publishing). doi: 10.1787/f1b0b29c-en

Ørskov, E. R., Flatt, W. P., Moe, P. W., Munson, A. W., Hemken, R. W., and Katz, I. (1969). The influence of ruminal infusion of volatile fatty acids on milk yield and composition and on energy utilization by lactating cows. *Br. J. Nutr.* 23, 443–453. doi: 10.1079/BJN19690054

Ørskov, E. R., and McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* 92, 499–503. doi: 10.1017/S0021859600063048

Ospina, P. A., Nydam, D. V., Stokol, T., and Overton, T. R. (2010). Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. *J. Dairy Sci.* 93, 1596–1603. doi: 10.3168/jds.2009-2852

Palmquist, D. L., Denise Beaulieu, A., and Barbano, D. M. (1993). Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* 76, 1753–1771. doi: 10.3168/jds.S0022-0302(93)77508-6

Patra, A. K. (2015). ""Urea/ammonia metabolism in the rumen and toxicity in ruminants,"," in *Rumen microbiology: from evolution to revolution*. Eds. A. K. Puniya, R. Singh and D. N. Kamra (New Delhi: Springer India), 329–341. doi: 10.1007/978-81-322-2401-3_22

Pengpeng, W., and Tan, Z. (2013). Ammonia assimilation in rumen bacteria: A review. *Anim. Biotechnol.* 24, 107–128. doi: 10.1080/10495398.2012.756402

Piantoni, P., Ylioja, C. M., and Allen, M. S. (2015). Feed intake is related to changes in plasma nonesterified fatty acid concentration and hepatic acetyl CoA content following feeding in lactating dairy cows. *J. Dairy Sci.* 98, 6839–6847. doi: 10.3168/jds.2014-9085

Pichler, M., Damberger, A., Schwendenwein, I., Gasteiner, J., Drillich, M., and Iwersen, M. (2014). Thresholds of whole-blood β-hydroxybutyrate and glucose concentrations measured with an electronic hand-held device to identify ovine hyperketonemia. *J. Dairy Sci.* 97, 1388–1399. doi: 10.3168/jds.2013-7169

Ream, C. N., Stevens, A. V., Hall, J. B., and Chibisa, G. E. (2020). Harvest maturity of Eragrostis tef 'Moxie': Effects on ruminal fermentation, total-tract nutrient digestibility, and growth performance in backgrounding beef cattle. *Appl. Anim. Sci.* 36, 600–609. doi: 10.15232/aas.2020-02008

Reynolds, C. K., Aikman, P. C., Lupoli, B., Humphries, D. J., and Beever, D. E. (2003). Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *J. Dairy Sci.* 86, 1201–1217. doi: 10.3168/jds.S0022-0302(03)73704-7

Rezac, D. J., Grigsby, K. N., Bello, N. M., and Bradford, B. J. (2012). Effects of varying rates of tallgrass prairie hay and wet corn gluten feed on productivity of lactating dairy cows. *J. Dairy Sci.* 95, 842–849. doi: 10.3168/jds.2011-4752

Roseberg, R., Norberg, S., and Charlton, B. (2018)Teff Grass for Forage: Nitrogen and irrigation requirements (Corvallis, Oregon). Available at: https://catalog.extension.oregonstate.edu/pnw709/html (Accessed May 15, 2023).

Russell, J. B., Sniffen, C. J., and Van Soest, P. J. (1983). Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. *J. Dairy Sci.* 66, 763–775. doi: 10.3168/jds.S0022-0302(83)81856-6

Satter, L. D. (1986). Protein supply from undegraded dietary protein. *J. Dairy Sci.* 69, 2734–2749. doi: 10.3168/jds.S0022-0302(86)80722-6

Saylor, B. A., Min, D. H., and Bradford, B. J. (2018). Productivity of lactating dairy cows fed diets with teff hay as the sole forage. *J. Dairy Sci.* 101, 5984–5990. doi: 10.3168/jds.2017-14118

Schaefer, D. M., Davis, C. L., and Bryant, M. P. (1980). Ammonia saturation constants for predominant species of rumen bacteria. *J. Dairy Sci.* 63, 1248–1263. doi: 10.3168/jds.S0022-0302(80)83076-1

Seymour, W. M., Campbell, D. R., and Johnson, Z. B. (2005). Relationships between rumen volatile fatty acid concentrations and milk production in dairy cows: a literature study. *Anim. Feed Sci. Technol.* 119, 155–169. doi: 10.1016/j.anifeedsci.2004.10.001

Staniar, W. B., Bussard, J. R., Repard, N. M., Hall, M. H., and Burk, A. O. (2010). Voluntary intake and digestibility of teff hay fed to horses1. *J. Anim. Sci.* 88, 3296–3303. doi: 10.2527/jas.2009-2668

Stoop, W. M., Bovenhuis, H., Heck, J. M. L., and van Arendonk, J. A. M. (2009). Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *J. Dairy Sci.* 92, 1469–1478. doi: 10.3168/jds.2008-1468

Sudweeks, E. M., Ely, L. O., and Sisk, L. R. (1979). Effect of particle size of corn silage on digestibility and rumen fermentation. *J. Dairy Sci.* 62, 292–296. doi: 10.3168/jds.S0022-0302(79)83238-5

van Knegsel, A. T. M., van den Brand, H., Dijkstra, J., Tamminga, S., and Kemp, B. (2005). Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. *Reprod. Nutr. Dev.* 45, 665–688. doi: 10.1051/rnd:2005059

Van Soest, P. J. (1994) *Nutritional ecology of the ruminant.* (Soest Ithaca, New York: Cornell University Press). Available at: https://www.jstor.org/stable/10.7591/j.ctv5rf668 (Accessed June 20, 2023).

Van Soest, P. J., Robertson, J. B., and Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2

Vinyard, J. R., Hall, J. B., Sprinkle, J. E., and Chibisa, G. E. (2018). Effects of maturity at harvest on the nutritive value and ruminal digestion of Eragrostis tef (cv. Moxie) when fed to beef cattle. *J. Anim. Sci.* 96, 3420–3432. doi: 10.1093/jas/sky202

Wagali, P., Sabastian, C., Saranga, Y., Ben-Zeev, S., and Mabjeesh, S. J. (2023). The effects of irrigation, genotype and additives on tef silage making. *Animals* 13, 470. doi: 10.3390/ani13030470

Yansari, T. A., Valizadeh, R., Naserian, A., Christensen, D. A., Yu, P., and Eftekhari Shahroodi, F. (2004). Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of holstein dairy cows. *J. Dairy Sci.* 87, 3912–3924. doi: 10.3168/jds.S0022-0302(04)73530-4

Zebeli, Q., Tafaj, M., Weber, I., Dijkstra, J., Steingass, H., and Drochner, W. (2007). Effects of varying dietary forage particle size in two concentrate levels on chewing activity, ruminal mat characteristics, and passage in dairy cows. *J. Dairy Sci.* 90, 1929–1942. doi: 10.3168/jds.2006-354

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 11 December 2023
DOI 10.3389/fanim.2023.1233570

OPEN ACCESS

EDITED BY
Marcos Inacio Marcondes,
Washington State University, United States

REVIEWED BY
Marketta Rinne,
Natural Resources Institute Finland (Luke), Finland
Juana Catarina Cariri Chagas,
Swedish University of Agricultural Sciences, Sweden

*CORRESPONDENCE
Sanfo Abroulaye
z.nouhoun@ufl.edu

RECEIVED 02 June 2023
ACCEPTED 07 November 2023
PUBLISHED 11 December 2023

CITATION
Abroulaye S, Nouhoun Z, Jethro DB, Abalo KE, Abdoulaye O, Esteban F. R, José D, Ken BJ and Adegbola A (2023) Sorghum [*Sorghum bicolor* (L.) Moench] and cowpea [*Vigna unguiculata (L.)* Walpers] intercropping improves grain yield, fodder biomass, and nutritive value. *Front. Anim. Sci.* 4:1233570. doi: 10.3389/fanim.2023.1233570

Sorghum [*Sorghum bicolor* (L.) Moench] and cowpea [*Vigna unguiculata (L.)* Walpers] intercropping improves grain yield, fodder biomass, and nutritive value

Sanfo Abroulaye[1,2]*, Zampaligré Nouhoun[1], Delma B. Jethro[1], Kulo E. Abalo[2], Ouédraogo Abdoulaye[1], Rios Esteban F.[3], Dubeux José[3], Boote J. Ken[3] and Adesogan Adegbola[4]

[1]Centre National de Recherche Scientifique et Technologique, Institut de l'Environnement et de Recherches Agricoles, Station de Farako-Ba, Bobo-Dioulasso, Burkina Faso, [2]Université de Lomé, Ecole Supérieure d'Agronomie, Lomé, Togo, [3]Agronomy Department, Institute of Food and Agricultural Sciences (IFAS), University of Florida, Gainesville, FL, United States, [4]Feed the Future Innovation Lab for Livestock Systems, Department of Animal Sciences, Institute of Food and Agricultural Sciences (IFAS), University of Florida, Gainesville, FL, United States

Burkina Faso livestock feeding is characterized by a hot dry season fodder deficit, which affects animal performance and causes economic losses. To overcome this challenge, improving quality fodder production through the use of dual-purpose crops is a potential alternative. Hence, this study aimed at testing dual-purpose cultivars of sorghum and cowpea under monoculture and intercropping in the North Sudan zone in Burkina Faso. To do this, a "*Mother and Baby trials*" approach was adopted. The mother trial was designed as a randomized complete block with eight treatments (combinations of monoculture and intercropping systems for two cowpeas and two sorghum cultivars) and four replications during two cropping seasons (2019 and 2020) at the INERA research station in Saria. The on-farm "*baby*" trials involved 30 farmers during two cropping seasons (2019 and 2020) in four communes: *Koudougou, Poa, Nandiala, and Kokologo*. Data were collected on weed biomass and density, fodder biomass and grain yield, intercropping efficiency, and fodder nutritive value. The results of the mother trial showed that intercropping significantly ($p \leq 0.05$) reduced weed density and weed biomass. Sorghum cultivar *Ponta Negra* had the highest fodder biomass yield (10.05 kg DM/ha) while sorghum Sariaso16 had the highest grain yield (4.42 kg/ha). Cowpea cultivar KVx745-11P had greater fodder biomass (4.72 kg DM/ha) than Tiligré (3.28 kg DM/ha) with similar grain yield (2.17 and 2.17 kg/ha). Intercropping was the most efficient land-use cropping system for fodder biomass and grain yield improvement both in mother and baby trials. For fodder nutritive value, cultivars Sariaso16 and Ponta Negra had similar crude protein concentrations (ranging from 4.1 to 5.4%), and cowpea cultivar KVx745-11P haulms had greater crude protein (ranging from 16.9 to 20.3%). The use of Ponta Negra and KVx745-11P and Sariaso16 and KVx745-11P under intercropping is likely to optimize grain and quality fodder production for crop-livestock farmers in the North Sudan zone.

KEYWORDS

crop-livestock system, cowpea, burkina faso, food-feed crops, sorghum

1 Introduction

Three main livestock systems used in Burkina Faso for ruminant production include extensive, semi-intensive, and intensive systems (Kristjanson et al., 2012). Local breeds of cattle (*Bos taurus*), sheep (*Ovis aries*), and goats (*Capra hircus*) are the dominant species. The semi-intensive system involves crop-livestock integration, which includes agro-pastoralists, sedentary crop-livestock farmers, and some peri-urban dairy farmers, and it is the most dominant system (MRA, 2015). In this system, livestock do not move too far from the production site because of the use of manure for soil fertility management and crop residues as livestock feed. Animals are grazed on natural pastures with a little feed complementation during the hot dry season using stored fodder such as legume haulms and cereal straws (Kiéma et al., 2019). The crop-livestock system enhances farmers' resilience to environmental risks and reduces conflicts for the use of natural resources (FAO, 2014; Sanfo et al., 2015; Kiéma et al., 2019).

The availability of feed resources in the hot dry season is the main constraint of this livestock system. The distances traveled for pasture by livestock are becoming longer because of the decline in pasture productivity and larger livestock herds (Boote et al., 2021). This induces a systematic use of crop residues as feed (Zampaligré et al., 2013; Amole & Ayantunde, 2016; Duncan et al., 2016). These crop residues are either directly grazed on farms after grain harvest or are collected and stored for later use during the hot dry season (Sanou et al., 2011; Cuvelier & Dufrasne, 2014; FAO, 2014; Kiéma et al., 2019). Cereal straw (sorghum [*Sorghum bicolor* (L.) Moench], millet [*Pennisetum glaucum* (L.) R. Br.], and maize [*Zea mays* L)] have low crude protein concentrations (2-9%) compared to legume haulms such as cowpea [*Vigna unguiculata (L.)* Walpers], groundnut [*Arachis hypogaea* L], and Bambara bean [*Vigna subterranea* (L.) Verdc.] (Nantoumé et al., 2000; Palé, 2017; Zampaligré et al., 2021). The use of cereal straw and legume haulms respectively at 40% straw and 10 to 60% legume haulm in local and hybrid sheep diets provided a daily weight body gain of 92 to 206 g/day (Somda, 2001; Kiéma, 2008).

Forage crop production for livestock feed has been sparsely adopted by farmers despite the efforts of extension and research (Bayala et al., 2014; Boote et al., 2021). This reflects the lack of farmers' interest in those forages due to land tenure, cropping calendar, lack of technical skills, and seed availability issues (Kagoné, 2001; Bayala et al., 2014). Urbanization and demographic pressure on croplands favor food-feed cereal and legume cropping to the detriment of strict forages. This risk management strategy for small crop-livestock farmers benefits from crop residues as livestock feed and grains for food using the same unit of area (Sanfo et al., 2015). The majority of these crop residues used by farmers are from local cultivars with low nutritive value, in addition to their poor preservation causing a loss of nutritive value over time (Feyissa et al., 2014; Akakpo et al., 2020). Research on crop breeding has enabled improved food-feed cultivars that have good grain yield and fodder biomass with better quality (Cesar and Guiro, 2004). A recent study by Zampaligré et al. (2021) identified Sariaso16 for Sorghum and KVX74511P for cowpea to be among the best bet food-feed cultivars for Burkina Faso's different agro-ecologies. Their introduction of appropriate cropping systems among the smallholder farmers is key for their adoption. Due to a lack of land area and poor soil fertility, smallholder farmers are increasing the use of intercropping of legumes and cereals for their crop production.

Intercropping is an arrangement of crops on the same plot and at the same time for complementarity in order to make the system more resilient to physico-chemical soil conditions (Matusso et al., 2014). Cereal and legume intercropping is more suitable for agro-pastoralists and sedentary crop-livestock farmers because of this dual need for food and feed for human beings and livestock, respectively (Nasir et al., 2019). The use of improved food-feed cultivars, which can fit into smallholder farmer's cropping calendar, is likely to provide greater benefits both for grains and fodder biomass (Mbaye et al., 2014; Louarn et al., 2016; Abera et al., 2021; Sanfo et al., 2023). Previous studies revealed that intercrop of maize-cowpea-sorghum-cowpea led to an increase in grain yield and fodder biomass in the range of 30-60% with better weed control (Matusso et al., 2014; Obulbiga et al., 2015; Coulibaly et al., 2017; Sanfo et al., 2023), and land equivalent rations were greater in cowpea-maize intercropping systems (Sanfo et al., 2023). Considering the multiple benefits of using improved cultivars and appropriate cropping systems, we hypothesized that intercropping of best-bet sorghum and cowpea cultivars will provide greater grain yield and fodder biomass as well as quality fodder in smallholder farming systems in Burkina Faso. Thus, this study was conducted to assess the agronomic performance and fodder quality of sorghum Sariaso16 and cowpea KVX74511P in intercrop by comparison to monoculture in the crop-livestock system.

2 Materials and methods

2.1 Site description: location, rainfall, soil, vegetation, crop, and livestock systems

The mother study was conducted in Burkina Faso (Figure 1). A central trial was established at INERA (Institut de l'Environnement et de Recherches Agricoles) research station, Saria, located in Boulkiemdé province and Nandiala commune located 80 km southwest of Ouagadougou with coordinates 12° 15′ 57″ N, 2° 08′ 47″ W. On-farm trials were conducted in four other communes, namely, Koudougou, Poa, Nandiala, and Kokologo within 40 km maximum radius from the Saria research station (Figure 1).

The climate is part of the Köppen climate zone B classification (Beck et al., 2018). The annual rainfall is 600-900 mm/year with 6 to 7 months of dry season lasting from November to May, with the rainy season occurring from June to October and the number of rainy days ranging from 60 to 68 a year. The monthly rainfall recorded in 2019 and 2020 in the study site is indicated in Figure 2. The average annual temperature in Saria is approximately 28°C, with a low temperature (12°C) occurring in December-January and a high temperature (40°C) in March-April.

Soils in the study sites are classified as Ferric Lixisol (Traoré, 2012). They are sandy-silty textured and have acid with low nitrogen, organic matter, available phosphorus, and available potassium contents (Table 1). The vegetation is dominated by agroforestry parklands and annual grasses. Main woody species are *Vittelaria paradoxa* C.F.Gaertn, *Faidherbia albida*, *Parkia biglobosa* Jacq, *Tamarindus indica* L., *Combretum nigricans* Lepr, and *Piliostigma recticulatum* (DC.) Hochst. The dominant grasses are *Andropogon gayanus* Kunth, *Loudecia togoensis* Hubb, and *Dactyloctenium aegyptium* Beauv (Ouédraogo, 2019; Zampaligré et al., 2021).

The cropping system is mainly rain-fed, extensive subsistence agriculture with small farms averaging 2 to 5 ha. It is dominated by cereal-based systems that integrate crops and livestock. The main crops are sorghum for cereals and groundnut and cowpea for legumes, accounting for 61, 69, and 56% of the national area sown for each crop type, respectively (MA, 2015). The dominant livestock systems are the integrated sedentary crop-livestock and agro-pastoral systems with local breeds of cattle, sheep, and goats (Mulumba et al., 2008). The livestock population in this zone is estimated at approximately 11,836,020 for small ruminants and 3,683,125 for large ruminants which represent 50 and 40% of the national flock, respectively (MRA, 2015).

2.2 Methodology

2.2.1 Conceptual framework

This research used the participatory "*Mother and Baby trials*" approach (Snapp, 2002). It has two steps which are central (Mother) and on-farm (Baby) trials. The central trial is conducted in a village with a leader and innovative farmer or in a nearby research station. On-farm trials are farmers' individual tests conducted by themselves under the research team's supervision (Rusike et al., 2004; Gonsalves et al., 2005). Each farmer is a replication and linked to the central trial in order to compare a subset of innovations from the central trial (Snapp, 2002; Gonsalves et al., 2005). This approach allows farmers to evaluate the innovations that are most appropriate for their production system and resource endowment. It is an approach that initiates dialogue and collaboration between farmers, extension workers, policymakers, and researchers. In this way, rapid transformation and dissemination of the innovation are achieved through the snowball effect (Snapp, 2002).

2.2.2 Sampling method and plant materials

In 2018 - 2019, prior to the start of the experiment, a baseline survey with 250 farmers in the same four communes was conducted on fodder production in crop-livestock systems. A reasoned and stratified sampling approach was used regarding these criteria: (i) farmers' willingness to conduct food-feed trials on at least 0.1 ha, (ii) farm accessibility, (iii) a minimum of three sheep availability for fattening trials, and (iv) gender with at least 30% of women participation. Based on the results of this study, 30 farmers (20 men and 10 women) were selected for a second survey on the evaluation of their preferences for crop species, cultivars, cropping systems, and fodder conservation methods (Sanfo et al., 2020). Following the results of these surveys, the same 30 farmers were chosen for this participatory research on food-feed crop production

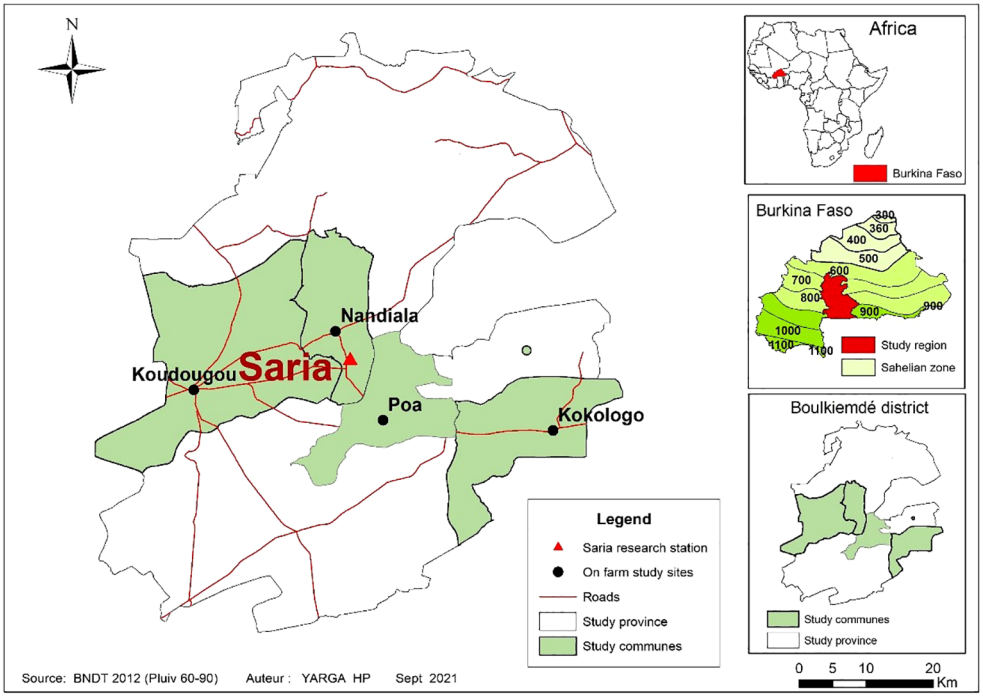


FIGURE 1
Map of the study location.

10.3389/fanim.2023.1233570

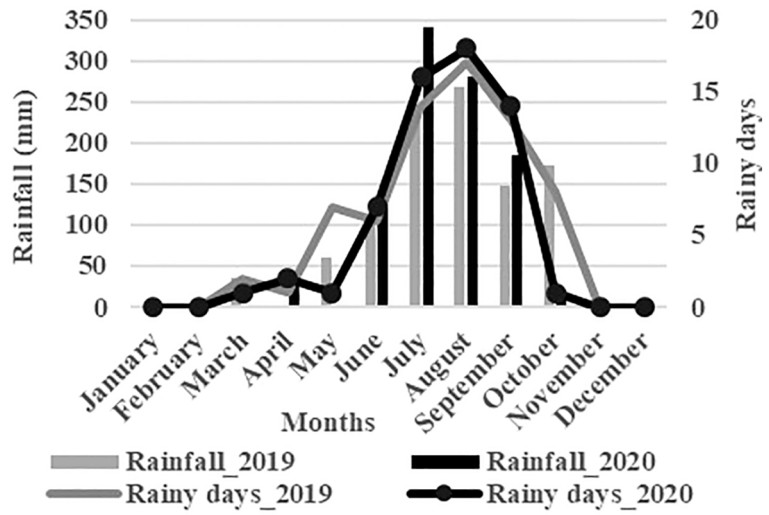


FIGURE 2
Monthly rainfall recorded in the study site in 2019 and 2020. Source, INERA FARAKOBA Pluviométries de l'année 2019 et 2020.

based on improved cultivars of sorghum and cowpea in intercropping systems.

The choice of cultivars was done according to the farmers' preferences (Sanfo et al., 2020) and included improved cultivars of sorghum (Sariaso16 and Ponta Negra) and cowpea (KVx745-11P and Tiligré). These cultivars were released by INERA (*Institut de l'Environnement et de Recherches Agricoles*) and EMBRAPA (Brazil) research. The INERA cultivars are the ones currently being promoted in Burkina Faso (Zampaligré et al., 2021). These cultivars are described in Table 2 (MRSI, 2014; Palé, 2017; Ramdé, 2019; Zampaligré et al., 2021).

2.2.3 Experimental design

The on-station trials were laid out as a completely randomized block design with eight treatments and four replications: two sorghum cultivars, two cowpea cultivars, and two cropping systems (monocultures versus intercropping). The size of each subplot was 35 m² (7 m x 5 m). Below are the treatments:

(T1) Sorghum Ponta Negra only; (T2) Sorghum Sariaso16 only; (T3) Cowpea Tiligré only;

(T4) Cowpea KVx745-11P only; (T5) Sorghum Ponta Negra intercropped with cowpea Tiligré;

(T6) Sorghum Ponta Negra intercropped with cowpea KVx745-11P;

(T7) Sorghum Sariaso16 intercropped with Cowpea Tiligré; and

(T8) Sorghum Sariaso16 intercropped with Cowpea KVx745-11P.

The on-farm trials involved 30 farmers with 0.1 ha as individual plot size. The farmers were all trained on farm agricultural practices and management on 14 and 15 June 2019 at Saria and Kokologo,

TABLE 1 Study sites soils' chemical characteristics.

Sample	pH H2O	pH KCl	OM (%)	N (%)	C/N	Total P	P Bray1	Total K	K Av.	Clay (%)	Silt (%)	Sand (%)
						mg/kg						
A	4.78	4.06	0.46	0.02	10.5	90	3.9	1012	43	16.4	21.3	62.3
B	5.00	4.19	0.49	0.03	10.9	99	5.2	1063	45	17.2	19.4	63.5
C	4.73	4.33	0.43	0.02	10.4	94	4.9	952	37	15.4	20.8	63.7
D	4.95	4.07	0.53	0.03	10.7	99	6.2	1049	42	17.2	19.6	63.2
E	5.01	4.14	0.49	0.03	10.4	85	4.1	1011	41	17.4	17.7	64.9
F	4.90	4.12	0.43	0.02	10.6	83	4.3	975	42	16.7	19.1	64.2
G	5.01	4.18	0.39	0.02	10.4	88	3.5	976	43	16.4	17.2	66.4
H	5.09	4.22	0.43	0.02	10.5	87	4.7	1123	38	19.4	16.2	64.5
Mean	**4.93**	**4.16**	**0.46**	**0.02**	**10.6**	**91**	**4.6**	**1020**	**41**	**17.0**	**18.9**	**64.1**

OM, Organic Matter; N, Nitrogen; C, Carbon; P, Phosphorus; K, Potassium; Av, Available. *(Soils samples from study site in 2019.
NB: Composite samples from 3 sampling points on the diagonal of each plot.

TABLE 2 Plant materials characteristics.

Species	Cultivar	Origin	Cycle (days)	Grain yield (t/ha)	Biomass (t DM/ha)	CP (%)	IVOMD (%)
Sorghum (*Sorghum bicolor* L. Moensh)	Ponta Negra Sariaso16	EMBRAPA INERA	130 105-110	0.4 - 3 1.5 - 4.4	5.3 - 10 3 - 4.7	5 - 5.5 3.9 - 4.2	48 - 49 49 - 50
Cowpea (*Vigna unguiculata* L. Walpers)	Tiligré KVx745-11P	INERA INERA	70 75	1.5 - 2.3 0.8 - 2.1	2.5 - 3 3 - 5	13 - 15.4 16 - 21.6	64 - 65 64 - 65

CP, Crude protein; IVOMD, In vivo organic matter digestibility (Source: Ramdé, 2019 and Ilboudo, 2020).

respectively. Then, they were provided with seed, fertilizer, and pest control products by the research team. Trial implementation was facilitated by a team of extension workers (crop and livestock agents) under the supervision of the research team. Three treatments/options were selected from the on-station trial and tested by 10 farmers per treatment, each farmer representing a replicate for each option:

(T1) Sorghum Sariaso16 only;

(T4) Cowpea KVx745-11P only; and

(T8) Sorghum Sariaso16 intercropped with Cowpea KVx745-11P.

The study was done during two consecutive rainy seasons in 2019 and 2020 on-farm and on-station.

2.2.4 Trial establishment and agronomic management

2.2.4.1 Mother trial established at Saria research station

The trial was implemented on fallow land that had cowpea as the previous crop after soil analysis (Table 1). Flat plowing with a tractor followed by leveling was done after rainfall prior to sowings from 5 to 12 July. These sowings were done manually from 15 to 19 and 18 to 22 July for sorghum in the 2019 and 2020 cropping seasons, respectively. For cowpea, they were done from 25 to 30 July and 28 July to 3 August in 2019 and 2020, respectively. Cowpea sowings were shifted approximately 10 days later from those of sorghum in order to optimize its grain yield and biomass (Mbaye et al., 2014). Spacing was 80 cm between rows and 40 cm between plants within rows for all cultivars. Thinning was done 15-20 days after sowing (DAS) to obtain the needed densities: 62,500 plants/ha for sorghum and cowpea in monocultures plots; and 20 833 plants/ha for cowpea and 41 666 for sorghum in the intercropping plots.

Weeding was done twice (15-20 and 25-35 DAS) followed by hoeing at 40-45 DAS. Cattle manure was applied in the first year at the rate of 5 t/ha before planting. Mineral fertilization was done annually with NPK (14-23-14) at a rate of 100 kg/ha for all sorghum and cowpea crops at 15-20 DAS, corresponding to 14 kg of nitrogen, 10 kg of potassium, and 12 kg of phosphorus (Zampaligré et al., 2021). In addition to NPK, only sorghum plots received 50 kg/ha of urea annually at 40-45 DAS (23 kg of nitrogen). For pest control, two treatments were done on cowpea plots with *Acetamiprid16g/l +Indoxacarb 30g/l* at the rate of 1 l/ha at flowering and pod formation stages. Sorghum plots were treated specifically with *Lambda-cyhalothrin 15 g/l + Acetamiprid 20 g/l* against armyworm (*Spodoptera frugiperda)* attack.

2.2.4.2 On-farm trials (baby trials)

At the on-farm plots, previous crops included cowpea, Bambara bean, millet, and sorghum. Flat plowing with animal traction was done after rainfall followed by sowings. Plantings were done manually by farmers themselves in lines from 14 to 25 July for sorghum and from 22 July to 5 August for cowpea in 2019 and 2020. Planting spacing and thinning were performed following the method described above for the mother trial. Weeding and hoeing were done similarly as in the mother trial.

Mineral fertilization was done annually with NPK (14-23-14) at a rate of 100 kg/ha for all crops at 15 - 20 DAS. In addition to NPK, sorghum plots received 50 kg/ha of urea annually at 40 - 45 DAS. For pest control, two (02) treatments were done on cowpea plots with *Acetamiprid16g/l +Indoxacarb 30g/l* at the rate of 1 l/ha at flowering and pod formation stages. Sorghum plots were treated specifically with *Lambda-cyhalothrin 15 g/l + Acetamiprid 20 g/l* against armyworm attack.

2.3 Data collection

2.3.1 Grain yield and fodder biomass evaluation

Grain yield (GY) was evaluated at sorghum panicle and cowpea pod maturity stages using the yield square method in 1m². Three yield squares were placed along the diagonal of each plot; sorghum panicles and cowpea pods were harvested separately and sun-dried for 10 days before being shelled/threshed and winnowed. The obtained grains were further sun-dried to constant weight and then weighed using a small scale (2 kg ± 5 g) to get grain yield for each crop. The fodder biomass was evaluated right after grain harvest (same day) using the same three yield squares. Fresh fodder weight in each square was taken using a 10 kg ± 10 g sensitive scale; a sample of 500 g from each square was collected and oven-dried at 105°C for 24 hours to determine fodder biomass on a dry matter basis (kg DM/ha).

2.3.2 Intercropping efficiency assessment

Three (03) parameters were used to assess the intercropping efficiency: (i) Weed Control (WC) based on Weed Density (WD) and Weed Biomass (WB), (ii) Land Equivalent Ratio (LER), and (iii) System Productivity Index (SPI).

WD was assessed at 70-80 DAS using three (03) yield squares of 1m² in each plot along the diagonal and all weeds within the square were counted.

➢WB was evaluated using the same three yield squares and all the weeds in the square were collected and weighed. Then, samples were oven-dried at 105°C for 24 h. After dry matter determination, weed biomass was calculated by extrapolation (kg DM/ha).

➢LER is the relative land area under sole crops required to produce the yield achieved in intercropping and determined by the following formula (Wiley, 1979 *in* N'Goran et al., 2011):

$$LER = (Ya / YA) + (Yb / YB)$$

Ya and Yb are the yields of sorghum and cowpea in intercropping, respectively. YA and YB are also the yields of sole cultures of sorghum and cowpea on a similar unit area, respectively.

SPI is the standardization of the yields of the secondary crop "b" into those of the main crop "a" in intercropping according to the formula (Agegnehu et al., 2008; Khan et al., 2020): =

$$SPI = (Rs / Rc) \times Yc + Ys)$$

Rs and Rc are the average yields of sorghum and cowpea in monoculture, respectively. Ys and Yc are also the average yields of sorghum and cowpea in intercropping, respectively.

The indexes WC, LER, and SPI were used to identify the relative advantages of intercropping, and their appropriate spatial arrangement is described in Table 3.

2.3.3 Fodder nutritive value assessment

Two composite samples of 500 g of the whole plant (stems + leaves) were taken in each plot replication from the three yield squares on the same day of fodder biomass and grain yield assessment. Samples were pre-dried and then shade-dried and ground at 1 mm size. The Near Infrared Spectrometry (NIRS) method was used for nutritive value analysis. Sample spectra were collected using the NIRS FOSS DS 2500 F and the *International Livestock Research Institute* (ILRI) *Global Mixed Feed* calibration was used to predict the nutritional quality. Nutritive value parameters assessed were: Dry Matter (DM), ash, Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), Metabolizable Energy (ME), and *In Vitro* Organic Matter Digestibility (IVOMD).

TABLE 3 Description of indexes used for intercropping efficiency assessment.

Index	Definition	Interpretation	Reference
WC	Weed density (WD) and biomass (WB) per unit area of land	Comparison of different weed densities and biomass regarding cropping systems	Ekeleme et al., 2019
LER	Land area under sole crops required to produce the yields achieved in intercropping	An LER of 1 indicates equal advantages for intercropping and monoculture; a value more than 1 means more advantage for intercropping than for monoculture; and an LER less than 1 means less advantage for intercropping than for monoculture	N'Goran et al., 2011
SPI	Standardization of the yields of the secondary crop "b" into those of the main crop "a" in the intercropping	Comparison between yields of the main crop in monoculture and the one in intercropping after standardization	Khan et al., 2020

2.4 Data analysis

Data entry as well as tables and graphs were created using Excel. The general linear model of SPSS Statistic 20.0 was used for performing an analysis of variance considering all factors as fixed effects. Three-way ANOVA (year, cultivar, and cropping system) was applied to the on-station trial data and two-way ANOVA (cropping system and year) for on-farm trial data. One-way ANOVA was performed for fodder nutritive value analysis. Mean comparisons for significant effects were done using the LSD (Least Significant Difference) test and significance was declared at $p \leq 0.05\%$.

3 Results and discussion

3.1 Results

3.1.1 Grain yield, fodder biomass, weed density, and biomass

On-station (mother trial) data analysis of variance (ANOVA) showed that the cultivar effect was significant for fodder biomass, weed density, and biomass of cowpea, while it was only significant for grain yield and fodder biomass of sorghum (Table 4). The cropping system effect was significant for grain yield and fodder biomass for cowpea and sorghum but was significant for only weed density and biomass for sorghum (Table 4).

For the on-farm trial, only the cropping system effect was significant for sorghum grain yield and fodder biomass (Table 4).

3.1.2 Cropping systems and weed control

Low weed density (12 plants/m²) and low weed biomass (36.2 kg DM/ha) were obtained with cowpea KVx74511P in monoculture (Table 5). In addition, regardless of sorghum cultivar, weed density and biomass were lower in the intercropping system than in monoculture except for Sariaso16 weed biomass in its intercropping with Tiligré (Table 5).

3.1.3 Grain yield and fodder biomass of tested cultivars

Sorghum Sariaso16 had the greatest grain yield for the on-station trial (4415 kg/ha) and also had a high yield in the on-farm trials (3458 kg/ha) across two rainy seasons (2019 and 2020) (Table 6). Ponte Negra had the highest fodder biomass yield (10051 kg DM/ha). For

10.3389/fanim.2023.1233570

TABLE 4 ANOVA results for grain yield, fodder biomass, weeds density, and biomass for cowpea and sorghum (2019 and 2020).

Trial	Source of variation	Cowpea				Sorghum			
		Grain Yield (kg ha^{-1})	Fodder Biomass (kg DM ha^{-1})	Weed Density (plant/ m^2)	Weed Biomass (kg ha^{-1})	Grain Yield (kg ha^{-1})	Fodder Biomass (kg DM ha^{-1})	Weed Density (plant/ m^2)	Weed Biomass (kg ha^{-1})
Mother trial (On Station)	Year	NS	NS	NS	NS	*NS*	*NS*	NS	NS
	Cultivar	NS	*	**	*	*	*	NS	NS
	Cropping system	***	***	NS	NS	***	***	*	*
	Year*cultivar	NS	NS	NS	NS	NS	NS	NS	NS
	Year*cropping system	NS	NS	NS	NS	NS	NS	NS	NS
	cultivar*cropping system	NS	*	NS	NS	***	**	NS	NS
	Year*cultivar*cropping system	NS	NS	NS	NS	NS	NS	NS	NS
Baby trials (On Farm)	Year	NS	NS	–	–	NS	NS	–	–
	Cropping system	NS	NS	–	–	***	***	–	–
	Year* Cropping system	NS	NS	–	–	NS	NS	–	–

*=p ≤ 0.05, **=p ≤ 0.01, ***=p ≤ 0.001, NS, Not significant at p ≤ 0.05. DM, Dry Matter.

cowpea cultivars in the on-station trials, KVx745-11P and Tiligré had similar grain yield (2116-2169 kg/ha), while KVx745-11P fodder biomass (4721 kg DM/ha) was higher than Tiligré (3283 kg DM/ha). In the on-station trials, greater fodder biomass was obtained for monoculture of sorghum cultivars compared to intercropping with cowpea. This appeared to repeat for the on-farm trials but was not significant because of large farm-to-farm variability. For intercropping in the on-station trial, the fodder biomass of Ponte Negra was greater than for Sariaso16 regardless of cowpea cultivars used, but Sariaso16 had greater grain yield regardless of cowpea cultivars. For cowpea cultivars tested on-station, grain yield and fodder biomass were greater in monoculture than for intercropping. However, no significant differences were found between the two cultivars for grain yield and fodder biomass in intercropping.

TABLE 5 Effect of cropping systems on weeds biomass and density for the mother trial (average of 2019 and 2020).

Cropping system	Cultivar	Density (plant/ m^2)	Biomass (kg DM/ ha)
Monoculture	Ponta Negra	60^{a} ± 41	115.2^{a} ± 97
	Sariaso16	67^{a} ± 52	137.0^{a} ± 63
	Tiligré	46^{ab} ± 47	76.5^{ab} ± 44
	KVx745-11P	12^{c} ± 10	36.2^{c} ± 40
Intercropping	Ponta Negra and Tiligré	32^{b} ± 12	80.7^{ab} ± 40
	Ponta Negra and KVx745-11P	28^{bc} ± 22	40.7^{c} ± 22
	Sariaso16 and Tiligré	58^{ab} ± 26	117.3^{a} ± 24
	Sariaso16 and KVx745-11P	32^{b} ± 9	87.3^{ab} ± 80
Statistic	F	2.84	3.28
	P-Value	0.013	0.005

Values with the same letters in the same column are equal (LSD; P = 0.05).

3.1.4 Intercropping efficiency for land use, grain yield, and fodder biomass

LER and SPI were used to assess land use, grain yield, and fodder biomass efficiencies regarding cropping systems (Table 7). Sorghum and cowpea intercropping LERs for fodder biomass (1.01-1.37) and grain yield (1.02-1.65) were greater than one. Sorghum Sariaso16 intercropped with cowpea Tiligré had the greatest LER for fodder biomass, while the highest LER was obtained for Ponta Negra intercropped with Tiligré for grain yield.

Intercropping SPIs were higher than those of the corresponding sorghum cultivar (main crop) monoculture evaluated. The highest SPI values for fodder biomass were obtained by Ponta Negra intercropped with either cowpea cultivar (10539 and 12587). In terms of grain yield, Sariaso16 intercropped with cowpea had the highest index (4517 - 5438) regardless of the cowpea cultivar.

3.1.5 Sorghum straw and cowpea haulm nutritive value

Sariaso16 had greater fodder DM, NDF, and ADL, and lower fodder ME and IVOMD compared to Ponta Negra regardless of the cropping system (monoculture and intercropping) and cowpea cultivar used in intercropping (Table 8). Nevertheless, the two sorghum cultivars had similar CP (4.1 – 5.1%) concentrations regardless of the cropping system and cowpea cultivar used in intercropping. There were differences between the nutritive value of sorghum fodder in monoculture and intercropping. Sariaso16 intercropped with cowpea KVx745-11P had lower fodder ADF compared to Sariaso16 in monoculture. Ponta Negra intercropped with Tiligré had greater ME than in monoculture.

TABLE 6 Sorghum and cowpea grain yield and fodder biomass regarding the interaction between cropping systems and cultivars across two years.

	Cropping system	Fodder biomass (kg DM/ha)		Grain yield (kg/ha)	
		Sorghum	Cowpea	Sorghum	Cowpea
Mother trial (On-station)	Sorghum Ponta Negra monoculture	$10051^{a} \pm 1169$	–	$1863^{c} \pm 934$	–
	Sorghum Sariasso16 monoculture	$4447^{bc} \pm 407$	–	$4415^{a} \pm 593$	–
	Cowpea Tiligré monoculture	–	$3283^{ab} \pm 556$	–	$2116^{a} \pm 457$
	Cowpea KVx745-11P monoculture	–	$4721^{a} \pm 1550$	–	$2169^{a} \pm 341$
	Ponta Negra intercropped with Tiligré	$5735^{b} \pm 838$	$2238^{b} \pm 405$	$2015^{c} \pm 325$	$1193^{b} \pm 391$
	Ponta Negra intercropped with KVx745-11P	$6230^{b} \pm 1276$	$2025^{b} \pm 517$	$1345^{d} \pm 309$	$834^{b} \pm 224$
	Sariasso16 intercropped with Tiligré	$2832^{c} \pm 388$	$2420^{b} \pm 380$	$2365^{bc} \pm 145$	$1207^{b} \pm 241$
	Sariasso16 intercropped with KVx745-11P	$2313^{c} \pm 434$	$2288^{b} \pm 386$	$2315^{bc} \pm 457$	$1082^{b} \pm 475$
Baby trials (On-farm)	Sariasso16 only	$6414^{b} \pm 2817$	–	$3458^{ab} \pm 1175$	
	KVx745-11P only	–	$3860^{ab} \pm 1888$	–	$1268^{b} \pm 635$
	Sariasso16 intercropped with KVx745-11P	$3084^{bc} \pm 1232$	$3251^{ab} \pm 1265$	$2394^{bc} \pm 1089$	$1116^{b} \pm 401$
Statistic	F	2.21	2.21	4.48	3.58
	P-Value	0.035	0.039	0.000	0.002

Values with the same letters in the same column are equal (LSD; P = 0.05).

Cowpea fodder ME and IVOMD were lower in intercropping than their monoculture regardless of sorghum cultivar used for the intercropping. Cowpea Tiligré fodder ash, ADF, and ADL were greater in intercropping than in monoculture regardless of the companion sorghum cultivar in intercropping (Table 9). Cowpea KVx745-11P intercropped with sorghum Sariaso16 had greater fodder CP than in monoculture, while Tiligré intercropped with Ponta Negra had lower CP compared to monoculture. For cowpea cultivars, KVx745-11P had greater ash (10.6-12.5%) and CP (17-20%) regardless of the cropping system.

3.2 Discussion

3.2.1 Sorghum and cowpea intercropping reduced weed density and biomass

Cowpea and sorghum intercropping reduced weed density and biomass compared to sorghum monocultures. KVx745-11P was the most effective cowpea cultivar to improve weed control. Cowpea Tiligré was less effective, possibly because it is a semi-erect plant that also exhibits defoliation in the later stages of the reproductive period. This cultivar may be more sensitive to dry spells explaining

TABLE 7 Cropping systems System Productivity Index and Land Equivalent Ratio (mother and baby trials).

Trial	Treatment	LER For forage biomass	LER For grain yield	SPI For forage Biomass	SPI For grain yield
Mother trials (On- station)	Tiligré monoculture	1.00	1.00	3283	2116
	KVx745-11P monoculture	1.00	1.00	4721	2169
	Ponta Negra monoculture	1.00	1.00	10051	1863
	Sariaso16 monoculture	1.00	1.00	4447	4415
	Ponta Negra intercropped with Tiligré	1.25	1.65	12587	3064
	Ponta Negra intercropped with KVx745-11P	1.05	1.11	10539	2061
	Sariaso16 intercropped with Tiligré	1.37	1.11	6110	4883
	Sariaso16 intercropped with KVx745-11P	1.01	1.02	4468	4517
Baby trials (On-farm)	KVx745-11P monoculture	1.00	1.00	3860	1268
	Sariaso16 monoculture	1.00	1.00	6414	3458
	Sariaso16 intercropped with KVx745-11P	1.32	1.57	8486	5438

TABLE 8 Proximate composition and *in vitro* digestibility of the dry matter of sorghum straws from the mother trial.

Cropping system		DM (%)	Ash (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/kg)	IVOMD (%)
Monoculture	Ponta Negra	91.7^{b}± 0.3	5.7^{b} ± 0.9	4.1 ± 1.5	65^{b} ± 2	37^{c} ± 2	4.7^{b} ± 0.1	7.8^{ab} ± 0.2	51^{a} ± 2
	Sariasso16	92.3^{a} ± 0.3	6.2^{b} ± 1.0	4.5 ± 0.8	68^{ab} ± 2	40^{ab} ± 3	6.4^{a} ± 0.4	7.3^{b} ± 0.3	48^{b} ± 1
	Sariasso16*	92.3^{a} ± 0.2	8.6^{a} ± 1.0	4.4 ± 1.1	70^{a} ± 2	43^{a} ± 2	6.3^{a} ± 0.7	6.9^{bc} ± 0.3	46^{b} ± 2
Intercropping	Ponta Negra and KVx745-11P	91.7^{b} ± 0.8	5.5^{b} ± 0.9	5.1 ± 1.5	65^{b} ± 3	37^{c} ± 5	5.2^{b} ± 1.4	7.6^{ab} ± 1.0	51^{a} ± 3
	Sariasso16 and Tiligré	92.6^{a} ± 0.3	6.6^{b} ± 1.5	4.8 ± 0.6	68^{ab} ± 3	41^{ab} ± 2	6.2^{a} ± 0.4	7.2^{b} ± 0.4	47^{b} ± 2
	Sariasso16 and KVx745-11P	92.4^{a} ± 0.1	7.6^{ab} ± 1.17	5.4 ± 1.2	66^{ab} ± 1	40^{b} ± 1	6.1^{a} ± 0.6	7.2^{b} ± 0.2	48^{b} ± 1
	Ponta Negra and Tiligré	91.7^{b} ± 0.4	5.1^{b} ± 0.9	5.1 ± 0.7	64^{b} ± 2	35^{c} ± 1	4.7^{b} ± 0.9	8.1^{a} ± 0.2	52^{a} ± 1
	Sariasso16 and KVx745-11P*	92.2^{a} ± 0.4	8.8^{a} ± 1.1	4.9 ± 1.1	67^{ab} ± 4	42^{a} ± 4	6.1^{a} ± 0.6	7.0^{bc} ± 0.3	47^{b} ± 2
Statistic	F	4.26	10.10	0.73	3.69	7.34	4.56	5.30	3.94
	P-Value	0.002	0.000	0.64	0.004	0.000	0.001	0.000	0.003

*= On-farm trials. Values with the same letters in the same column are identical (LSD; $p \leq 0.05$).
DM, Dry Matter; CP, Crude Protein, NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; ADL, Acid Detergent Lignin; EM, Metabolizable Energy; IVOMD, In Vitro Organic Matter Digestibility.

the loss of leaves unfavorable to the process of smothering weeds (Ekeleme et al., 2019). Both sorghum cultivars were effective in controlling weeds, but Ponta Negra was somewhat more effective in relation to its great capacity to produce aerial biomass with large and long leaves that could play the role of smothering weeds. Allelopathy associated with competition for light, water, and mineral elements would influence weed photosynthesis, inducing a decrease in their growth (Kruk et al., 2006; Cordeau et al., 2015). Crop shading also creates unfavorable conditions for weed seed germination (Barro et al., 2016; Benider, 2018). Other authors also showed that cowpea intercropping with sorghum or maize reduced weed density and biomass compared to cereal monocultures and manual weeding (Bybee and Ryan, 2018; Ekeleme et al., 2019). Better weed control would lead to increased grain yield and fodder biomass and save time for plot weeding (Odhinambo and Ariga, 2001; Muhammad et al., 2013). Intercropping could therefore be an acceptable biological weed control approach in cropping systems instead of using chemical weed management strategies.

3.2.2 Grain yield and fodder biomass improvement

Sorghum Sariaso16, being a genetically improved grain type, had the greatest grain yield as expected. By comparison, Ponta Negra being a forage-type sorghum had the greatest fodder biomass. Those cultivars were selected as the best bet for this experiment based on prior research in Burkina Faso (Zampaligré et al., 2021). For cowpea, KVx745-11P and Tiligré had similar grain yield, while KVx745-11P fodder biomass was greater than for Tiligré, which is consistent with its spreading vegetative growth habit (Ramdé, 2019). The results are influenced by the combined effect of genetic, agro-climatic factors and various steps

TABLE 9 Proximate composition and *In vitro* digestibility of the dry matter of cowpea haulms tested from the mother trial.

Cropping system		DM (%)	Ash (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/kg)	IVOMD (%)
Monoculture	Tiligré	91.1 ± 0.1	8.8^{c} ± 1.7	15^{bc} ± 0.8	36^{bc} ± 2	23^{c} ± 2	4.6^{c} ± 0.3	10.0^{a} ± 1.0	67^{a} ± 0.5
	KVX745-11P	91.1 ± 0.3	10.6^{b} ± 0.7	17^{b} ± 1.4	36^{bc} ± 1	28^{b} ± 1	5.1^{bc} ± 0.1	9.8^{a} ± 1.0	67^{a} ± 0.7
	KVx745-11P*	91.1 ± 0.2	12.5^{a} ± 1.3	18^{b} ± 1.6	41^{a} ± 4	37^{a} ± 4	6.5^{a} ± 0.9	8.7^{c} ± 0.4	59^{c} ± 2.6
Intercropping	KVx745-11P and Ponta Negra	91.3 ± 0.3	11.1^{b} ± 1.0	17^{b} ± 2.2	35^{bc} ± 2	30^{b} ± 1	5.4^{bc} ± 0.4	9.5^{b} ± 0.1	65^{ab} ± 0.8
	Tiligré and Sariasso16	91.4 ± 0.2	9.9^{bc} ± 0.3	15^{bc} ± 0.2	34^{c} ± 4	27^{bc} ± 3	5.4^{bc} ± 0.7	9.7^{b} ± 0.2	66^{ab} ± 1.5
	KVx745-11P and Sariasso16	91.2 ± 0.2	10.6^{b} ± 0.2	20^{a} ± 2.6	37^{b} ± 2	31^{b} ± 1	5.8^{bc} ± 0.3	9.6^{b} ± 0.4	66^{ab} ± 2.4
	Tiligré and Ponta Negra	91.1 ± 0.1	9.9^{bc} ± 1.5	13^{c} ± 1.0	37^{b} ± 2	28^{b} ± 2	5.5^{b} ± 0.3	9.5^{b} ± 0.2	64^{ab} ± 1.1
	KVx745-11P and Sariasso16*	91.2 ± 0.3	13.2^{a} ± 0.8	19^{a} ± 1.5	39^{ab} ± 2	38^{a} ± 2	6.1^{a} ± 0.5	8.6^{c} ± 0.3	60^{c} ± 1.6
Statistic	F	1.53	9.23	7.93	4.18	21.12	6.05	22.41	18.05
	P-Value	0.19	0.001	0.001	0.002	0.001	0.001	0.001	0.001

*= On-farm trials. Values with the same letters in the same column are identical (LSD; $p \leq 0.05$).
DM, Dry Matter; CP, Crude Protein, NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; ADL, Acid Detergent Lignin; EM, Metabolizable Energy; IVOMD, In Vitro Organic Matter Digestibility.

of crop management (Coulibaly et al., 2012; Alidu et al., 2013; Coulibaly et al., 2020; Ouédraogo et al., 2021). Some authors identified Sariaso16 and Ponta Negra as the best cultivars performing well in the North Sudan zone of Burkina Faso respectively for grain yield and fodder biomass even though yields were lower compared to the results presented in this study (Zampaligré et al., 2021).

These results highlight that the choice of cultivar by farmers would depend on their primary production objective (grain or biomass). Sariaso16 and cowpea KVx745-11P cultivars were locally developed at Saria (North Sudan zone of Burkina Faso) by INERA for food and food-feed purposes, respectively, even though Sariaso16 has acceptable fodder biomass (Kondombo, 2001; Palé, 2017; Zampaligré et al., 2021). Sorghum Ponta Negra (EMBRAPA-Brazil) and cowpea Tiligré (INERA) cultivars were released for fodder production and grain purposes, respectively, with more or less intermediate performances for food (Palé, 2017; Zampaligré et al., 2021).

This study was performed in the 2019 and 2020 cropping seasons which were wet years (rainfall deviating from the 1990-2020 series) (Figure 3). The genetic effect and pedo-climatic conditions influenced crop cultivar performance. Indeed, water and soil fertility which were the most limiting factors of crop production affected crop genetic potential (Alidu et al., 2013; Lalsaga and Drabo, 2017). Greater grain yield and fodder biomass were associated with rainy years, moderate temperatures, and normal rainfall distributions with better soil fertility conditions (Ishiyaku and Aliyu, 2013; Kihindo et al., 2015; Obulbiga et al., 2015). Hence, location-specific characteristics and production objectives were important in cultivar selection and recommendation (Zampaligré et al, 2021).

3.2.3 Sorghum and cowpea intercrop improves grain and fodder biomass yield

Sorghum and cowpea intercropping LERs for fodder biomass and grain yield were usually greater than one. This showed an advantage in terms of total production for intercropping. The capacity production of one sorghum stand grown with cowpea was higher than the one of sorghum or cowpea stands grown in monoculture. Thus, sorghum and cowpea intercropping would save 1 to 65% of land use for the overall production (grain and biomass) compared to the monoculture of each crop. This would lead to an increase in grain yield and fodder biomass in the same order of magnitude (Obulbiga et al., 2015; Akanza & N'guessan, 2017; Diatta et al., 2019). The SPI values for sorghum-cowpea intercropping were also higher than those of the corresponding sorghum monocultures in the intercropping (main crop). Then, the conversion of grain yield and fodder biomass of the secondary crop (cowpea) to the main crop (sorghum) in the intercropping are all higher than those of the corresponding sorghum monocultures. The best fodder biomass indexes (10539 and 12587) were obtained by the intercropping of Ponta Negra and KVx745-11P, respectively; Ponta Negra and Tiligré. For grain yield, the best values of SPI (4517 and 4883) are recorded respectively by the intercropping of Sariaso16 and KVx745-11P; Sariaso16 and Tiligré. These results showed the advantages of grain yield and fodder biomass production with sorghum-cowpea intercropping and were corroborated by other authors (Agegnehu et al., 2008; Khan et al., 2020). These comparative advantages can be explained by the beneficial relationships of complementarity between the two associated crops for the use of nitrogen resources, space (very different aerial architectures), and growth peaks (Justes et al., 2014; Barro et al., 2016; Louarn et al., 2016). Shifting the sowing date between the two associated crops reduces the interspecific competition (Mbaye et al. (2014). Indeed, the symbiotic fixation of atmospheric nitrogen by cowpeas limits its competition with sorghum, which uses mineral nitrogen in the soil. In addition, a greater interception of radiation and a reduction in weed incidence by intercropping

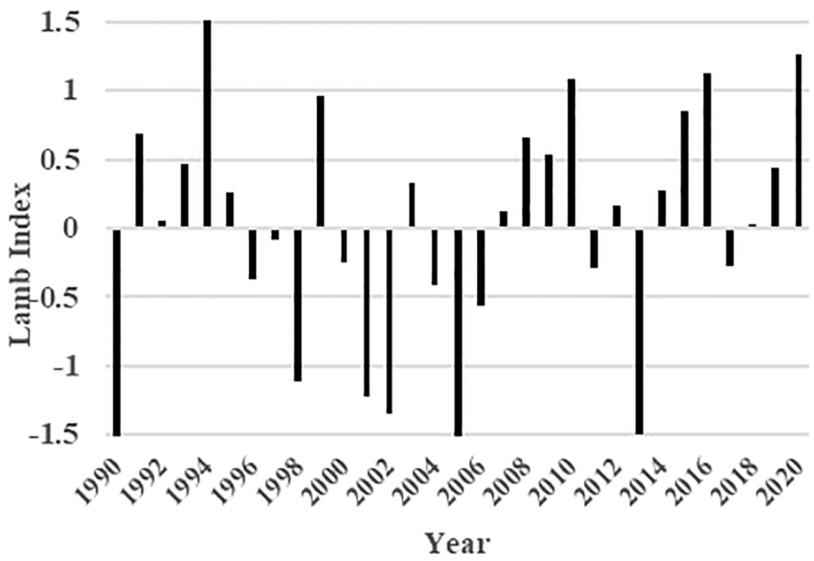


FIGURE 3
Saria rainfall Lam Index (1990-2020).

could further explain these advantages (Muhammad et al., 2013). Sorghum and cowpea intercropping is a very efficient and appropriate cropping system to optimize fodder biomass production and grain yield for crop-livestock farmers.

3.2.4 Sorghum and cowpea nutritive value as fodder

Sorghum Sariaso16 fodder content of two anti-nutritional factors NDF and ADL was greater than those of Ponta Negra, implying a slightly better nutritive value for Ponta Negra. Sariaso16 and Ponta Negra both had similar CP regardless of the cropping system. The fodder of Ponta Negra had higher IVOMD than Sariaso16, regardless of the cropping system, confirming its higher nutritive value. In addition, fodder of sorghum Ponta Negra intercropped with Tiligré had greater ME content. For cowpea cultivars, KVx745-11P had the greatest content of ash and CP regardless of cropping systems. Intercropping also differentially affected cowpea fodder's nutritive value for ash, ME, CP, and IVOMD. Finally, on-farm sorghum Sariaso16 and cowpea KVx745-fodder concentration in ash and ADF were greater than those on-station with the lowest content being ME. These results could be explained by the combined effect of genetic, agro-climatic factors and cropping systems (Cesar et al., 2009; Python and Boessinger, 2012; Mehdadi et al., 2013; Schlegel and Wyss, 2013; Louarn et al., 2016).

Cowpea KVx745-11P has better *stay-green attributes* at pod maturity with low leaf losses, resulting in greater CP content than many cultivars, which do not have these genetic characteristics (Obulbiga et al., 2015; Simian, 2017). Many cultivars of sorghum fodder showed variation of their CP content with ranges between 4.3 to 10.2%, (Simian, 2017; Zampaligré et al., 2021). This variability is mostly correlated to the harvest period; better CP concentration occurs during the heading phase and the CP begins to decline as the grains continue their maturation process (Simian, 2017). However, fodder cultivars such as Sariaso16 are more lignified with high concentrations of ADF and ash (Cesar et al., 2009). Some authors have shown that agro-ecological conditions and the duration time between fodder sample collection at farms and their pre-drying or drying would influence ash and crude cellulose content (Python and Boessinger, 2012; Mehdadi et al., 2013; Schlegel and Wyss, 2013). It has also been shown that cereal-legume intercropping would improve their total fodder quality in terms of protein and energy (Louarn et al., 2016). Sorghum fodder cultivars with CP concentration lower than 7% can be intercropped with cowpea in order to obtain an improved diet for livestock. Sorghum Ponta Negra intercropped with cowpea KVx745-11P would be the most appropriate cropping system for agro-pastoralists in Burkina Faso (livestock production as the main goal); whereas for crop-livestock farmers, it would be sorghum Sariaso16 intercropped with cowpea KVx745-11P (food production as the main goal).

For the extension of quality and quantity fodder production based on food-feed crops to meet human and livestock needs in Burkina Faso northern Sudan zone, we suggested emphasizing sorghum cultivar Ponta Negra and cowpea cultivar KVx745-11P intercropping and adoption of best fodder conservation technics in order to preserve fodder nutritive value for dry season utilization in Burkina Faso.

4 Conclusions

Sorghum and cowpea intercropping with improved cultivars resulted in optimized grain and fodder biomass production while effectively controlling weeds when compared to monoculture of either sorghum or cowpea. For intercropping systems, sorghum cultivar Ponta Negra and cowpea cultivars had the greatest fodder production whereas sorghum cultivar Sariaso16 and cowpea cultivars had the greatest grain yields. The two cultivars of sorghum had similar fodder nutritive value in terms of ash, CP, and ME although Ponta Negra had higher IVOMD and lower ADF. Cowpea KVx745-11P fodder nutritive value was the best for ash, CP, and ADF content. Sorghum-Cowpea intercropping differentially affected fodder nutritive value in terms of ash, ME, CP, and IVOMD. In summary, intercropping was more efficient and appropriate for fodder biomass and grain yield compared to monoculture with equivalent or better fodder quality.

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

SA: Contribution to study's conceptual and methodological design, as well as first manuscript drafting, revision, and editing. ZN: Contribution to study's conceptual and methodological design, as well as first manuscript drafting, revision, editing, and country project coordination. KA: Contribution to study's conceptual and methodological design, as well as manuscript revision and editing; this PhD research supervisor. DJ and OA: Contributed to data collection and curation. RF, DJ, JB, and BK: Contributed to the study's conceptual and methodological design, as well as manuscript revision and editing. AA: Contributed to manuscript revision and editing, project coordination, and fundraising. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was funded in whole or part by the United States Agency for International Development (USAID) Bureau for Resilience and Food Security under Agreement # AID-OAA-L-15-00003 as part of Feed the Future Innovation Lab for Livestock Systems. Additional funding was received from the Bill & Melinda Gates Foundation OPP#1175487. Any opinions, findings, conclusions, or recommendations expressed here are those of the authors alone. Under the grant conditions of the Foundation, a Creative Commons Attribution 4.0 Generic License has already been assigned to the

Author Accepted Manuscript version that might arise from this submission.

Conflict of interest

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

Publisher's note

References

Abera, A., Mekonnen, K., Duncan, A. J., Kamal, S., Ergano, K., Thorne, P., et al. (2021). Redesigning traditional weed management practices in faba bean production to optimize food-feed production in the smallholder system. *Agron. J.*, 248–258. doi: 10.1002/agj2.20779

Agegnehu, G., Ghizaw, A., and Sinebo, W. (2008). Yield potential and land-use efficiency of wheat and faba bean mixed intercropping. *Agron. Sustain. Dev.* 28 (2), 257–263. doi: 10.1051/agro:2008012

Akakpo, D. B., de Boera, I. M., Adjei-Nsiahc, S., Duncand, A. J., Giller, K. E., and Oosting, S. J. (2020). Evaluating the effects of storage conditions on dry matter loss and nutritional quality of grain legume fodders in West Africa. *Anim. Feed Sci. Technol.* 262. doi: 10.1016/j.anifeedsci.2020.114419

Akanza, K. P., and N'guessan, N. E. (2017). *Performances agronomique et économique des systèmes de culture à base de maïs (Zea mays L.) et d'arachide (Arachis hypogaea L.) au nord de la Côte d'Ivoire* Vol. 5 (REV. CAMES).

Alidu, M. S., Atokple, I. D., and Akromah, R. (2013). Genetic analysis of vegetative-stage drought tolerance in cowpea. *Greener J. Agric. Sci.* 3 (6), 481–496. doi: 10.15580/GJAS.2013.3.030613516

Amole, T., and Ayantunde, A. A. (2016). Improving livestock productivity: Assessment of feed resources and livestock management practices in Sudan-Savanna zones of West Africa. *Afr. J. Agric. Res.* 11, 422–440. doi: 10.5897/AJAR2015.10460

Barro, A., Sangaré, M., Coulibaly, K., Koutou, M., and Diallo, M. A. (2016). Etude des modalités d'association maïs/niébé dans les villages de Koumbia et Gombêlédougou en zone cotonnière de l'Ouest du Burkina Faso. *Sci. Technique Spécial hors-série*, 151–163.

Bayala, J., Ky-Dembele, C., Kalinganire, A., Olivier, A., and Nantoume, H. (2014). *A review of pasture and fodder production and productivity for small ruminants in the Sahel* (Nairobi: ICRAF/World agroforestery Center).

Beck, H. E., Zimmermann, N. E., McVicar, R. T., Vergopolan, N., Berg, N. A., and Wood, F. E. (2018). Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Sci. Data* 5. doi: 10.1038/sdata.2018.214

Benider, C. (2018). *Performances de l'association céréales-légumineuses en systèmes fourragers des régions semi-arides (Algérie). thèse de doctorat en biologie végétale* Vol. 1 (Université Ferhat Abbas Sétif).

Boote, J. K., Adesogan, T. A., Balehegn, M., Duncan, A., Muir, P. J., Dubeux, C. J. Jr., et al. (2021). Fodder development in Sub-Saharan Africa: an introduction. *Agron. J.* 114, 1–7. doi: 10.1002/agj2.20924

Bybee, K. A., and Ryan, M. R. (2018). Advancing intercropping research and practices in industrialized agricultural landscapes. *Agriculture* 8, 80–96. doi: 10.3390/agriculture8060080

Cesar, J., and Guiro, A. (2004). *Les légumineuses fourragères 'herbacées. Production animale en Afrique de l'Ouest. Fiche technique NO 7* (Bobo-Dioulasso: CIRDES).

Cesar, J., Kanwe, A., Zongo, L., and Akoudjim, M. (2009). *EXPERIMENTATIONS EN CULTURES FOURRAGERES dans la region de Bobo-Dioulasso* (Bobo-Dioulasso: CIRDES - Urpan/CIRAD).

Cordeau, S., Guillemin, J. P., Reibel, C., and Chauvel, B. (2015). Weed species differ in their ability to emerge in no-till systems that include cover crops. *Ann. Appl. Biol.* 166, 444–455. doi: 10.1111/aab.12195

Coulibaly, Z., Barro, A., Tignegré, J. B., and Kiébré, Z. (2020). Évaluation des performances agronomiques de douze (12) variétés de niébé vert [Vigna unguiculata (L.) walp.] au Burkina Faso. *J. Appl. Biosci.* 153, 15745–15755.

Coulibaly, K., Gomgnimbou, A. P., Traore, M., Nacro, H. B., and Sédogo, M. (2017). Effets des associations maïs-légumineuses sur le rendement du maïs (Zea mays L.) et la fertilité d'un sol ferrugineux tropical à l'Ouest du Burkina Faso. *Afrique Sci.* 13 (6), 226–235. doi: 85597201

Coulibaly, K., Vall, E., Autfray, P., and Sedogo, P. M. (2012). Performance technico-économique des associations maïs-niébé et maïs-mucuna en situation réelle de culture au Burkina Faso: potentiels et contraintes. *Tropicultura* 30 (3), 147–154. doi: 10.35759/JABS.153.2

Cuvelier, C., and Dufrasne, I. (2014). *L'alimentation de la vache laitière: Aliments, calculs de ration, indicateurs d'évaluation des déséquilibres de la ration et pathologies d'origine nutritionnelle. Livret de l'agriculteur* (Liège: Université de Liège). 105p.

Diatta, A. A., Abaye, O., Thomason, W. E., Modou, L. O., Guèye, F., Baldé, A. B., et al. (2019). Effet de l'association du haricot mungo sur le rendement du mil dans le Bassin arachidier. *Innov. Agronomiques* 74, 69–81. doi: 10.15454/T7GWAX

Duncan, A. J., Bachewe, F., Mekonnen, K., Lule, D., Bahta, M., and Erenstein, O. (2016). Crop residue allocation to livestock feed, soil improvement and other uses along a productivity gradient in East Africa. *Agric. Ecosyst. Environ.* 228, 101–110. doi: 10.1016/j.agee.2016.05.011

Ekeleme, F., Atser, G., Dixon, A., Hauser, S., Chikoyé, D., Olorunmaiye, P. M., et al. (2019). Assessment of weeds of cassava and farmers mannagement practices in Nigeria. *Tropicultura* 37 (2), 12–25.

FAO (2014). *Résidus agricoles et sous-produits agro-industriels en Afrique de l'ouest: état des lieux et perspectives pour l'élevage* (Accra: Bureau Régional pour l'Afrique de la FAO). 73P.

Feyissa, F., Prasad, S., Assefa, G., Bediye, S., Kitaw, G., Kehaliew, A., et al. (2014). Dynamics in nutritional characteristics of natural pasture hay as affected by harvesting stage, storage method and storage duration in the cooler tropical highlands. *Afr. J. Agric. Res.* 9, 3233–3244. doi: 10.5897/AJAR2014.8652

Gonsalves, J., Becker, T., Braun, A., Campilan, D., de Chavez, H., Fajber, E., et al. (2005). *Participatory research and development for sustainable agriculture and natural resource management: A sourcebook. Volume 2: enabling participatory research and development* (Laguna, Philippines; Ottawa, Canada: International Potato Center-Users' Perspectives With Agricultural Research and Development and International Development Research Centre).

Ilboudo, M. (2020). *Optimisation de la production fourragère à base d'association culturale de variétés améliorées de sorgho et d'arachide à double usage dans la zone Nord Soudanienne du Burkina Faso* (Bobo Dioulasso: Mémoire de fin de cycle/Université Nazi Boni-IDR).

Ishiyaku, M. F., and Aliyu, H. (2013). Field evaluation of cowpea genotypes for drought tolerance and Striga resistance in the dry savanna of north-west Nigeria. *Int. J. Plant Breed. Genet.* 7 (1), 47–56.

Justes, E., Bedoussac, L., Corre-Hellou, G., Fustec, J., Hinsinger, P., and Pelzer, E. (2014). Les processus de complémentarité de niche et de facilitation déterminant le fonctionnement des associations végétales et leur efficacité pour l'acquisition des ressources abiotitiques. *Innov. agronomiques* 40, 1–24.

Kagoné, H. (2001). *Profil fourrager – Burkina Faso* (Rome, Italie: FAO). Available at: https://docplayer.fr/51588104-Profil-fourrager-burkina-faso-par-dr-ir-hamade-kagone.html.

Khan, M. N., Shoaib, M., Ashraf, M. S., Qamar, R., Mahboob, A., and Ijaz, S. (2020). Mungbean (Vigna radiata) intercropping enhances productivity of late season irrigated cotton in Punjab. *Asian J. Agric. Biol.* 8 (4), 472–479. doi: 10.35495/ ajab.2020.03.187

Kiéma, A. (2008). *Effets des techniques de restauration et d'exploitation des pâturages naturels sahéliens sur la dynamique de la production fourragère* (Bobo-Dioulasso: Université Polytechnique de Bobo-Dioulasso).

Kiéma, A., Bambara, G. T., and Zampaligré, N. (2019). *Transhumance et gestion des ressources naturelles au Sahel: contraintes et perspectives face aux mutations des systèmes de productions pastorales* Vol. 14 (La revue électronique en sciences de l'environnement).

Kihindo, A. P., Bazie, R. H., Ouedraogo, R. F., Somé, P. P., Zombré, G., and Tozo, K. (2015). Effets de la date de semis et du régime hydrique sur la réponse agro morphologique de deux variétés de niébé (KN1 ET KVX 61-1) au Burkina Faso. *Intrenational J. Innovation Appl. Stud.* 12 (3), 564–573.

Kondombo, C. P. (2001). *Evaluation agronomique et fourragère de 194 lignées recombinantes de sorgho. Mémoire de fin de cycle d'Ingénieur du Développement Rural, option: Agronomie* (Burkina Faso: Institut du développement rural, Université polytechnique de Bobo Dioulasso). 89p.

Kristjanson, P., Neufeldt, H., Gassner, A., Mango, J., Kyazze, B. F., Desta, S., et al. (2012). Are food insecure smallholder households making changes in their farming practices? *Evidence East Africa Food Secur.* 4 (3), 381–397. doi: 10.1007/s12571-012-0194-z

Kruk, B., Insausti, P., Razul, A., and Benech-Arnold, R. (2006). Light and thermal environments as modified by a wheat crop: effects on weed seed germination. *J. Appl. Ecol.* 43, 227–236. doi: 10.1111/j.1365-2664.2006.01140.x

Lalsaga, W. J., and Drabo, I. (2017). Évaluation de quinze génotypes de niébé [Vigna unguiculata (L.) Walp.] sous conditions pluviales dans le Nord et le Centre Ouest du Burkina Faso. *Int. J. Biol. Chem. Sci.* 11 (6), 2756–2763. doi: 10.4314/ijbcs.v11i6.16

Louarn, G., Faverjon, L., Bijelić, Z., and Julier, B. (2016). Dynamique de l'azote dans les associations graminées -légumineuses: quels leviers pour valoriser l'azote fixé? *Fourrages Fourrages* 226, 135–142.

MA (2015). *Resultats definitifs de la campagne agricole 2014/2015 et perspectives de la situation alimentaires et nutritionelle* (Ouagadougou: Ministère de l'agriculture).

Matusso, J. M., Mugwe, J. N., and Mucheru-Muna, M. (2014). Potential role of cereal-legume intercropping systems in integrated soil fertility management in smallholder farming systems of Sub-Saharan Africa. *Res. J. Agric. Environ. Manage.* 3 (3), 162–174. doi: 46301268

Mbaye, M. S., Gueye, M., Bassence, C., Ba, N., Diop, D., Sylla, S. N., et al. (2014). Date et densité optimales de semis du niébé [Vigna unguiculata (L.) Walp.] en association avec le mil [Pennisetum glaucum (L.) R. Br.]. *J. Appl. Biosci.* 76, 6305–6315. doi: 10.4314/jab.v76i1.4

Mehdadi, Z., Benaouda, Z., Latreche, A., Benhassaini, H., and Belbraouet, S. (2013). Évolution saisonnière de la composition foliaire de Stipa tenacissima L. en éléments minéraux et en fibres pariétales. *Acta Botanica Gallica* 155 (3), 435–445. doi: 10.1080/12538078.2008.10516123

MRA (2015). *Rapport statistique annuel* (Ouaougougad: MRA).

MRSI (2014). *Catalogue national des espèces et variétés agricoles du Burkana Faso* (Ouagadougou: MASA/MEDD).

Muhammad, A. K., Khan, B. M., UMM, E. K., Zahid, H., Saima, H., Abdur, R., et al. (2013). Weed contol effects on Wheat-Pea intercropping. *Pak. J. Bot.* 45 (5), 1743–1748.

Mulumba, J. B., Somda, J., Sanon, Y., and Kagoné, H. (2008). *Elevage et marché régional au Sahel et en Afrique de l'Ouest* (Potentialités et défis. CSAO/OCDE/CEDEAO). 163 p.

Nantoumé, H., Kouriba, A., Togola, D., and Ouologuem, B. (2000). Mesure de la valeur alimentaire des fourrages et sous-produits utilisés dans l'alimentation des petits ruminants. *Rev. d'Elevage Médicine Vétérinaire Des. Pays Tropicaux* 53, 279–284.

Nasir, I., Sajad, H., Zeeshan, A., Feng, Y., Xiaochun, W., Weiguo, L., et al. (2019). Comparative analysis of maize–soybean strip intercropping systems. *Plant Production Sci.* 22 (2), 131–142.

N'Goran, K. E., Kaassin, K. E., Zohouri, G. P., N'Gbesso, M. F., and Yoro, G. R. (2011). Performances agronomiques des associations culturales igname légumineuses alimentaires dans le Centre- Ouest de Côte d'Ivoire. *J. Appl. Biosci.* 43, 2915–2923.

Obulbiga, M. F., Bougouma, V., and Sanon, O. H. (2015). Amélioration de l'offre fourragère par l'association culturale céréale-légumineuse à double usage en zone nord soudanienne du Burkina Faso. *Int. J. Biol. Chem. Sci.* 9 (3), 1431–1439. doi: 10.4314/ijbcs.v9i3.26

Odhinambo, G. D., and Ariga, E. S. (2001). Effet of intercropping maize and bean on striga incidence and grain yield. *Seventh Eastern South. Afr. Rrgional Maize Conf.*, 183–186.

Ouédraogo, S. (2019). *Performances agronomiques et fourragères des variétés améliorées de Sorgho à double usage en zone nord soudanienne du Burkina Faso* (Bobo Dioulasso: Mémoire de fin de cycle. Université Nazi Boni-IDR).

Ouédraogo, N., Thio, G. I., Sanou, A., Kouraogo, I., Barro, O., Sawadogo1, A. P., et al. (2021). Agronomic performance and adaptability study of New Guinea lines in Sudanian and Sudano-sahelian zones. *J. Appl. Biosci.* 167, 17320–17334.

Palé, K. (2017). *Evaluation des performances agronomiques de cinq lignées de niébé et de leurs qualités fourragères à la Station expérimentale de Saria, Burkina Faso* (Dedougou: IISEDR-CUPD).

Python, P., and Boessinger, M. (2012). Facteurs d'influence sur les valeurs nutritives des fourrages secs ventilés/Production végétale. *Recherche Agronomique Suisse* 3 (1), 36–43.

Ramdé, A. (2019). *Performances agronomiques et fourragères de variétés améliorées de niébé (Vigna unguiculata (L.) Walpers) à double usage dans les zones agro écologiques du Burkina Faso* (Université Ouaga1 Pr. Joseph Ki Zerb: Mémoire de fin de cycle. ISEDR).

Rusike, J. S., Snapp, S. S., and Twomlow, S. (2004). *Mother-Baby trial approach for developing soil water and fertility management technologies. Volume 2* (Lima, Peru: Field Tested Practices in Participatory Research and Development International Potato Center (CIP-UPWARD).

Sanfo, A., Savadogo, I., Kulo, E. A., and Zampaligre, N. (2015). Climate change: A driver of crop farmers and agro-pastoralists conflicts in Burkina Faso. *Int. J. Appl. Sci. Technol.* 5 (3), 92–104. doi: 10.35759/JAnmPlSci.v46-3.4

Sanfo, A., Zampaligré, N., and Kulo, E. A. (2020). Analyse des préférences des agropasteurs pour la production et la conservation du fourrage à base devariétés améliorées de cultures à double objectifs dans deux zones agro-écologiques au Burkina Faso. *J. Anim. Plant Sci.* 46 (3), 8318–8335.

Sanfo, A., Zampaligré, N., Kulo, A. E., Somé, S., Traoré, K., Rios, E. F., et al. (2023). Performance of food–feed maize and cowpea cultivars under monoculture and intercropping systems: Grain yield, fodder biomass, and nutritive value. *Front. Anim. Sci.* 3, 998012. doi: 10.3389/fanim.2022.998012

Sanou, K. F., Nacro, S., Ouédraogo, M., Ouédraogo, S., and Kaboré-Zoungrana, C. (2011). La commercialisation de fourrages en zone urbaine de Bobo-Dioulasso (Burkina Faso): pratiques marchandes et rentabilité économique. *Cah Agric.* 20, 487–493. doi: 10.1051/cagri/2016007

Schlegel, P., and Wyss, Y. ,. U. (2013). Teneur en oligo-éléments des fourrages. *Renc. Rech. Ruminants* 20, 45–48.

Simian, A. (2017). *Production et valeur alimentaire des résidus de culture des variétés à double objectifs utilisés dans l'alimentation animale dans la province du Yatenga* (Bobo Dioulasso: Mémoire de fin de cycle. Université Nazi Boni-IDR).

Snapp, S. (2002). "Quantifying farmer evaluation of technologies: the mother and baby trial design," in *Quantitative analysis of data from participatory methods in plant breeding*. Ed. D. M.a. Bellon (Mexico, DF: CIMMYT).

Somda, J. (2001). Performances zootechniques et rentabilité financière des ovins en embouche au Burkina Faso. *Biotechnol. Agron. Soc Environ.* 5 (2), 73–78.

Traoré, S. H. (2012). Etude des inlpacts agro-pédologiques des apports continus et en rotation de fertilisants organo-minéraux sur le cotonnier en stations de recherche : cas de Sana et Farako-Bâ. Bobo-Dioulasso: IDR-Université polytechnique de Bobo-Dioulasso.

Willey, R. W. (1979). Intercropping—It's Important and Research Needs. Part 1. Competition and Yield Advantages. *Field Crop Abstracts* 32, 1–10.

Zampaligré, N., Dossa, H. L., and Schlecht, E. (2013). Contribution of browse to ruminant nutrition across three agro-ecological zones of Burkina Faso. *J. Arid Environments* 95, 55–64. doi: 10.1016/j.jaridenv.2013.03.011

Zampaligré, N., Yoda, G., Delma, J., Sanfo, A., Balehegn, M., Rios, E., et al. (2021). Yield and fodder nutritive value of Pearl Millet, Sorghum, and Maize cultivars in Burkina Faso. *Agron. J.* 114, 115–125. doi: 10.1002/agj2.20860

frontiers | Frontiers in Animal Science

TYPE Review
PUBLISHED 19 December 2023
DOI 10.3389/fanim.2023.1274473

OPEN ACCESS

EDITED BY
Juana Catarina Cariri Chagas,
Swedish University of Agricultural Sciences, Sweden

REVIEWED BY
Sakine Yalçin,
Ankara University, Türkiye
Gabriel Da Silva Viana,
Natural Resources Institute Finland (Luke), Finland

*CORRESPONDENCE
Muzi M. Ginindza
gininmm@unisa.ac.za

RECEIVED 08 August 2023
ACCEPTED 07 November 2023
PUBLISHED 19 December 2023

CITATION
Ginindza MM (2023) Lucerne meal in the diet of indigenous chickens: a review. *Front. Anim. Sci.* 4:1274473. doi: 10.3389/fanim.2023.1274473

Lucerne meal in the diet of indigenous chickens: a review

Muzi M. Ginindza*

College of Graduate Studies, School of Interdisciplinary Research and Graduate Studies, University of South Africa, Pretoria, South Africa

Indigenous chicken production consists of an array of activities important to smallholder poultry farmers in Africa. One of the many factors influencing their production and threating the local food security is in the area of nutrition, particularly, that related to protein supplementation. The available feed resources to farmers are not enough to sustain the productivity of the chickens. Hence, the chickens' diets often require nutritional supplementation. There is therefore an urgent need for the validation of locally grown feed ingredients to improve the sustainability of poultry production in sub-Saharan Africa. A dietary ingredient that may be used in the diets of chickens is lucerne (*Medicago sativa*), which is also known as alfalfa. In South Africa, lucerne is the most cultivated forage legume and approximately 1.3 million metric tonnes of lucerne are produced per year. Lucerne has high nutritional value, as it is a source of protein, amino acids, vitamins, and fatty acids. The potential of lucerne as a feed resource for indigenous chickens should, therefore, be investigated so that strategies to improve the nutrition of such chickens can be developed. The purpose of this review was to highlight lucerne as a potential dietary ingredient for indigenous chickens and discuss its effects on the productivity of broilers, egg-layers, and dual-purpose chickens.

KEYWORDS

***Medicago sativa*, poultry, diet, protein source, fibre, saponins**

1 Introduction

Food production must increase to meet global nutritional needs, particularly in the sub-Saharan countries, where economic and health challenges negatively contribute to food insecurity (Bjornlund et al., 2022). In this area of the globe, sustainable agriculture proves to be critical, as population growth rates continue to increase while arable land availability has been declining due to industrialization (Caillouet et al., 2019). Poultry producers are faced with a plethora of challenges related to the health, nutrition, and predation of their flocks. This has been exacerbated by the increase in ambient temperatures in communities where indigenous chickens are kept (Mapiye et al., 2008; Nyoni et al., 2022). Indigenous chickens have been exposed to unfavourable environmental conditions for centuries, which has resulted in their developing traits to cope with these harsh conditions, which include food scarcity (Köhler-Rollefson, 2004). Such birds can convert a wide range of agri-food

by-products into edible products for humans (Mottet and Tempio, 2017). Therefore, indigenous chicken rearing could be an alternative means of maintaining and increasing sustainable food production in Africa. Meat and egg production are crucial for the nutrition of families in Africa (Alabi et al., 2012; Motsepe et al., 2016) and they are marketed as enhancing incomes (Nondzutha et al., 2020). Furthermore, indigenous chickens are used in communities for traditional events (Herd-Hoare and Shackleton, 2020).

In a country such as South Africa, the production of indigenous chickens predominantly takes place under subsistence farming of the extensive type, which is characterised by low-input systems (Mtileni et al., 2016; Idowu et al., 2021). Several researchers have found that poultry farmers in rural communities in South Africa rear from 5 to 30 chickens per cycle (Guèye, 1998; Idowu et al., 2019; Nxumalo et al., 2020). The chickens are often left to search for food that meets their nutritional requirements on their own (Raphulu et al., 2015; Yusuf et al., 2017), especially when supplementary grains are out of season. Chickens reared under subsistence farming in South Africa have been found to suffer greatly because of poor nutrition (Alabi et al., 2013). The rising cost of feed constrains production and is, therefore, a key motivation to seek alternative forms of feed resources for poultry farmers and producers. The main source of protein in poultry diets for the commercial sector, soyabean meal, remains expensive for smallholder farmers. The range of locally available feed resources is wide in terms of dietary ingredients; however, it does not yield enough of the nutrients required by chickens for sustaining production (Rashid et al., 2003; Ncobela and Chimonyo, 2015; Hayat et al., 2016). Protein, which is a limiting factor for growth and productivity, is often inadequate in the diet of chickens (Ncobela and Chimonyo, 2015). Due to the scarcity of some grains, the potential of locally grown alternative feed resources could and should, therefore, be thoroughly explored in African communities to develop nutritional strategies to optimize the nutrition of indigenous birds and consequently ensure local food security.

Plant species such as *Carica papaya* (Banjoko et al., 2020), *Moringa oleifera* (Sebola et al., 2015), *Morus alba* (Mwai, 2021), *Ipomoea batatas* (Bekele and Sintayehu, 2021), *Amaranthus cruentus* (Manyelo et al., 2022), and *Azadirachta indica* (Ubua et al., 2019) are useful in chicken diets as sources of protein (Sugiharto et al., 2019). However, the usage of some of these plants by subsistence farmers of indigenous chickens is limited, not solely because of antinutritional factors, but also due to their limited availability and their high cost of acquisition. The cost of agricultural inputs and of establishing some of these recommended plants in yields may also not be practical. Therefore, the need to further explore adapted and hardy forage crops for indigenous chickens is pertinent.

Lucerne (*Medicago sativa*), also known as alfalfa, is commonly used as a forage for ruminants as hay or silage, and, to a lesser extent, as grazing material (Suwignyo et al., 2021). However, when harvested at the appropriate growth stage, literature shows that it may be of great importance for poultry feeding (Tkáčová et al., 2011; Jiang et al., 2018; Paredes and Risso, 2020; He et al., 2021; Vlaicu et al., 2023). For a long period lucerne has been used in poultry diets as a source of fibre for egg-laying chickens, and it was used to induce moulting (Donalson et al., 2005; Glatz and Tilbrook, 2021). However, research has indicated that it possesses not only favourable amino acid (Homolka et al., 2008) and fatty acid contents (Bashir et al., 2023), but also pharmacological benefits (Bora and Sharma, 2011). The potential of lucerne as a feed resource, therefore, needs to be investigated to map strategies for improving the nutrition of indigenous chickens, especially in developing countries such as South Africa. The purpose of this review was to assess the available literature on the utilization of lucerne as a dietary ingredient for indigenous chickens and its effects on chickens (broilers, egg-layers, and dual-purpose breeds).

2 Description of lucerne

Lucerne is known as the "queen of forages" because of its ability to sustain growth and adapt to a wide range of climatic conditions (Russelle, 2001). It is a forage that is grown in many parts of the world (Mielmann et al., 2017). It is cultivated in more than 80 countries and in all continents, over areas which exceed 35 million ha (Radović et al., 2009). It is grown in the frozen regions of southern Canada and eastern China, and from the mild climates of Chile to the searing deserts of Mexico and Africa.

Lucerne belongs to the class Magnoliopsida, in the order Fabales, in the family Fabaceae, which are also known as legumes and belong to the genus *Medicago*, species *sativa* (Burezq, 2021). The plant has a "crown" that can generate much foliage through bud formation, having many stems at the base of the plant, which grow to the height of about 1 m, and by having a deep tap root system. The leaves of lucerne are trifoliate with smooth, slightly tooth-edged, and oval-shaped leaflets. The flower is mostly purple in colour, and approximately 0.8 cm wide, and has a cluster up to 4 cm long at the top of its branches. Lucerne develops pods in its reproductive stage. Each plant may have four or five coiled spiral pods with a hard outer surface, which are typically hairy or leathery. Each pod may have between three and seven seeds.

Lucerne is a perennial legume crop that may be harvested three to five times per year depending on the harvesting interval and cultivar (Palmonari et al., 2014; Xu et al., 2021; Eckberg et al., 2022). It can survive defoliation through its ability to quickly regenerate shoots. It can respond quickly to significant summer rainfall (>10 mm) but requires 20 mm–25 mm of rainfall to produce substantial growth.

In South Africa, lucerne is the most cultivated forage legume and the country produces approximately 1.3 million tonnes of lucerne per year, which is planted in all provinces (STATSSA, 2017). The western to central regions of the country (i.e., the Northern, Western, and Eastern Capes, the Free State, and the North West provinces) produce a greater amount of lucerne (129,986 to 487, 181 tonnes per year) under dry and irrigated farms. In contrast, the eastern to central regions (i.e., the Mpumalanga, Limpopo, KwaZulu-Natal, and Gauteng provinces) produce smaller amounts of lucerne (1,019 to 4,767 tonnes) per year under dry and irrigated farms. These areas contain a wide range of soil characteristics (texture, organic matter, nitrogen and

phosphorus content, and pH) and farm management practices that can influence yields.

3 Nutritional value

The nutritive value of lucerne is affected by its nutritional components, which tend to vary as the plant grows. This is illustrated by the nutrient composition harvested at the different physiological stages (Fan et al., 2018) (Table 1). As the plant matures, the crude protein (CP) content decreases, while the fibre content increases. Thus, the stage of harvesting is important for lucerne. Other climatic factors and edaphic factors (i.e., soil conditions, storage, diseases and insects, weeds, cultivar, water supply, and fertilisation) affect the nutritional quality of lucerne (Scholtz et al., 2009). Xu et al. (2021) have illustrated that the harvesting interval (28 day or 35 days) in a given year also affects the plant's nutrient composition.

The nutrient composition of lucerne meal is presented in Table 2. Indigenous chickens (growers and adults) require 12% to 16% CP dry matter (DM) in their diets (NRC, 1994; Kingori et al., 2007; Alabi et al., 2013; Kalinda and Tanganyika, 2017). However, Goromela et al. (2006) and Hayat et al. (2016) reported that indigenous chicken diets are provided with only 10% CP DM under free-range systems. This, therefore, indicates that there is a deficit in the diet of chickens in free-range systems which has to be supplemented through various interventions. Lucerne can be utilized as a dietary ingredient providing protein for chickens as it contains 16% to 24% CP (Table 2).

The crude fibre (CF) level of lucerne meal ranges between 20.3% and 30.0% DM (Table 2), while its neutral detergent fibre content ranges between 36.7% and 53.7% according to He et al. (2021) and Hidosa and Biru (2021). When harvested during the budding stage, lucerne will contain a higher moisture content and less fibre [neutral detergent fibre (NDF) and acid detergent fibre (ADF)]. As plants age, they tend to have a higher CF content (Fan et al., 2018). Laudadio et al. (2014) lowered the fibre content in lucerne meal by fractionation and classification process to achieve 15.3% CF in DM. However, such technologies might be inaccessible to, and impractical for, smallholder farmers in Southern Africa.

The ash content of lucerne meal ranges between 9.2% and 12.9% DM (Table 2). The ash content indicates the concentration of minerals in the feed ingredient. Mwalusanya et al. (2002) and Hayat et al. (2016) showed that scavenging chickens do not suffer from the lack of minerals. Raphulu et al. (2015) demonstrated that South African indigenous chickens meet dietary mineral requirements determined by the ash levels of the crop contents for growing and adult indigenous chickens which ranged between 10.5 to 12.3%. Therefore, lucerne may be utilized by subsistence farmers to meet the mineral growth and production requirements of indigenous chickens.

TABLE 1 Crude protein and fibre contents (% DM) of lucerne harvested at different stages of physiological growth (Fan et al., 2018).

Growth stage	Crude protein	Neutral detergent fibre	Acid detergent fibre
Budding	29.00	19.36	12.14
Early flowering	27.69	21.58	12.16
Middle flowering	26.84	22.43	12.21

DM, Dry matter.

Lucerne meal has a good balance of amino acids (Ensminger, 1992; Sen et al., 1998; Ponte et al., 2004; Markovic et al., 2007). Table 3 shows the concentration of 17 amino acids reported in lucerne meal and other proteinous dietary ingredients (*Moringa oleifera*, sunflower, and soyabean meals). Lucerne contains high proportions of aspartic acid, glutamic acid, and leucine, but low proportions of methionine and cysteine (Homolka et al., 2008; Pleger et al., 2021; Luo et al., 2022). When compared with the other plant species, lucerne and moringa leaf meal have a similar lysine content (5.4 g/100 g to 5.6 g/100 g CP), which is higher than that of sunflower meal (3.6g/100 g CP). Lucerne and soyabean meal also contain similar methionine levels (1.4 g/100 g CP). However, sunflower seed meal has a slightly higher methionine content (1.7 g/100 g CP) than lucerne meal. The threonine level of lucerne is similar to that of soyabean (3.8 g/100 g CP). However, moringa leaf meal contains a higher (4.5 g/100 g CP) threonine content than lucerne meal.

3.1 Secondary metabolites in lucerne

Secondary metabolites are a diverse group of compounds with a low molecular weight and are distinct from primary metabolites. They do not play a crucial role in the normal growth and development of plants; they are, however, synthesized for defence purposes and other forms of interspecies protection (Bennett and Wallsgrove, 1994). Secondary metabolites have a myriad of properties including antioxidative, pharmacological, antifungal, anti-inflammatory, and phytoestrogenic properties (Wang et al., 2021). The secondary metabolites of plants can be classified into three broad groups: phenolic compounds, terpenoids, and nitrogen-containing compounds.

Lucerne contains different types of phenolic compounds (Krakowska et al., 2017; Horvat et al., 2022) (Table 4). Phenolics may further be classified as either simple phenols (phenolic acids) or polyphenols (flavonoids and non-flavonoids), as shown in Vardhan and Shukla, 2017. Dietary supplementation with lucerne flavonoids (15 mg/kg diet) improved the growth performance of chickens (Surai, 2014; Ouyang et al., 2016). The factor responsible for the increased growth performance of chickens was explained as the combination of the growth and hepatic growth hormone receptor upregulated by isoflavone (Elkomy and Elghalid, 2014). Additionally, isoflavones promote protein synthesis in the muscles, which in turn leads to increased growth (Kamboh and Zhu, 2013). In egg layers, Iskender et al. (2017) and Prihambodo et al. (2022) showed that the use of dietary supplementation flavonoids (0.5 g/kg of hesperidin, naringin, or quercetin) had no effect on laying performance; however, the egg yolk cholesterol level decreased while the yolk protein percentage increased. Improvements in the

TABLE 2 Nutrient composition (% DM) of lucerne (*Medicago sativa*) meal.

Nutrient component							Reference
DM	EE	CP	Ash	CF	NDF	ADF	
90.1	–	17.3	11.1	–	–	–	Pleger et al. (2021)
79.5	–	18.5	–	28.9	41.3	33.3	Homolka et al. (2008)
92.7	–	20.7	13.0	–	44.1	33.2	Scholtz et al. (2009)
85.0	1.3	22.5	9.5	30.0	49.3	37.5	McDonald et al. (2011)
89.7	–	20.1	9.2	–	53.7	38.5	Hidosa and Biru (2021)
92.0	1.4	21.5	11.0	28.2	36.7	25.3	He et al. (2021)
–	–	16.4	–	25.7	–	–	Tkáčová et al. (2011)
–	–	23.6	–	–	49.1	30.2	Tessema and Baars (2006)
–	1.8	24.1	–	25.8	49.3	43.8	Stavarache et al. (2012)
–	–	18.1	–	–	42.3	37.4	Liu et al. (2018)
89.6	–	17.0	11.3	20.3	–	–	Zheng et al. (2019)
84.0	1.83	13.5	12.4	28.1	48.3	36.7	Koçer et al. (2018)
92.5	–	16.0	9.1	27.3	–	–	Aganga and Tshwenyane (2003)
89.4	2.1	19.2	–	–	–	–	Laudadio et al. (2014)

DM, dry matter; EE, ether extract; CP, crude protein; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre.

TABLE 3 Crude protein and amino acid (g/100 g protein) contents of lucerne, sunflower, soyabean, and moringa meals.

Amino acid	Amino acid in lucerne			*Moringa oleifera* leaf meal (Moyo et al., 2011)	Sunflower meal (Rosa et al., 2009)	Soyabean meal (Lagos and Stein, 2017)
	Homolka et al. (2008)	Luo et al. (2022)	Pleger et al. (2021)			
Crude protein (g/kg DM)	230	196.1	219	302.9	182	473
Histidine	4.09	1.84	2.33	2.36	1.96	2.90
Isoleucine	2.91	3.67	3.97	3.86	3.38	4.74
Leucine	8.26	6.27	7.21	6.47	4.98	7.74
Lysine	5.13	5.66	5.98	5.41	3.59	6.49
Methionine	1.39	1.17	1.64	0.99	1.78	1.37
Phenylalanine	2.39	4.03	4.70	5.41	3.83	5.12
Threonine	3.04	3.93	4.43	4.49	2.90	3.76
Valine	4.30	4.64	5.02	4.66	3.95	4.93
Arginine	5.04	3.72	4.52	5.88	6.49	7.23
Serine	3.96	4.13	4.16	3.60	3.44	4.40
Aspartic acid	10.35	10.96	12.65	4.72	7.28	11.04
Cystine	1.30	1.12	1.32	0.03	1.93	1.33
Glutamic acid	8.22	8.41	10.09	8.35	17.21	17.82
Glycine	3.65	4.03	4.75	5.05	4.38	4.19
Alanine	3.96	4.79	5.53	10.00	3.41	4.29
Tyrosine	2.70	2.45	3.11	8.75	1.72	3.55
Proline	4.96	6.07	5.21	3.96	3.35	4.82

DM, Dry matter.

TABLE 4 Phenolic acids and flavonoids found in lucerne (Srisaikham, 2021).

Compound		µg/g DM
Phenolic acids	Gallic acid	3.14
	Protocatechuic acid	–
	p-hydroxybenzoic acid	74.39
	Chlorogenic acid	–
	Vanillic acid	4.24
	Caffeic acid	11.48
	Syrigic acid	2.79
	p-coumaric acid	64.82
	Ferulic acid	22.25
	Sinapic acid	24.36
Flavonoid	Catechin	6,780
	Rutin	270
	Myricetin	3,910
	Luteolin	450
	Quercetin	–
	Apigenin	280
	Genistein	104
	Daizein	131

DM, Dry matter.

growth performance and meat cholesterol have also been reported when 10 mg/kg–15 mg/kg of alfalfa flavonoids was supplemented (Ouyang et al., 2016; Prihambodo et al., 2021).

An important category of non-flavonoids that were also identified in lucerne are tannins. Tannins are prominent metabolites widely distributed in forages such as trees, shrubs, and legumes (including lucerne). These have been implicated in limiting the digestibility of amino acids and energy (Getachew et al., 2008). Tannins exert negative effects on the feed intake, weight gain, and feed conversion ratio of broiler chickens (Hidayat et al., 2021). However, other reports indicate beneficial effects of tannins because they exert antimicrobial, antioxidant, anti-inflammatory, and gut health-promoting effects (Medugu et al., 2012). The literature is inconsistent in terms of the effect of tanniniferous feed ingredients on poultry. There is therefore a need to ascertain the appropriate dosages for lucerne meal supplementation, especially in the diets of indigenous chickens (Wonkyun et al., 2020).

Lucerne possesses antioxidative properties. It has been investigated because of the health benefits it is believed to confer (Bora and Sharma, 2011). One method of measuring the antioxidative capacity of organic material is to determine its 2,2-diphenyl-1-picrylhydrazyl (DHHP) scavenging activity, as outlined by Krakowska et al. (2017). The results are expressed as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) equivalence per gram DM of the sample. The same authors, Krakowska et al. (2017), reported that the antioxidative capacity of lucerne ranges between 15.6 mg and 48.3 mg trolox equivalence (TE)/g DM depending on the part of the plant (i.e., leaves, stems, or flowers) studied. Horvat et al. (2022) indicated that lucerne leaf meal had 32.2 mg TE/g DM, while Vlaicu et al. (2023) reported that lucerne meal from ground lucerne cuttings had 17.78 mg TE/g DM. Hence, dietary lucerne supplementation could be useful in enhancing the antioxidant levels in poultry products.

There are phenolic compounds in lucerne which possess powerful phytoestrogenic properties. Phytoestrogens accumulate in the plant during times of environmental stress and disease (Křížová et al., 2019). They contribute to the plant's defence mechanisms, hence they do not have hormonal functions (Shah and Smith, 2020). These compounds have the capacity to influence growth and reproduction in animals. Phenolic compounds that possess phytoestrogenic properties include non-flavonoids (coumestrol 3-methoxycoumestrol and 4-methoxycoumestrol), flavonoids (luteolin, apigenin, quercetin, genistein, and daidzein) and lignans (Srisaikham, 2021; Wyse et al., 2021) (Table 5). These compounds vary in quantity and oestrogenic activity levels depending on the lucerne cultivar, environmental factors, and stage of growth.

Lucerne contains a particular class of secondary metabolites classified under triterpenes saponins (Bera et al., 2019; Szumacher-Strabel et al., 2019; Nagy and Makleit, 2021). Lucerne leaf meal is composed of different types of saponins (Table 6) (Pleger et al., 2021). Saponins are also capable of forming insoluble complexes with dietary cholesterol in the intestines, which are then excreted (Zheng et al., 2019; Pleger et al., 2021). It has been reported that levels and types of saponins could improve meat quality in poultry by enhancing its lipid oxidative stability (Bera et al., 2019). Dietary saponins, however, have adverse effects on chickens' performance due to reduced amino acid digestibility (Pleger et al., 2020). Hence, they are considered the main antinutritional component in lucerne. There is a need to determine the type of saponins responsible for poor performance (i.e, reduced digestibility) in poultry.

3.2 Vitamins and carotenoids

Vitamins are useful in preventing oxidative stress, regulating the immune response, and maintaining normal physiological, biochemical, and homeostatic mechanisms in animals (Alagawany et al., 2021) (Table 7). Lucerne contains vitamins A, D, E, and K (fat soluble) and also vitamin B and vitamin B complex with vitamin C

TABLE 5 Phytoestrogens (coumestans and isoflavone) identified in lucerne (Wyse et al., 2021).

Phytoestrogen		mg/kg DM
Coumestans	Coumestrol (µg/g DM)	17.5
	3′-methoxycoumestrol	5.1
	4′-methoxycoumestrol	12.5
Isoflavone	Formononetin	29.4

DM, dry matter.

TABLE 6 Relative contents of putatively identified saponins expressed as equivalent umbelliferone in *Medicago sativa* (lucerne) leaves (Pleger et al., 2021).

Saponin	μg/g DM
Hexose hederagenin	11.3
3-glucose-medicagenic acid	44.6
Medicagenic acid derived	51.4
Hexose-hexuronic acid-aglycone A	61.4
Azukisaponin II	62.7
Medicoside H	84.2
Medicagenic acid 3-O-β-D-glucuronide	89.3
3-glucose-glucose-28-arabinose-rhamnose-medicagenic acid	101
Hexuronic acid-d-hexose-pentose-pentose-pentose-zanhic acid	200

DM, dry matter.

(fat soluble) (González-Calvo et al., 2015; Englmaierová et al., 2019; Jia et al., 2019). Lucerne contains higher levels of vitamin E (205.9 mg/kg) and precursors of vitamin A (carotenoids: zeaxanthin, lutein, and β-carotene). These vitamins have significant roles in egg production, and, therefore, the consumption of lucerne could enhance the egg-laying potential of birds.

3.3 Fatty acids

Lucerne contains a range of fatty acids that may be impactful to poultry (Table 8). The fatty acid profile of lucerne varies with varieties, seasons, and other agronomic factors. Lucerne contains a higher proportion of unsaturated fatty acids than saturated fatty acids. Unsaturated fatty acids are required for the biosynthesis of oxylipids (Raphael and Sordillo, 2013). Some of the polyunsaturated fatty acids contained include linoleic acid and α-linolenic acid, which are essential fatty acids (Nakamura and Nara, 2003). In terms of saturated fatty acids, palmitic acids are the major fatty acids that occur in lucerne. This indicates that lucerne meal supplementation in poultry diets could be useful in manipulating the fatty acid profile in poultry products (i.e., meat and eggs) (Grela et al., 2020; Kop-bozbay et al., 2021). However, in the case of South African indigenous chickens, the effects of dietary lucerne supplementation on the fatty acid content of meat and eggs have not been investigated.

TABLE 7 Vitamins and carotenoids found in Lucerne.

	Compound	mg/kg DM	Source
Vitamin C	Ascorbic acid (mg/kg)	77.6	Englmaierová et al. (2019)
Vitamin B complex	Thiamine	8.9	Jia et al. (2019)
	Riboflavin	90.4	
	Niacin	2.0	
	Pantothenic acid	31.8	
	Pyridoxine	2.7	
Vitamin E	α-tocopherol	205.9	
Carotenoid	Zeaxanthin (mg/kg)	90.3	Englmaierová et al. (2019)
	Lutein (mg/kg)	86.3	
	β-carotene (mg/kg)	24.8	
	Retinol (mg/kg)	–	

DM, dry matter.

4 Lucerne in broiler chicken diets

Tkáčová et al. (2011) found that lucerne inclusion, even at a level of 20 g/kg, increased the body weight of Ross 308 broiler chickens. In addition, Shirzadegan and Taheri (2017), showed that the inclusion of lucerne meal at a level of 30 g/kg improved the body weight gain, feed intake, and feed conversion ratio (FCR) in Ross 308 broiler chickens. However, Varzaru et al. (2020) and Sánchez et al. (2022) found that lucerne meal inclusion levels of as high as 50 g/kg in the diet of Hubbard broiler chickens and of 40 g/kg in that of Cobb 500 broiler chickens did not affect either group's feed intake, daily weight gain, or FCR. The inclusion of dietary lucerne meal at a level ranging from 50 g/kg to 200 g/kg resulted in poor production performance (Tkáčová et al., 2011). This was also supported by the findings of Paredes and Risso (2020), who reported that the body weight of Hubbard broiler chickens decreased sequentially in groups fed 0 g/kg, 50 g/kg, and 100 g/kg lucerne meal, and found no benefits in terms of feed intake and FCR. Pleger et al. (2020) demonstrated that Hubbard broiler chickens consuming dietary lucerne meal at a level between 100 g/kg and 200 g/kg resulted in poor growth rate and feed intake. Therefore, the lower inclusion (<40 g/kg) of dietary lucerne meal may be beneficial to broiler chickens for optimal growth performance.

Dietary lucerne, with its high fibre content, has effects on the development and performance of digestive organs, which ultimately influences productivity (JøRgensen et al., 1996). According to Shirzadegan and Taheri (2017), when lucerne meal content is increased in the diet of chickens, their proventriculus, gizzard, and small intestine increase in weight to adapt to the stimulation of the organs to facilitate thorough mechanical and chemical digestion (Abdollahi et al., 2018). This adaptation is not beneficial to broiler chickens because the nutrients meant for growth and muscle accretion are utilized for this instead.

Varzaru et al. (2020) showed that the inclusion of fibrous ingredients such as lucerne (at a level of 50 g/kg) resulted in the increased composition of beneficial microbial organisms in the intestines of Cobb 500 broiler chickens. This indicates that lucerne meal in the diet of chickens helps to lower the incidence of diseases, thus preventing gut health issues and promoting animal performance. Picoli et al. (2014) supported this benefit by reporting that the histomorphology of the intestines was enhanced by lucerne meal inclusion in the diet of chickens. The intestinal villi height and crypt depth increased with the inclusion of dietary lucerne meal. This response is advantageous because the available surface area for

TABLE 8 Total fatty acid content and fatty acid composition (g/100 g total fatty acids) of Lucerne.

Item	Systematic name	González-Calvo et al. (2015)	Toral et al. (2016)	Liu et al. (2018)	Vlaicu et al. (2023)
Total fatty acids (g/kg DM)		–	–	36.9	–
Saturated fatty acids (g/100 g total fatty acids)		–	–	24.4	29.7
Unsaturated fatty acids (g/100 g total fatty acids)		–	–	75.6	69.6
Caproic	C6:0	–	–	0.00	0.34
Caprylic	C8:0	–	–	–	0.53
Capric	C10:0	–	–	–	0.40
Lauric	C12:0	–	0.7	–	0.83
Myristic	C14:0	–	2.4	0.00	1.46
Pentadecanoic	C15:0	–	–	–	0.44
Palmitic	C16:0	17.7	25.0	19.9	22.08
Palmitoleic	C16:1	–	–	1.90	2.30
Stearic	C18:0	3.02	5.6	4.50	3.18
Oleic	C18:1	4.52	2.3	5.87	4.05
Linoleic	C18:2n6	22.28	14.0	16.20	16.88
α-linolenic	C18:3n3	37.93	41.1	43.1	41.36
γ-linolenic	C18:3n6	–	–	6.60	0.28
Eicosanoic	C20:0	4.46	–	–	–
Eicosadienoic	C20:2n6	–	–	0.00	0.28
Arachidonic	C20:4n6	–	–	–	0.30
Docosanoic	C22:0	3.03	–	–	–
Tricosanoic	C23:0	–	–	–	0.15

DM, dry matter.

effective nutrient digestion and absorption is increased (Incharoen, 2013; Adibmoradi et al., 2016). Further studies are necessary to elucidate the merits of having increased intestinal villi length and deeper crypt depth when chickens are fed lucerne meals supplemented diets, especially in indigenous chickens.

The carcass traits of broiler chickens were also affected by the dietary inclusion of lucerne meal (Varzaru et al., 2020). Low inclusion levels (30 g/kg lucerne) improved the carcass weight, breast weight, and thigh weight (Shirzadegan and Taheri, 2017) of chickens, while higher levels (of 40 g/kg to 100 g/kg) dietary lucerne meal negatively affected these traits (Shirzadegan and Taheri, 2017; Paredes and Risso, 2020; Varzaru et al., 2020; Sánchez et al., 2022). This might be due to lowered feed intake, with higher dietary lucerne meal content resulting in the lowered body weight of chickens (Paredes and Risso, 2020). The taste of the carcass is also altered by lucerne supplementation. Ponte et al. (2004) reported that lucerne-treated birds had a better taste than their non-lucerne-treated counterparts and indicated that this was due to the antioxidant content of lucerne. Lucerne meal is rich in carotenoids (Englmaierová et al., 2019; Pleger et al., 2020). The carcasses of chickens consuming diets supplemented with lucerne meal were found to have higher yellowness (b*) values (Pleger et al., 2021). Lucerne meal also influenced the cholesterol content of broiler chicken meat, and increasing its supplementation level from 0 g/kg to 75 g/kg in broiler chickens' diet resulted in their meat having lower cholesterol levels.

5 Lucerne in egg-laying chicken diets

Al-shami et al. (2011) reported that, in White Hisex laying hens, lucerne supplementation at a level of 50 g/kg and 70 g/kg decreased the production performance (i.e., the feed intake and egg production) of layers, while their egg yolk parameters (yolk yellowness, β-carotene content, yolk percentage, and cholesterol levels) improved. The yolk colour and β-carotene content improved due to the presence of carotenoids in lucerne, while the lowered yolk cholesterol content is consistent with the presents of phenolic compounds and saponins in diets supplemented with lucerne. Similar findings were reported by Laudadio et al. (2014), who fed Isa Brown laying hens diets supplemented with as much as 150 g/kg lucerne, and noted no adverse effects on egg production traits but found this to have positive

effects on yolk quality. Therefore, improvements on the quality of the eggs occur at the expense of the quantity of eggs produced. The utilization of lucerne in the diets of laying hens to achieve production goals therefore has to be strategic.

6 Lucerne in dual-purpose chicken diets

Dual-purpose chickens are typically indigenous or local/native, and the same can be true of hybrid chickens that are bred selectively because of their possessing good meat and egg production traits. The native chicken breed Zhuanghe Dagu, also called the grass chicken, has the characteristic of herbivorous traits such as strong tolerance to dietary ingredients high in CF (Cui et al., 2022). There is a paucity of research information on the effects of lucerne on the production performance of dual-purpose chickens.

Beijing-You indigenous chickens aged 20 weeks to 28 weeks fed diets supplemented with 50 g/kg to 80 g/kg lucerne did not gain body weight or exhibit improved growth rate and feed intake (Zheng et al., 2019). Instead, their FCR and mortality rates improved when their diets were supplemented with 50 g/kg lucerne. Chinese indigenous chickens (Guangxi-Tiejiaoma broilers) aged 3 weeks to 12 weeks fed diets supplemented with 25 g/kg to 75 g/kg lucerne meal exhibited improved growth performance (in terms of feed intake and body weight) (He et al., 2021). Similarly, Cui et al. (2022) observed that lucerne meal inclusion in the diet of 35-week-old Zhuanghe Dagu chickens at a level of 60 g/kg was beneficial to their production performance. Therefore, the age or stage of growth of these chickens is a critical factor that influences the effects of lucerne on their growth production performance. As chickens aged, their growth performance was less affected by the inclusion of lucerne in their diet. Jiang et al. (2018) reported similar results, namely that the growth productivity of yellow feathered Chinese broiler chickens (aged 6 weeks to 9 weeks) fed diets supplemented with 80g/kg lucerne meal were not affected also indicated that an energy source needs to be provided to complement lucerne.

Zheng et al. (2019) showed that the carcass, breast, and thigh percentages; the albumen protein, breast muscle content of inosine monophosphate; abdominal fat yield; yolk protein; and total amino acid, and cholesterol levels were improved in chickens fed diets supplemented with lucerne compared with those fed no lucerne meal. However, the chemical compositions of breast and thigh meat were not influenced by lucerne supplementation. Jiang et al. (2018) reported improvements in the taste of yellow-feathered broiler chickens aged 6 weeks to 7 weeks when lucerne meal at a level of 40 g/kg was included in their diet. Therefore, the taste of the meat improved with the dietary inclusion of lucerne meal in chickens.

Lucerne had positive effects on the egg production rate, FCR, egg mass, yolk colour, albumen height, and haugh unit (Cui et al., 2022). Previous studies (Al-shami et al., 2011) have shown that lucerne supplementation is beneficial in terms of the internal contents of the egg, i.e., the egg yolk. Zheng et al. (2019) reported that albumen protein was improved by lucerne supplementation, and also that the yolk colour, yolk protein, and cholesterol levels also improved. This indicates the high potential of dual-purpose local chickens when offered lucerne.

7 Use of lucerne in indigenous chicken production

In South Africa, the need for alternative sources of protein is critical for production, especially by smallholder farmers. The lucerne produced for ruminants may also be useful in indigenous chicken diets. Plant-based ingredients, also referred to as "greens", including lucerne, are being consumed by indigenous chickens (Raphulu et al., 2015; Admasu et al., 2019). There is, however, a knowledge gap concerning the utilization of lucerne for the productivity of indigenous chickens. The impact of lucerne on the production performance, nutrient digestibility, haematology, health status, and meat and egg quality of indigenous chickens is not sufficiently known or understood. Smallholder farmers have not been properly informed about the benefits of using lucerne in the feed of indigenous chickens since it is well established as feed for ruminants, and much benefit could be conferred from its utilization in the diets of indigenous chickens.

8 Conclusion

South African smallholder farmers involved in indigenous poultry production are faced with the challenge of finding local and sustainable feed resources that will improve the production of indigenous chickens. Lucerne, grown in most parts of the world, including South Africa has to be explored as an option for indigenous poultry. Lucerne contains crude protein at sufficient-enough levels to contribute towards meeting the requirements of slow-growing poultry such as indigenous chicken. The high fibre content of lucerne, however, limits dietary intake because it acts as a nutrient diluent. This may be mitigated by harvesting when the forage is still young (in the budding stage) or implementing other mechanical means to varying degrees thereby enabling access to the amino acid contents of this ingredient. Phenolics and saponins are secondary metabolites present in lucerne as antinutritional factors, and there needs to be conclusive evidence about the extent to which these might have beneficial effects in indigenous chickens. These secondary metabolites together with vitamins in lucerne meal may be useful in enhancing the antioxidant content of chicken products. Furthermore, lucerne may be useful in improving the fatty acid profile of poultry products because it has a higher proportion of unsaturated fatty acids (omega-3 and -6 fatty acids). When included at levels up to 50 g/kg, lucerne has shown to be beneficial for broilers, layers, and Asian indigenous chickens. Such findings indicate that lucerne has significant potential as a feed ingredient and should be explored in South African chicken nutrition.

Author contributions

MG: Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declares that financial support was received for the research, authorship, and/or publication of this article. The author wishes to acknowledge the School of Interdisciplinary Research and Graduate Studies in the College of Graduate Studies at the University of South Africa for the support of the publication of this research manuscript.

Acknowledgments

The author would like to acknowledge the School of Interdisciplinary Research and Graduate Studies, in particular Professor KR Mbatha for the tremendous support in this manuscript.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abdollahi, M. R., Zaefarian, F., and Ravindran, V. (2018). Feed intake response of broilers: Impact of feed processing. *Anim. Feed Sci. Technol.* 237, 154–165. doi: 10.1016/j.anifeedsci.2018.01.013

Adibmoradi, M., Navidshad, B., and Jahromi, M. F. (2016). The effect of moderate levels of finely ground insoluble fibre on small intestine morphology, nutrient digestibility and performance of broiler chickens. *Ital. J. Anim. Sci.* 15, 310–317. doi: 10.1080/1828051X.2016.1147335

Admasu, S., Solomon, D., and Meseret, M. (2019). Poultry feed resources and chemical composition of crop content of scavenging indigenous chicken. *Online J. Anim. Feed Res.* 9 (6), 247–255. doi: 10.36380/scil.2019.ojafr34

Aganga, A. A., and Tshwenyane, S. O. (2003). Lucerne, lablab and *Leucaena leucocephala* forages: Production and utilization for livestock production. *Pakistan J. Nutr.* 2, 46–53. doi: 10.3923/pjn.2003.46.53

Alabi, O. J., Ng'ambi, J. W., and Norris, D. (2012). Effect of egg weight on physical egg parameters and hatchability of indigenous Venda chickens. *Asian J. Anim. Veterinary Adv.* 7, 166–172. doi: 10.3923/ajava.2012.166.172

Alabi, O. J., Ng'ambi, J. W., and Norris, D. (2013). Dietary energy level for optimum productivity and carcass characteristics of indigenous venda chickens raised in closed confinement. *South Afr. J. Anim. Sci.* 43 (1), 75–80. doi: 10.4314/SAJAS.V43I5.14

Alagawany, M., Elnesr, S. S., Farag, M. R., Tiwari, R., Yatoo, M. I., Karthik, K., et al. (2021). Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health – a comprehensive review. *Veterinary Q.* 41, 1. doi: 10.1080/01652176.2020.1857887

Al-shami, M. A., Salih, M., and Abbas, T. (2011). Effects of dietary inclusion of alfalfa (Medicago sativa L.) leaf meal and Xylam enzyme on laying hens' performance and egg quality. *Res. Vet. Sci.* 11, 754–759. doi: 10.1080/01652176.2020.1857887

Banjoko, O., Adebayo, I., Osho, I., Olumide, M., Ajayi, O. A., and Akinboye, O. (2020). Evaluation of varying levels of *Carica papaya* leaf meal on growth, carcass, haematological parameters and its use as anticoccidial for broiler chicken. *Nigerian J. Anim. Sci.* 22 (3), 229–241.

Bashir, N., Şekeroğlu, A., Tainika, B., and Özer, C. O. (2023). Effect of different pasture species on growth performance, carcass traits, internal organ weights, and meat quality of slower growing broilers in free-range production system. *Trop. Anim. Health Production* 55, 1–14. doi: 10.1007/S11250-023-03581-9/TABLES/9

Bekele, B., and Sintayehu, S. (2021). Effect of various levels of sweat potato (hawassa-83) leaf meal on the laying performance and egg quality of koekoek dual purpose chicken. *Poultry Fisheries Wildlife Sci.* 9, 1–4.

Bennett, R. N., and Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New Phytol.* 127, 617–633. doi: 10.1111/j.1469-8137.1994.tb02968.x

Bera, I., Tyagi, P. K., Mir, N. A., Tyagi, P. K., Dev, K., Sharma, D., et al. (2019). Dietary supplementation of saponins to improve the quality and oxidative stability of broiler chicken meat. *J. Food Sci. Technol.* 56, 2063–2072. doi: 10.1007/s13197-019-03683-z

Bjornlund, V., Bjornlund, H., and van Rooyen, A. (2022). Why food insecurity persists in sub-Saharan Africa: A review of existing evidence. *Food Secur.* 14, 845–864. doi: 10.1007/S12571-022-01256-1/FIGURES/4

Bora, K. S., and Sharma, A. (2011). Phytochemical and pharmacological potential of *Medicago sativa*: a review. *Pharm. Biol.* 49 (2), 211–220. doi: 10.3109/13880209.2010.504732

Burezq, H. (2021). Reproductive ecology of forage alfalfa (*Medicago sativa* L.): Plant Reproductive Ecology. *Recent Adv.* doi: 10.5772/intechopen.100640

Caillouet, O. C., Nalley, L. L., and Farmer, A. L. (2019). Increasing economic resilience through mixed farming with maize and poultry in Northern Mozambique. *Acta Hortic.* 1246, 93–102. doi: 10.17660/ACTAHORTIC.2019.1246.13

Cui, Y., Diao, Z., Fan, W., Wei, J., Zhou, J., Zhu, H., et al. (2022). Effects of dietary inclusion of alfalfa meal on laying performance, egg quality, intestinal morphology, caecal microbiota and metabolites in Zhuanghe Dagu chickens. *Ital. J. Anim. Sci.* 21 (1), 831–846. doi: 10.1080/1828051X.2022.2067009

Donalson, L. M., Kim, W. K., Woodward, C. L., Herrera, P., Kubena, L. F., Nisbet, D. J., et al. (2005). Utilizing different ratios of alfalfa and layer ration for molt induction and performance in commercial laying hens. *Poultry Sci.* 84, 362–369. doi: 10.1093/ps/84.3.362

Eckberg, J. O., Wells, S. S., Jungers, J. M., Lamb, J. F. S., and Sheaffer, C. C. (2022). Alfalfa forage yield, milk yield, and nutritive value under intensive cutting. *Agrosystems Geosciences Environ.* 5, e20246. doi: 10.1002/agg2.20246

Elkomy, A. E., and Elghalid, O. A. (2014). Physiological performance of broiler chicks fed on *Medicago sativa* seeds as natural source of isoflavones. *Asian J. Poultry Sci.* 8, 97–105. doi: 10.3923/AJPSAJ.2014.97.105

Englmaierová, M., Skřivan, M., and Vít, T. (2019). Alfalfa meal as a source of carotenoids in combination with ascorbic acid in the diet of laying hens. *Czech J. Anim. Sci.* 64, 17–25. doi: 10.17221/116/2018-CJAS

Ensminger, M. E. (1992). *Poultry Science. 3rd edition* (New Jersey: Prentice Hall).

Fan, W., Ge, G., Liu, Y., Wang, W., Liu, L., and Jia, Y. (2018). Proteomics integrated with metabolomics: Analysis of the internal causes of nutrient changes in alfalfa at different growth stages. *BMC Plant Biol.* 18, 1–15. doi: 10.1186/S12870-018-1291-8/TABLES/5

Getachew, G., Pittroff, W., DePeters, E. J., Putnam, D. H., Dandekar, A., and Goyal, S. (2008). Influence of tannic acid application on alfalfa hay: *In vitro* rumen fermentation, serum metabolites and nitrogen balance in sheep. *Animal* 2, 381–390. doi: 10.1017/S1751731107001486

Glatz, P. C., and Tilbrook, A. J. (2021). Welfare issues associated with moulting of laying hens. *Anim. Production Sci.* 61, 1006–1012. doi: 10.1071/AN19700

González-Calvo, L., Joy, M., Blanco, M., Dervishi, E., Molino, F., Sarto, P., et al. (2015). Effect of vitamin E supplementation or alfalfa grazing on fatty acid composition and expression of genes related to lipid metabolism in lambs1. *J. Anim. Sci.* 93, 3044–3054. doi: 10.2527/jas.2014-8758

Goromela, E. H., Kwakkel, R. P., Verstegen, M. W. A., and Katule, A. M. (2006). Strategies to optimize the use of scavengeable feed resource base by smallholders in traditional poultry production systems in Africa: a review. *Afr. J. Agric. Res.* 1, 91–100.

Grela, E. R., Knaga, S., Winiarska-Mieczan, A., and Zięba, G. (2020). Effects of dietary alfalfa protein concentrate supplementation on performance, egg quality, and fatty acid composition of raw, freeze-dried, and hard-boiled eggs from Polbar laying hens. *Poultry Sci.* 99, 2256–2265. doi: 10.1016/J.PSJ.2019.11.030

Guèye, E. H. F. (1998). Village egg and chicken meat production in Africa. *World's Poultry Sci. J.* 54, 73–85. doi: 10.1079/wps19980007

Hayat, N., Solomon, D., and Meseret, M. (2016). Chemical composition of scavenging feed resource of indigenous chickens. *Asian J. Anim. Sci.* 10, 182–188. doi: 10.3923/AJAS.2016.182.188

He, G., Zhao, L., Shishir, M. S. R., Yang, Y., Li, Q., Cheng, L., et al. (2021). Influence of alfalfa meal, as a source of dietary fibre, on growth performance, development, pH of gastrointestinal tract, blood biochemical profile, and meat quality of broilers. *J. Appl. Anim. Res.* 49, 431–439. doi: 10.1080/09712119.2021.2000417

Herd-Hoare, S. C., and Shackleton, C. (2020). Integrating ecosystem services and disservices in valuing smallholder livestock and poultry production in three villages in South Africa. *Land* 9 (9), 294. doi: 10.3390/LAND9090294

Hidayat, C., Irawan, A., Jayanegara, A., Sholikin, M. M., Prihambodo, T. R., Yanza, Y. R., et al. (2021). Effect of dietary tannins on the performance, lymphoid organ weight, and amino acid ileal digestibility of broiler chickens: A meta-analysis. *Veterinary World* 14, 1405–1411. doi: 10.14202/vetworld.2021.1405-1411

Hidosa, D., and Biru, S. K. (2021). Dry matter yield and chemical composition of alfalfa (*Medicago sativa*) varieties as animal feed in the South Omo Zone of South-western Ethiopia. *Acta Veterinaria Brno* 3, 2582–3183.

Homolka, P., Koukolová, V., Němec, Z., Mudřík, Z., Hučko, B., and Sales, J. (2008). Amino acid contents and intestinal digestibility of lucerne in ruminants as influenced by growth stage. *Czech J. Anim. Sci.* 53, 499–505. doi: 10.17221/367-CJAS

Horvat, D., Viljevac Vuletić, M., Andrić, L., Baličević, R., Kovačević Babić, M., and Tucak, M. (2022). Characterization of forage quality, phenolic profiles, and antioxidant activity in alfalfa (*Medicago sativa* L.). *Plants* 11, 2735. doi: 10.3390/plants11202735

Incharoen, T. (2013). Histological adaptations of the gastrointestinal tract of broilers fed diets containing insoluble fiber from rice hull meal. *Am. J. Anim. Vet. Sci.* 8 (2), 79–88. doi: 10.3844/ajavsp.2013.79.88

Idowu, P. A., Mpayipheli, M., and Muchenje, V. (2019). Original research paper a survey study on productive and reproductive performance of indigenous poultry. *Am. J. Anim. Veterinary Sci.* 14, 33–39. doi: 10.3844/ajavsp.2019.33.39

Idowu, P. A., Zishiri, O., Nephawe, K. A., and Mtileni, B. (2021). Current status and intervention of South Africa chicken production-A review. *World's Poultry Sci. J.* 77, 115–133. doi: 10.1080/00439339.2020.1866965

Iskender, H., Yenice, G., Dokumacioglu, E., Kaynar, O., Hayirli, A., and Kaya, A. (2017). Comparison of the effects of dietary supplementation of flavonoids on laying hen performance, egg quality and egg nutrient profile. *Br. Poultry Sci.* 58, 550–556. doi: 10.1080/00071668.2017.1349297

Jia, T., Sun, Z., Gao, R., and Yu, Z. (2019). Lactic acid bacterial inoculant effects on the vitamin content of alfalfa and Chinese leymus silage. *Asian-Australasian J. Anim. Sci.* 32, 1873. doi: 10.5713/AJAS.19.0135

Jiang, S., Gou, Z., Li, L., Lin, X., and Jiang, Z. (2018). Growth performance, carcass traits and meat quality of yellow-feathered broilers fed graded levels of alfalfa meal with or without wheat. *Anim. Sci. J.* 89, 561–569. doi: 10.1111/ASJ.12968

JøRgensen, H., Zhao, X.-Q., Knudsen, K. E. B., and Eggum, B. O. (1996). The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. *Br. J. Nutr.* 75, 379–395. doi: 10.1079/BJN19960141

Kalinda, W., and Tanganyika, J. (2017). Effects of protein levels on growth and laying performance of the Malawian indigenous chicken. *Int. J. Avian Wildlife Biol.* 2, 169–171. doi: 10.15406/IJAWB.2017.02.00037

Kamboh, A. A., and Zhu, W. (2013). Individual and combined effects of genistein and hesperidin supplementation on meat quality in meat-type broiler chickens. *J. Sci. Food Agric.* 93, 3362–3367. doi: 10.1002/jsfa.6185

Kingori, A. M., Tuitoek, J. K., Muiruri, H. K., Wachira, A. M., and Birech, E. K. (2007). Protein intake of growing indigenous chickens on free-range and their response to supplementation. *Int. J. Poultry Sci.* 6, 617–621. doi: 10.3923/IJPS.2007.617.621

Koçer, B., Bozkurt, M., Ege, G., Tüzün, A. E., Konak, R., and Olgun, O. (2018). Effects of a meal feeding regimen and the availability of fresh alfalfa on growth performance and meat and bone quality of broiler genotypes. *Br. poultry Sci.* 59.3, 318–329. doi: 10.1080/00071668.2018.1440378

Köhler-Rollefson, I. (2004). "Farm animal genetic resources: Safeguarding national assets for food security and trade," in *A summary of workshops on FAnGR held in the Southern African Development Community (SADC)*. Eschborn, Germany: GTZ; Rome, Italy: FAO and Wageningen; Netherlands: CTA.

Kop-bozbay, C., Akdag, A., Bozkurt-kiraz, A., Gore, M., Kurt, O., and Ocak, N. (2021). Laying performance, egg quality characteristics, and egg yolk fatty acids profile in layer hens housed with free access to chicory- and/or white clover-vegetated or non-vegetated areas. *Animals* 11, 1708. doi: 10.3390/ANI11061708

Krakowska, A., Rafińska, K., Walczak, J., Kowalkowski, T., and Buszewski, B. (2017). Comparison of various extraction techniques of *Medicago sativa*: yield, antioxidant activity, and content of phytochemical constituents. *J. AOAC Int.* 100, 1681–1693. doi: 10.5740/jaoacint.17-0234

Křížová, L., Dadáková, K., Kašparovská, J., and Kašparovský, T. (2019). Isoflavones. *Molecules* 24, 1076. doi: 10.3390/molecules24061076

Lagos, L. V., and Stein, H. H. (2017). Chemical composition and amino acid digestibility of soybean meal produced in the United States, China, Argentina, Brazil, or India1. *J. Anim. Sci.* 95, 1626–1636. doi: 10.2527/jas.2017.1440

Laudadio, V., Ceci, E., Lastella, N. M. B., Introna, M., and Tufarelli, V. (2014). Low-fiber alfalfa (Medicago sativa L.) meal in the laying hen diet: Effects on productive traits and egg quality. *Poultry Sci.* 93, 1868–1874. doi: 10.3382/PS.2013-03831

Liu, Q. H., Dong, Z. H., and Shao, T. (2018). Effect of additives on fatty acid profile of high moisture alfalfa silage during ensiling and after exposure to air. *Anim. Feed Sci. Technol.* 236, 29–38. doi: 10.1016/j.anifeedsci.2017.11.022

Luo, C., Wang, D., Lu, N., Li, H., Liu, G., Cao, Z., et al. (2022). Analysis of chemical composition, amino acid content, and rumen degradation characteristics of six organic feeds. *Animals* 12, 682. doi: 10.3390/ani12060682

Manyelo, T. G., Sebola, N. A., and Mabelebele, M. (2022). Effect of amaranth leaf meal on performance, meat, and bone characteristics of Ross 308 broiler chickens. *PLoS One* 17 (8). doi: 10.1371/JOURNAL.PONE.0271903

Mapiye, C., Mwale, M., Mupangwa, J. F., Chimonyo, M., Foti1, R., and Mutenje1, M. J. (2008). A research review of village chicken production constraints and opportunities in Zimbabwe. *Asian-Australian J. Anima Sci.* 21, 1680–1688. doi: 10.5713/ajas.2008.r.07

Markovic, J., Radovic, J., Lugic, Z., and Sokolovic, D. (2007). The effect of development stage on chemical composition of alfalfa leaf and stem. *Biotechnol. Anim. Husbandry* 23, 383–388. doi: 10.2298/BAH0702383M

McDonald, P., Greenhalgh, J. F. D., Morgan, C. A., Edwards, R., Sinclair, L., and Wilkinson, R. (2011). *Animal Nutrition. 7th ed* (London: Pearson).

Medugu, C. I., Saleh, B., Igwebuike, J. U., and Ndirmbita, R. L. (2012). Strategies to improve the utilization of tannin-rich feed materials by poultry. *Int. J. Poultry Sci.* 11, 417. doi: 10.3923/ijps.2012.417.423

Mielmann, A., Bothma, C., Steyn, F., Hugo, C. J., Mielmann, A., Bothma, C., et al. (2017). Consumers' knowledge about and attitudes towards lucerne (*Medicago sativa* L.). *J. Consumer Sciences Special Edition Food Nutr. challenges South. Afr.* 2, 69–82.

Motsepe, R., Mabelebele, M., Norris, D., Brown, D., Ngambi, J., and Ginindza, M. (2016). Carcass and meat quality characteristics of South African indigenous chickens. *Indian J. Anim. Res.* 50, 580–587. doi: 10.18805/ijar.11159

Mottet, A., and Tempio, G. (2017). Global poultry production: Current state and future outlook and challenges. *World's Poultry Sci. J.* 73, 245–256. doi: 10.1017/S0043933917000071

Moyo, B., Masika, P. J., Hugo, A., and Muchenje, V. (2011). Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves. *Afr. J. Biotechnol.* 10, 12925–12933. doi: 10.5897/AJB10.1599

Mtileni, B., Dzama, K., Nephawe, K., and Rhode, C. (2016). Estimates of effective population size and inbreeding in South African indigenous chicken populations: implications for the conservation of unique genetic resources. *Trop. Anim. Health Production* 48, 943–950. doi: 10.1007/s11250-016-1030-9

Mwai, L. M. (2021). *Mulberry (Morus alba) leaf meal in indigenous chicken layer diets effect on egg production and quality. MSc thesis* (Kenya: Egerton University).

Mwalusanya, N. A., Katule, A. M., Mutayoba, S. K., Mtambo, M. M. A., Olsen, J. E., and Minga, U. M. (2002). Productivity of local chickens under village management conditions. *Trop. Anim. Health Production* 34, 405–416. doi: 10.1023/a:1020048327158

Nagy, B., and Makleit, P. (2021). Total saponin content (TSC) of different alfalfa (*Medicago sativa* l.) cultivars cultivated in field experiment. *Rev. Agric. Rural Dev.* 10, 176–180. doi: 10.14232/rard.2021.1-2.176-180

Nakamura, M. T., and Nara, T. Y. (2003). Essential fatty acid synthesis and its regulation in mammals. *Prostaglandins Leukotrienes Essential Fatty Acids* 68, 145–150. doi: 10.1016/S0952-3278(02)00264-8

Ncobela, C. N., and Chimonyo, M. (2015). Potential of using non-conventional animal protein sources for sustainable intensification of scavenging village chickens: A review. *Anim. Feed Sci. Technol.* 208, 1–11. doi: 10.1016/J.ANIFEEDSCI.2015.07.005

Nondzutha, T., Mdoda, L., and Qange, S. (2020). The profitability and contribution of indigenous chicken production towards improving household well-being in Lusikisiki. *J. Hum. Ecol.* 71, 212–221. doi: 10.31901/24566608.2020/71.1-3.3257

NRC (National Research Council) (1994). *Nutrient requirements of poultry. 9th ed* (Washington DC: National Academy Press).

Nxumalo, N., Ceccobelli, S., Cardinali, I., Lancioni, H., Lasagna, E., and Kunene, N. W. (2020). Genetic diversity, population structure and ancestral origin of KwaZulu-Natal native chicken ecotypes using microsatellite and mitochondrial DNA markers. *Ital. J. Anim. Sci.* 19, 1277–1290. doi: 10.1080/1828051X.2020.1838350

Nyoni, N. M. B., Grab, S., Archer, E., and Hetem, R. (2022). Perceived impacts of climate change on rural poultry production: a case study in Limpopo Province, South Africa. *Climate Dev.* 14, 389–397. doi: 10.1080/17565529.2021.1929803

Ouyang, K., Xu, M., Jiang, Y., and Wang, W. (2016). Effects of alfalfa flavonoids on broiler performance, meat quality, and gene expression. *Can. J. Anim. Sci.* 96, 332–341. doi: 10.1139/cjas-2015-0132

Palmonari, A., Fustini, M., Canestrari, G., Grilli, E., and Formigoni, A. (2014). Influence of maturity on alfalfa hay nutritional fractions and indigestible fiber content. *J. Dairy Sci.* 97, 7729–7734. doi: 10.3168/jds.2014-8123

Paredes, M., and Risso, A. L. (2020). Effects of dietary inclusion of alfalfa meal on productive performance, carcass and weight of digestive and lymphoid organs in organic type broiler chicken. *Rev. Investigaciones Veterinarias del Peru* 31 (2). doi: 10.15381/rivep.v31i2.17846

Picoli, K. P., Murakami, A. E., Duarte, C. R. A., Eyng, C., Ospina-Rojas, I. C., and Massuda, E. M. (2014). Effect of dietary restriction and hay inclusion in the diet of slow-growing broilers. *Ital. J. Anim. Sci.* 13, 771–775. doi: 10.4081/IJAS.2014.3216

Pleger, L., Weindl, P. N., Weindl, P. A., Carrasco, L. S., Leitao, C., Zhao, M., et al. (2021). Precaecal digestibility of crude protein and amino acids from alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*) leaves and silages in broilers. *Anim. Feed Sci. Technol.* 275, 114856. doi: 10.1016/J.ANIFEEDSCI.2021.114856

Pleger, L., Weindl, P. N., Weindl, P. A., Carrasco, L. S., Leitao, C., Zhao, M., et al. (2020). Effects of increasing alfalfa (*Medicago sativa*) leaf levels on the fattening and slaughtering performance of organic broilers. *J. Anim. Physiol. Anim. Nutr.* 104, 1317–1332. doi: 10.1111/JPN.13353

Ponte, P. I. P., Mendes, I., Quaresma, M., Aguiar, M. N. M., Lemos, J. P. C., Ferreira, L. M. A., et al. (2004). Cholesterol levels and sensory characteristics of meat from broilers consuming moderate to high levels of alfalfa. *Poultry Sci.* 83, 810–814. doi: 10.1093/PS/83.5.810

Prihambodo, T. R., Sholikin, M. M., Nahrowi, N., Batubara, I., Utomo, D. B., and Jayanegara, A. (2022). Flavonoids as dietary additives in laying hens: a meta-analysis of production performance, egg quality, liver, and antioxidant enzyme profile. *Poultry Sci. J.* 10 (1), 27–34. doi: 10.22069/PSJ.2022.19393.1714

Prihambodo, T. R., Sholikin, M. M., Qomariyah, N., Jayanegara, A., Batubara, I., Utomo, D. B., et al. (2021). Effects of dietary flavonoids on performance, blood constituents, carcass composition and small intestinal morphology of broilers: a meta-analysis. *Anim. Bioscience* 34, 434–442. doi: 10.5713/ajas.20.0379

Radović, J., Sokolović, D., and Marković, J. (2009). Alfalfa-most important perennial forage legume in animal husbandry. *Biotechnol. Anim. Husbandry* 25, 465–475. doi: 10.2298/BAH0906465R

Raphael, W., and Sordillo, L. M. (2013). Dietary polyunsaturated fatty acids and inflammation: the role of phospholipid biosynthesis. *Int. J. Mol. Sci.* 14, 21167–21188. doi: 10.3390/IJMS141021167

Raphulu, T., Jansen Van Rensburg, C., and Van Ryssen, J. B. J. (2015). Assessing nutrient adequacy from the crop contents of free-ranging indigenous chickens in rural villages of the Venda region of South Africa. *South Afr. J. Anim. Sci.* 45, 143–152. doi: 10.4314/sajas.v45i2.5

Rashid, M., Asaduzzaman,, and R., B. C. (2003). Chemical Composition of crop contents of local scavenging chickens. *Pakistan J. Nutr.* 3, 26–28. doi: 10.3923/PJN.2004.26.28

Rosa, P. M., Antoniassi, R., Freitas, S. C., Bizzo, H. R., Zanotto, D. L., Oliveira, M. F., et al. (2009). Chemical composition of Brazilian sunflower varieties. *Helia* 32, 145–156. doi: 10.2298/hel0950145r

Russelle, M. P. (2001). Alfalfa: After an 8,000-year journey, the "Queen of Forages" stands poised to enjoy renewed popularity. *Am. Scientist* 89, 252–261. doi: 10.1511/2001.3.252

Sánchez, Q. ,. A., Rodrigue, H. R., Pinza, D. V. C., Troya, G. A. P., Calco, A., and Diaz, C. A. A. (2022). Effect of the inclusion of *Medicago sativa* in feed chicken Cobb 500. *Revista Científica Facultad de Ciencias Veterinariasa* 32, 1–7. doi: 10.52973/RCFCV-E32108

Scholtz, G., Van der Merwe, H., and T., T. (2009). The nutritive value of South African *Medicago sativa* L. hay. *Afr. Journals Online* 39 (1), 179–182.

Sebola, N. A., Mlambo, V., Mokoboki, H. K., and Muchenje, V. (2015). Growth performance and carcass characteristics of three chicken strains in response to incremental levels of dietary M*oringa oleifera* leaf meal. *Livestock Sci.* 178, 202–208. doi: 10.1016/j.livsci.2015.04.019

Sen, S., Makkar, H. P. S., and Becker, K. (1998). Alfalfa saponins and their implication in animal nutrition. *J. Agric. Food Chem.* 46, 131–140. doi: 10.1021/JF970389I

Shah, A., and Smith, D. L. (2020). Flavonoids in agriculture: Chemistry and roles in, biotic and abiotic stress responses, and microbial associations. *Agronomy* 10, 1209. doi: 10.3390/agronomy10081209

Shirzadegan, K., and Taheri, H. R. (2017). Insoluble fibers affected the performance, carcass characteristics and serum lipid of broiler chickens fed wheat-based diet. *Iranian J. Appl. Anim. Sci.* 7, 109–117.

Srisaikham, S. (2021). A comparison of nutritional values, bioactive compounds, amino acids, and antioxidant activities of alfalfa (Medicago sativa) plant and Pellet for use as beneficial material ruminant feed. *Walailak J. Sci. Technol.* 18, 10312–10316. doi: 10.48048/wjst.2021.10312

STATSSA (2017). *Census of commercial agriculture* (Pretoria: Statistics South Africa).

Stavarache, M., Vintu, V., Samuil, C., Muntianu, I., Albu, A., Tarcău, D., et al. (2012). Quality of alfalfa (*Medicago sativa* L.), in the first year of vegetation. *Lucrări Ştiinţifice seria Agronomi* 1, 55–60.

Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H. I., and Sartono, T. A. (2019). Recent advances in the incorporation of leaf meals in broiler diets. *Livestock Res. Rural Dev.* 31 (7).

Surai, P. F. (2014). Polyphenol compounds in the chicken/animal diet: from the past to the future. *J. Anim. Physiol. Anim. Nutr.* 8, 19–31. doi: 10.1111/jpn.12070

Suwignyo, B., Rini, E. A., Fadli, M. K., and Ariyadi, B. (2021). Effects of alfalfa (*Medicago sativa* L.) supplementation in the diet on the growth, small intestinal histomorphology, and digestibility of hybrid ducks. *Veterinary World* 14, 2719. doi: 10.14202/VETWORLD.2021.2719-2726

Szumacher-Strabel, M., Stochmal, A., Cieslak, A., Kozłowska, M., Kuznicki, D., Kowalczyk, M., et al. (2019). Structural and quantitative changes of saponins in fresh alfalfa compared to alfalfa silage. *J. Sci. Food Agric.* 99, 2243–2250. doi: 10.1002/JSFA.9419

Tessema, Z., and Baars, R. (2006). Chemical composition, dry matter production and yield dynamics of tropical grasses mixed with perennial forage legumes. *Trop. Grasslands* 40 (3), 150.

Tkáčová, J., Angelovičová, M., Mrázová, Ľ., Kliment, M., and Král, M. (2011). Effect of different proportion of lucerne meal in broiler chickens. *Sci. Papers Anim. Sci. Biotechnologies* 44, 141–144. doi: 10.3/JQUERY-UI.JS

Toral, P., Hervás, G., Missaoui, H., Andrés, S., Giráldez, F., Jellali, S., et al. (2016). Effects of a tannin-rich legume (Onobrychis viciifolia) on in *vitro* ruminal biohydrogenation and fermentation. *Spanish J. Agric. Res.* 14, e0602. doi: 10.5424/sjar/2016141-8989

Ubua, J. A., Ozung, P. O., and Inagu, P. G. (2019). Dietary inclusion of neem (*Azadirachta indica*) leaf meal can influence growth performance and carcass characteristics of broiler chickens. *Asian J. Biol. Sci.* 12, 180–186. doi: 10.3923/ajbs.2019.180.186

Vardhan, P. V., and Shukla, L. I. (2017). Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production. *Int. J. Radiat. Biol.* 93 (9), 967–979. doi: 10.1080/09553002.2017.1344788

Varzaru, I., Panaite, T. D., and Untea, A. E. (2020). Effects of dietary supplementation of alfalfa meal and rice bran on growth performance, carcass characteristics and intestinal microbiota in broilers. *Archiva Zootechnica* 23, 117–128. doi: 10.2478/AZIBNA-2020-0017

Vlaicu, P. A., Untea, A. E., Turcu, R. P., Saracila, M., Varzaru, I., and Oancea, A. G. (2023). Chemical composition of dietary alfalfa and its effectiveness on broiler chicken thigh meat quality. *Czech J. Food Sci.* 41, 279–286. doi: 10.17221/79/2023-CJFS

Wang, E., Wang, J., Lv, J., Sun, X., Kong, F., Wang, S., et al. (2021). Comparison of ruminal degradability, indigestible neutral detergent fiber, and total-tract digestibility of three main crop straws with alfalfa hay and corn silage. *Animals* 11, 3218. doi: 10.3390/ani11113218

Wonkyun, C., Ryong, K., Song, L. H., and Ro, L. J. (2020). A Multiplex PCR method for the detection of genetically modified alfalfa (*Medicago sativa* L.) and analysis of feral alfalfa in South Korea. *Proc. Natl. Institute Ecol.* 1, 83–89. doi: 10.22920/PNIE.2020.1.1.83

Wyse, J. M., Latif, S., Gurusinghe, S., Berntsen, E. D., Weston, L. A., and Stephen, C. P. (2021). Characterization of phytoestrogens in *Medicago sativa* L. and grazing beef cattle. *Metabolites* 11, 550. doi: 10.3390/metabo11080550

Xu, X., Min, D., and McDonald, I. (2021). Effects of harvest intervals and seeding rates on dry matter yield and nutritive value of alfalfa cultivars. *J. Anim. Sci. Technol.* 63 (5), 1098–1113. doi: 10.5187/jast.2021.e97

Yusuf, S. F. G., Lategan, F. S., and Masika, P. J. (2017). Characterization of Indigenous poultry production systems in the Nkonkobe Municipality, Eastern Cape Province South Africa. *Kamla Raj Enterprises* 5, 31–44. doi: 10.1080/09766898.2014.11884710

Zheng, M., Mao, P., Tian, X., Guo, Q., and Meng, L. (2019). Effects of dietary supplementation of alfalfa meal on growth performance, carcass characteristics, meat and egg quality, and intestinal microbiota in Beijing-you chicken. *Poultry Sci.* 98, 2250–2259. doi: 10.3382/PS/PEY550

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 13 February 2024
DOI 10.3389/fanim.2024.1319150

OPEN ACCESS

EDITED BY
Marcos Inacio Marcondes,
Washington State University, United States

REVIEWED BY
M. Jordana Rivero,
Rothamsted Research, United Kingdom
Luc Delaby,
Institut National de la Recherche Agronomique (INRA), France
Pablo Modernel,
FrieslandCampina, Netherlands

*CORRESPONDENCE
Gastón Ortega
gortega@fagro.edu.uy

RECEIVED 11 October 2023
ACCEPTED 02 January 2024
PUBLISHED 13 February 2024

CITATION
Ortega G, Berberian N and Chilibroste P (2024) The effects of stocking rate, residual sward height, and forage supplementation on forage production, feeding strategies, and productivity of milking dairy cows.
Front. Anim. Sci. 5:1319150.
doi: 10.3389/fanim.2024.1319150

The effects of stocking rate, residual sward height, and forage supplementation on forage production, feeding strategies, and productivity of milking dairy cows

Gastón Ortega[1]*, Natalia Berberian[2] and Pablo Chilibroste[3]

[1]Departamento de Producción Animal, Facultad de Agronomía Universidad de la República, Canelones, Uruguay, [2]Departamento de Biometría y Estadística, Facultad de Agronomía Universidad de la República, Montevideo, Uruguay, [3]Departamento de Producción Animal, Facultad de Agronomía Universidad de la República, Paysandú, Uruguay

The intensification process in Uruguayan dairies over the last 30 years has involved increases in stocking rate (SR) and individual milk production. This research aimed to compare biophysical indicators along with the associated feeding strategies for Holstein–Jersey crossbred dairy cow systems. The comparison was conducted in farmlets representing a typical Uruguayan pasture-based dairy system on a grazing area. The study spanned from 2017 to 2019, combining 1.5 or 2.0 milking cows per hectare (SR), with two different residual sward heights (RH)—low (LR) and high (HR). These combinations resulted in four treatments: 1.5 LR, 1.5 HR, 2.0 LR, and 2.0 HR. A total of 96 cows were randomly allocated to each treatment based on parity, body weight (BW), and body condition score (BCS) for the years 2017, 2018, and 2019. The response variables per hectare were analyzed using a linear mixed model, including SR, RH, their interaction effect, year, and paddock as a repeated measurement. Results show that forage production did not differ between treatments, and forage directly harvested by cows was affected by SR, as well as milk and solids productivity. An interaction effect was detected between SR and RH on milk and milk solids production where 2.0 HR was higher than 2.0 LR, but treatments on SR 1.5 were not different between them. The consumption of concentrate, forage, and conserved forage per hectare was influenced by the level of SR. However, individual milk production was not influenced by SR or RH. Dry matter intake were affected by SR where 2.0 HR had higher consumption than 1.5 LR and 1.5 HR but was not different from 2.0 LR. The concentrate DMI per cow was not different between treatments, while the conserved forage DMI per cow was affected by SR and higher for SR 2.0 than that for SR 1.5. These combinations of feed determined varying proportions of time allocated for grazing, which were influenced by the SR and RH. This research highlights different approaches to enhance the competitiveness of Uruguayan grazing systems through improvements in forage harvest.

KEYWORDS

grazing, mechanic harvest, residual height, milk production, home-grown production

Introduction

The stocking rate (SR) is a crucial factor that influences system efficiency in grazing dairy where pastures serve as the primary feed source (McMeekan and Walshe, 1963; Baudracco et al., 2011). These systems are capable of achieving high milk outputs per hectare at low costs (Dillon et al., 2005). However, recent shifts in land competitiveness and fluctuations in international milk prices have compelled dairy farmers to enhance productivity, leading to increases in either SR or individual milk production or in both (Macdonald et al., 2017; Fariña and Chilibroste, 2019).

Dairy grazing systems have established their economic sustainability and competitiveness through effective pasture production and usage. Systems that maximize pasture utilization reap benefits in terms of productivity and profitability (Chataway et al., 2010; Ramsbottom et al., 2015), thereby maintaining resilience against market prices and climate threats (Fariña and Chilibroste, 2019). Strategies aimed at increasing the SR directly impact the amount of grass harvested (Baudracco et al., 2011), reducing the milk production costs without compromising home-grown forage production. However, the increase in productivity demands additional inputs (e.g., concentrates and silage) to meet the nutritional requirements of the cows (Baudracco et al., 2011; Macdonald et al., 2017). This heightened productivity also requires infrastructure designed to provide supplements, requiring significant investment and intensive labor (Dillon et al., 2005; Fariña and Chilibroste, 2019).

A higher SR in grazing systems typically results in lower forage allowances (FA) [in kg dry matter (DM) offered per cow], often leading to reduced post-grazing residuals, which can impact on animal performance (Ganche et al., 2013). As indicated by Merino et al. (2019), controlling the FA allows for the control of the impact of post-grazing height on animal consumption, subsequently influencing milk production. Higher FA and lower defoliation intensities have been associated with increased DM intake (DMI) and milk production in cows (Delaby and Peyraud, 2003; Menegazzi et al., 2021).

In Uruguayan dairy systems, particularly during autumn, which marks the sowing season of perennial and annual grasses, the SR may vary from 1,500 to 2,500 kg LW/ha on the grazing area. This intensifies pressure on pastures, leading to a greater defoliation intensity and immediate higher grass usage by cows (Chilibroste et al., 2003), where the accumulation of over-grazing events significantly contributes to reductions in home-grown forage production and persistence in grazing systems (Ganche et al., 2014).

The relationship between animals and plants during grazing events is so influential that mismatches can impact either the animal or the forage productivity. The effect of grazing residuals on subsequent pasture growth is temporary—for example, Chapman (2016) demonstrated that higher defoliation intensities extend the lag phase while plant energy status is rebuilt and the first re-growing leaf appears. Pastures managed with lower defoliation intensities contribute to improved forage and animal productivity responses, creating areas that are both over- and under-grazed, influencing growth and competitive relationships among plants (Hodgson and White, 2000). This heterogeneous configuration is the result of sectorized defoliation and recurrent decoupling between feeding supplies. Furthermore, low defoliation intensity might lead to reduced growth rates as more plant energy is directed into stem elongation. Dead matter, along with stem material, can shade out new tillers, thereby decreasing tiller density (McCarthy et al., 2014). However, since the timing of the maximum average growth rate depends on the relative rates of new leaf growth and senescence, the maximum forage mass (FM) target will be reached earlier.

Some farmlet studies have been conducted with grazing systems based on legumes such as *Medicago sativa* (Baudracco et al., 2011) or perennial grasses such as *Lolium multiflorum* (Fariña et al., 2011; Macdonald et al., 2017), exploring the effect of SR on systems. The plasticity of perennial pastures raises the question of whether the levels of harvest and animal performance could be affected by the application of grazing management practices that optimize forage production and quality. Notwithstanding, there is currently available information on long-term farm studies for a grazing area composed of a mixture of annual (*L. multiflorum*) and perennial (*Dactylis glomerata*) grasses with legumes (*T. repens*) for Uruguayan conditions (Stirling et al., 2021). There is no information available of how SR and residual sward height (RH) impact on biophysical indicators at the farm level.

The aim of this research was to evaluate the effect of SR and RH on milk, milk solids production and forage usage, along with the associated feeding strategies for crossbred Holstein–Jersey dairy cows over a farmlet study over 3 years. The cows were stocked at two different rates (SR, 1.5 and 2.0 cow per hectare) combined with two levels of post-grazing residual sward height (RH): high (HR) and low (LR) grazing annual and perennial grasses over a 3-year term.

Materials and methods

The investigation was undertaken in the dairy unit at the Centro Regional Sur research station, Agronomy College, located in Canelones, Uruguay (34°36.810 S, 56°13.088 W). The experiment involved four systems in a 3-year study (January 2017 to December 2019).

Description of the farmlet study

The study grazing areas consisted of a 4-year rotation combining annual grasses (*Avena sativa* with *L. multiflorum* and *Sorghum bicolor*) and three years of perennial pastures (*D. glomerata* with *T. repens*), a common characteristic of the national pasture-based dairy system. The study explored four intensification strategies to increase home-grown forage utilization and output per hectare through a twofold increase in SR and two post-grazing RH. During the first year, there was an area of 20% of third-year mixed *lucerne* (*M. sativa*) pasture to establish a stabilized rotation. A total of 24 cows were allocated to one of the four 2 × 2 factorial arrangements of treatments: two stocking rates (SR); 1.5 or 2.0 milking cows per hectare combined with two contrasting residual heights (RH): a conventional residual sward height of 6 to 7 cm was maintained all year round for low residual

(LR), and a high residual (HR) suggests a grazing management approach that allows for a higher residual of 9 to 10 cm on average throughout the year. Thus, four treatments resulted from the combination of these two factors: 1.5LR, 1.5HR, 2.0LR, and 2.0HR. The grazing area (e.g., the area where cows have direct access) integrated 56 ha assigned as follows: 32 ha for 1.5LR and 1.5HR divided into 16 ha each, and 24 ha for 2.0LR and 2.0HR representing 12 ha each. This area comprised five paddocks for treatments 1.5 LR and HR and four paddocks for 2.0 LR and HR. The paddocks' average size (3 ha) was evenly distributed across the entire area of the experimental farm to balance the four systems in terms of soil-type location, distance from the milking parlor, and pasture species.

Pasture sown and fertilizer

Each year, 50% of the grazing area (8 ha for 1.5 LR–1.5 HR and 6 ha for 2.0 LR–2.0 HR) was renewed with first year pasture (*D. glomerata* and *T. repens*) and annual grasses *(L. multiflorum*) in the autumn. The amounts of target seed at sowing were 18, 2.5, and 20 kg/ha for *D. glomerata*, *T. repens*, and *L. multiflorum*, respectively.

All systems were fertilized with nitrogen (N) after every grazing cycle (dependent on the growth rate in the grazing area) between July and November using urea (0.46 N) at a varying rate from 75 to 100 kg urea/ha per application. In autumn, 108 kg/ha of fertilizer mix (7 g/kg N and 40 g/kg P) was used for pastures and annual grasses at sowing. The perennial pasture re-fertilization rates varied depending on P Bray soil content, establishing minimum rates of 12 g/kg P for grasses and 20 g/kg P for *M. sativa* pastures.

Animals

Every year, in March, Holstein–Jersey dairy cows (n = 96) were randomly assigned to one of the four treatments (i.e., 1.5LR, 1.5HR, 2.0LR, and 2.0HR) of 24 cows each based on parity (2.1 ± 1.6, 2.5 ± 1.1, and 2.4 ± 1.2), body weight (BW) (520 ± 87, 548 ± 80, and 534 ± 77 kg), and body condition score (BCS) (2.9 ± 0.5, 3.7 ± 0.6, and 3.6 ± 0.4) for the years 2017, 2018, and 2019, respectively. Every year, 20% of the cows from each treatment were introduced as primiparous cows. The cows did not graze outside their grazing area at any stage of the lactation period (from March to December) and were dried off 60 days before their next expected calving date. Individual milk production was evaluated fortnightly each year until December. During the dry period, the cows were fed outside their grazing area on a natural grassland area (Allen et al., 2011) dominated by *Festuca arundinacea* and *Stipa setigera* until 25 days before calving, until the cows entered prepartum. The prepartum management consisted of offering a total mixed ration with corn silage, concentrate, and anionic salt (12, 4.0, and 0.3 kg DM/cow per day, respectively).

Grazing and feeding management

The animals grazed under rotational grazing on a new fresh strip of pasture after each milking, subdivided by electrified fences. The grazing management decision rules were based on matching the daily forage DM intake (FDMI) with the pasture growth rate (GR) while keeping the average grazing area FM at 1,800 kg DM/ha (measured through the COMPYLD method (details are provided in the section "Pasture determinations"). In addition, the phenological stage of grass in each paddock was monitored weekly to ensure adequate grazing intervals to maximize pasture growth and persistence. For this reason, the leaf growth stage was recorded on pre-grazing paddocks with three expanded leaves per tiller on *D. glomerata* and *L. multiflorum* as a pre-grazing benchmark (Donaghy and Fulkerson, 1998) and nine (for winter, spring, and summer) and 12 knots in autumn, for *M. sativa*. In early spring, occasional post-grazing slashing (the mechanical mowing of pasture to approximately 10 cm height) was performed when deemed necessary to maintain pasture quality and bloom control.

The energy demand of cows was determined by NRC (2001) on a weekly basis, contemplating BW, number of lactations, pregnancy, milk production, and milk fat content. As the objective was for cows to harvest directly as much grass as possible, in case the GR was higher than the cows' demand, paddocks were mechanically harvested (grass haylage), and if the GR on the grazing platform area was lower than the cows' demand, they were supplemented with sorghum or maize silage on the feeding yard with a mixer wagon (Mary, 2018). The amount of silage and hay that each treatment needed each year was imported outside of the grazing area. Additionally, part of the pasture haylage that treatments need was harvested within the grazing area, considered as mechanical harvest. The cows had access to one, two, or zero grazing sessions between milkings for an 8-h interval. When grazing sessions were banned due to low GR or strong rainy events, the cows stayed on a 2-ha resting paddock assigned to each treatment. The cows remained on those paddocks (between milking) with water, grass silage, or hay on bale hoops after being supplemented with silage in the feeding yard.

Concentrate was offered twice a day at the milking parlor through automatic feeders. The projected amount of concentrate delivered to the cows was the same for the four treatments (7.0, 5.0, and 3.0 kg DM/cow per day in early, mid, and late lactation, respectively), considering the requirements of a cow with a production of 6,000 L throughout its lactation. Every 15 days, the concentrate offered in the milking parlor was weighed to ensure that the amount of feed offered to the cows was correct.

Pasture determinations

The FM of each plot in each grazing area was assessed through the comparative yield method (COMPYLD) (Haydock and Shaw 1975). Every 15 days, five visual scales with three repetitions were defined in each paddock, ranging from 1 (low) to 5 (high), representing the yield scale. Before defining the scales on the terrain, the entire paddock was walked to collect representative samples of paddock yield. For each scale, a sample FM quadrant of 0.51 m × 0.30 m was placed. Five height determinations (in cm) were made using a ruler before removing the FM inside the quadrant with handled scissors at ground level. After removing the FM from each quadrant, fresh material was weighed and then

dried in an oven for 48 h at 60°C to estimate dry matter content. A regression equation was developed using the dry matter yield of each scale (15 points), rating, and sward height. This calibration scale was used weekly to estimate the FM and GR in each paddock by walking through the assigned transect. At least 20 determinations of scale per paddock were registered weekly by the same trained person walking alongside the paddock transect.

Based on these observations, weekly estimates of GR, paddock FM, and average FM for each grazing area were performed. Pre- and post-grazing sward heights, after each strip of graze, were measured using a ruler to monitor the management residue on pasture, with an average of 30 measurements per strip.

The annual forage production on the grazing area was calculated as the mean of the weekly paddocks GR, which were not under grazing, multiplied by 365 days.

Forage, conserved forage, and concentrate intake

The pre- and post-grazing sward heights of each grazing daily strip were determined and converted to FM with the information provided from paddock calibration. With this information, the FDMI was estimated based on pasture disappearance, contemplating pre- and post-grazing FM multiplied by strip area and divided by the number of grazing cows.

The amount of silage offered (in kg DM/per cow per day) was registered daily using a scale mounted on a mixer wagon. The scale was calibrated weekly to ensure the accurate offering of silage based on the weekly diet formulation. If the cows left silage on the feeder, the residual amount was weighed, and the daily consumption per cow was estimated. A sample of the material left by the cows was collected to determine the DM content. This value was then multiplied by the scale value and divided by the number of cows to calculate the silage consumption per cow. In the case of haylage and hay, supply was offered on a feeding ring in the resting paddock. The weight of each bale was previously recorded on the scale, and to estimate the cow DM intake, a visual scale was created based on the total weight divided into quarters. When the cows left the resting paddock, the scale was read and the difference between the feed offered and what was left in the feeding ring, divided by the number of cows, represented the consumption per animal. The total amount of conserved forage consumed by the cows was constructed from the sum of silage, hay, and haylage.

The amount of concentrate consumed by the animals was the same as that offered. It was delivered by automatic feeders into the milking parlor during milking.

Forage direct harvest (in kg DM/ha year) was calculated as the sum of FDMI multiplied by the number of milking cows per hectare grazing in that system each year.

Animal determinations

The cow's individual milk production was recorded fortnightly, and individual milk samples were taken during morning and evening milking on the same day. These samples were then analyzed for milk fat and milk protein content. Based on this, the milk solid production per cow was estimated. Milk at 305 days was calculated for each cow for each year as the sum of yields from calving to the last day of lactation, adjusting the lactation curve model of Wilmink (1987). The BW (in kg per cow) and BCS of all cows were recorded monthly following the morning milking. BCS was assessed by using the method of Ferguson et al. (1994), considering a scale from 1 to 5 points.

Variables at the farm scale expressed on the grazing area, such as milk and milk solids production per hectare per year, were calculated by summing the daily yields for each cow and multiplying the result by the number of milking cows per hectare on the grazing area for each year. For grazing time, the analysis was performed by integrating the proportion of days with zero, one, or two grazing turns as a percentage of the total grazing turns along the year.

Statistical analysis

The study was considered an unbalanced completely randomized block design with a factorial arrangement of two factors (SR and RH), each with two levels (1.5 and 2.0—LR and HR, respectively), considering the interaction effect between them. The paddock was considered as a repetition in space, with four repetitions for treatments 2.0 LR and 2.0 HR and five for treatments 1.5 LR and 1.5 HR. Additionally, the year was considered as a fixed effect to evaluate possible trends, and the experimental units were the groups of 24 cows. Variables related to system performance on grazing area, such as milk, milk solids, forage production and harvest, conserved forage production, forage, conserved forage, concentrate DM intake, and proportion of days with 0, 1, or 2 grazing turns were analyzed considering the effects of SR, RH, and their interaction effects as represented in Equation 1.

$$Y_{ijkl} = \mu + SR_i + RH_j + (SR \times RH)_{ij} + year_k + block_l + \varepsilon_{ijkl} \quad (1)$$

where μ is the mean of the variable;
SR is the effect of introducing 1.5 or 2.0 cows per hectare;
RH represents the effect of pasture residual height using 6 to 7 or 9 to 10 cm;
SR × RH is the interaction effect;
year denotes 2017, 2018, and 2019;
block represents the repetitions in space—four for 2.0 LR and 2.0 HR and five for 1.5 LR and 1.5 HR; and
ε is the experimental error.

For individual animal observations like BW, BCS, milk production, milk solids production, milk fat, and milk protein content, the cows were considered as the experimental unit. The model included SR, RH, their interaction effect (SR*RH), and year as the fixed effects. This model incorporated the group of cows (classified by parity, milk production, BW, and BCS 20 days before calving) and cows nested in year as random effects. The data were fitted with linear mixed models (lmer) using the CAR library on RStudio (1.0.153), as shown in Equation 2.

$$Y_{ijkl} = \mu + SR_i + RH_j + (SR \times RH)_{ij} + year_k + 1|cow(year_k) + 1|group_l + \varepsilon_{ijkl} \quad (2)$$

where μ is the mean of the variable;
SR is the effect of introducing 1.5 or 2.0 cows per hectare;
RH represents the effect of pasture residual height using 6 to 7 or 9 to 10 cm;
SR × RH is the interaction effect.
year denotes 2017, 2018, and 2019;
cow represents the ID cow nested in the year;
group refers to the block of cows grouped for productivity, calving date, number of lactations, BW, and BCS at calving; and
ε is the experimental error.

The lactcurves package (Strucken, 2021) for Rstudio (version 1.0.153) was employed to fit the data by running multiple lactation curve models and extracting the selection criteria for each model. The Wilmink function (1987) was chosen as the best fit based on the Akaike information criteria out of 32 lactation curve models. For the analysis of the lactation curves, parameters were estimated for each cow and lactation by fitting the exponential function of Wilmink (1987) for the fortnightly measurements of these variables on each cow as shown in Equation 3.

$$Y_{ijk} = a + b * exp^{-0.05dim} + c * dim \quad (3)$$

where a, b, and c are the estimated parameters that define the height of the lactation curve (a), the initial phase of post-calving inclining to peak (b), and the subsequent post-peak decline phase (c). The variable dim corresponds to days in milk for each cow. The effects of SR and RH were calculated for each parameter of the Wilmink (1987) model.

Results

Pasture growth rate and harvested forage

The results shown in Table 1 suggest that SR and RH did not have a significant impact on forage production. All treatments achieved similar levels of annual forage production, and the total amount of harvested forage (direct plus mechanical) was comparable across the different SR and RH treatments. However, the strategy employed by each system to harvest the highest amount possible of forage produced on the grazing area differed among treatments. There was an effect of SR on forage harvested directly by the animals, with SR 2.0 harvesting more forage than SR 1.5, but not on the total or mechanical harvested forage.

The average FM on grazing area was higher for the 1.5 and 2.0 HR treatments than for the 1.5 and 2.0 LR treatments. Despite the effect of RH on the average FM on grazing area, the pre-grazing FM was not different between SR or RH. Nevertheless, a trend towards the effect of RH on post-grazing FM was observed (p = 0.09). Additionally, an interaction effect between SR and RH was observed for pre-grazing height, where 2.0 LR showed higher values than 1.5 LR, 1.5 HR, and 2.0 HR which were not different between them (Table 1). As expected, there were significant differences in the post-grazing residual height between treatments, with HR being 1.6 cm higher than LR (Table 1).

As shown in Table 2, SR influenced the levels of conserved forage and concentrates required to meet animal demands. Systems with a SR of 2.0 required a higher supply of conserved forage and concentrate, increasing by 1,121 and 1,050 kg DM/ha year, respectively, compared to those with a SR of 1.5 (Table 2).

A positive effect of RH (17.2 vs. 15.1 for HR and LR, respectively) and SR (17.1 vs. 15.2 for SR 1.5 and 2.0, respectively) was observed for FA (Table 2). However, for FDMI, an interaction effect between SR and RH was significant, with cows on 1.5 HR consuming 0.9 kg DM/cow per day more forage than those on 1.5 LR. Conversely, the intake of conserved forage per cow was 0.8 kg DM/day lower for 1.5 SR than for 2.0 SR, while the RH effect was not significant in the amount of conserved forage consumed per cow. Regarding the concentrate, DMI per cow per day did not differ among the four treatments, as expected, based on the experimental setup.

For the cows' total DMI, the SR effect was significant, and there was a trend (p = 0.06) for RH effect. There were no differences between treatments for the same stocking rate, but differences were observed between 2.0 HR and 1.5 LR and HR and also 2.0 LR with 1.5 LR. As a result of how the feeding strategies were performed, the opportunities for grazing (days with either one or two grazing turns) were affected by SR. The cows in the 1.5 SR treatment had more grazing turns than those in the 2.0 SR treatment (68% vs. 61%, respectively). However, an interaction effect was reported for the proportion of time with no grazing, where higher SR levels and RH management led to longer periods with no grazing access. Furthermore, the time for one grazing turn was affected by the level of SR, with the 2.0 SR group spending 12% more time under one grazing turn than the 1.5 SR cows. Among the 2.0 SR treatments, the cows on 2.0 HR spent more time at one grazing turn compared to 2.0 LR, while there were no differences between 1.5 LR and 1.5 HR. The opportunities for two grazing sessions were affected by both SR and RH, being restricted by 14% for the 2.0 SR group in contrast to the 1.5 group, which spent 12% more time under one grazing turn than the 1.5 SR cows.

Animal response

A significant interaction effect was observed between SR and RH for milk and milk solids production per hectare per year (Table 3). Treatment 2.0 HR showed higher milk and milk solids production than 2.0 LR, and both of these treatments achieved higher production than 1.5 LR and 1.5 HR, with no significant differences among them. The individual performance in terms of milk production and milk protein content did not show significant effects of SR and RH.

Individual milk yield at 305 days was influenced by SR and RH (Table 3, Figure 1) and their interaction effects, where the performance of 2.0 HR cows was the highest of the four treatments (Table 3). For BCS and BW, the effects of stocking rate and management were not significant; however, there was a

10.3389/fanim.2024.1319150

TABLE 1 Effects of stocking rate (1.5 and 2.0) and residual height (LR and RH) on annual forage production, average forage mass, total forage harvested on the grazing area (direct and mechanical), and pre-grazing and post-grazing sward conditions for 1.5 LR, 1.5 HR, 2.0 LR, and 2.0 HR.

	Treatments					*p*-value		
	1.5 LR	1.5 HR	2.0 LR	2.0 HR	SEM	SR	RH	SR*RH
Harvest and forage production (kg DM/ha year)								
Annual forage production	7,393	7,712	7,962	7,535	685	0.70	0.97	0.44
Direct harvest	4,439b	4,743b	5,774a	5,724a	235	<0.001	0.32	0.13
Forage mechanic harvested[a]	2,327	2,720	919	706	1,804	0.19	0.87	0.69
Forage mass, kg DM/ha								
Average grazing area[b]	1,699b	1,751a	1,697b	1,755a	16.8	0.20	<0.001	0.74
Pre-grazing[c]	2,234	2,263	2,142	2,243	112.2	0.49	0.42	0.22
Post-grazing[c]	1,383	1,450	1,400	1,471	67.3	0.65	0.09	0.96
Pasture height (cm)								
Pre-grazing[c]	31.5b	31.9b	34.1a	32.3b	0.26	<0.001	0.01	<0.001
Post-grazing[c]	7.8b	9.5a	8.1b	9.6a	0.43	0.02	<0.001	0.53

For the same row, means with different letters differ (P< 0.05). Values in a row with different letters are significantly different between treatments (p< 0.05).
[a]Forage harvested on the grazing area.
[b]Area where cows graze directly.
[c]Average measures made with ruler from each grazing strip.
LR representing 6 to 7 cm was maintained all year round, and HR suggests a grazing management with a higher residual of 9 to 10 cm on average.

tendency for an interaction effect between factors for BCS (p = 0.09). Concerning BW per hectare, it was affected by the level of SR, with a difference of 218 kg ha−1 year−1 between the stocking rate of 2.0 compared to 1.5 cows per hectare.

The parameters of the estimated Wilmink curve fitting (Wilmink, 1987) are shown in Table 4. An effect of SR was detected on the initial phase of post-calving, inclining to peak (a) where the 2.0 cows group represented a positive difference of 1.50 L

TABLE 2 Effects of stocking rate (1.5 and 2.0 dairy cow/ha) and pasture residual height (LR or HR) on conserved forage and concentrate intake, forage allowance, forage, conserved forage, and concentrate intake for 1.5 LR, 1.5 HR, 2.0 LR, and 2.0 HR.

	Treatments					p-*value*		
	1.5 LR	1.5 HR	2.0 LR	2.0 HR	SEM	SR	RH	SR*RH
Supplement intake on grazing area kg DM/ha year								
Conserved forage	1,853b	1,883b	2,891a	3,090a	266	<0.001	0.83	0.39
Concentrate[a]	3,101c	3,111c	4,065b	4,247a	103	<0.001	0.16	0.45
Per cow per day, kg DM/cow per day								
Forage allowance	15.8b	18.4a	14.4c	16.0b	0.84	<0.001	<0.001	0.12
Forage DM intake (FDMI)	9.3b	10.2a	9.7b	9.7b	0.40	0.50	0.01	<0.001
Conserved forage intake	3.5b	3.7b	4.3a	4.5a	0.43	<0.001	0.29	0.84
Concentrate intake[a]	5.6	5.7	5.6	5.7	0.16	0.98	0.11	0.36
Total DM intake (DMI)	17.8c	18.1bc	18.3 ab	18.5a	0.16	<0.001	0.06	0.95
Proportion of time grazing	0.69a	0.66ab	0.64b	0.58c	0.01	<0.001	<0.001	0.36
Proportion days with no grazing	0.16b	0.18b	0.17b	0.21a	0.01	<0.001	<0.001	<0.001
Proportion days with one grazing	0.27c	0.28c	0.40a	0.39b	0.01	<0.001	0.65	0.08
Proportion days with two grazing	0.57a	0.54b	0.43c	0.40d	0.01	<0.001	<0.001	0.09

Values in a row with different letters are significantly different between treatments (p< 0.05).
[a]Refers to concentrate offered into the milking parlor.
LR representing 6 to 7 cm sward residual height was maintained all year round, and HR suggests a grazing management with a higher residual height of 9 to 10 cm on average.

cow^{-1} d^{-1} cow d in contrast to 1.5 cows per hectare, and the subsequent post-peak decline phase (c).

Discussion

To the best of our knowledge, no previous research has been published on the combined effects of SR and RH on forage supplementation, forage, and milk production evaluated at the farm level for 3 years. Most references related to RH on perennial grasses have been at the paddock level and have focused on the animal–plant relationship (Mezallira et al., 2013; Chapman, 2016; Menegazzi et al., 2021). In contrast, the effect of SR on biophysical variables at the farmlet level has been reported internationally (Macdonald et al., 2008; Baudracco et al., 2011; Mc Carthy et al., 2011; Patton et al., 2016) and more recently at the national level (Stirling et al., 2021).

Pasture production

The findings of the current study are consistent with those of Baudracco et al. (2011) on *M. sativa* pasture and those of Stirling et al. (2021) on a similar 4-year rotation combining perennial and annual grasses. As highlighted by McMeekan and Walshe (1963), the quantity of forage directly harvested increases as SR increases, improving the grazing efficiency (Macdonald et al., 2008; Baudracco et al., 2011). In this research, lower levels of forage directly harvested were reported for 1.5 LR and 1.5 HR compared to the 2.0 LR and 2.0 HR treatments. Despite this, the levels of forage harvested by treatment reflected the possibility of different strategies to achieve high proportions of home-grown harvested forage (directly and mechanically) in the grazing area where the total amount of forage harvested in all treatments (Table 1) was significantly higher than those reported at the national level (Fariña and Chilibroste, 2019).

The Uruguayan dairy system can be defined as a pasture-based dairy system that has the flexibility to reduce or increase external inputs. Systems like 1.5 LR or 1.5 RH presented an interesting strategy to increase milk solids productivity (900 kg/ha^{-1} year^{-1}) while supporting sustainable growth (Fariña and Chilibroste, 2019) based on the forage produced within its grazing area, without the need to import conserved forage from external areas (Chilibroste and Battegazzore, 2019).

The results of forage production in our study differed from those reported by Chapman (2016) who stated that a higher post-grazing height had a positive impact on forage production, affecting the length of the resting period. Other implications of working at low grazing depletion (14 cm post-grazing height) were reported by

TABLE 3 Effects of stocking rate (1.5 and 2.0) and pasture residual height (LR or HR) on milk (kg/cow per day) and milk solids production per cow and farm scale on.

	Treatments					*p*-value		
	1.5 LR	1.5 HR	2.0 LR	2.0 HR	SEM	SR	RH	SR*RH
Performance on grazing area								
Milk production (L/ha year)	12,886^{cd}	12,700^{d}	16,711^{b}	17,512^{a}	170	<0.001	0.002	<0.001
Milk solids production (kg/ha^{-1} year^{-1})[a]	992^{c}	980^{c}	1,314^{b}	1,353^{a}	10.5	<0.001	0.16	0.003
BW (kg/ha)	745^{b}	753^{b}	966^{a}	973^{a}	7.0	<0.001	0.87	0.25
Performance per cow								
305 d^{-1} milk yield (L/cow)[b]	6,330^{bc}	6,274^{c}	6,340^{b}	6,687^{a}	25.7	<0.001	<0.001	<0.001
Milk production (L/cow per day)	22.9	22.7	22.9	23.8	1.24	0.13	0.36	0.21
Milk protein production (kg/cow per day)	0.81	0.80	0.81	0.83	0.03	0.22	0.45	0.29
Milk fat production (kg/cow per day)	0.94^{b}	0.95^{b}	0.98^{a}	1.0^{a}	0.05	0.01	0.75	0.45
Milk solids production (kg/cow per day)[a]	1.76^{b}	1.75^{b}	1.79^{a}	1.84^{a}	0.07	0.03	0.59	0.34
Composition, g/kg								
Milk fat	42.3	41.9	43.0	42.8	0.05	0.13	0.13	0.86
Milk protein	35.7	35.8	35.7	35.7	0.03	0.46	0.97	0.69
BW (kg)	501	502	509	504	17.4	0.81	0.71	0.57
BCS	2.94	2.86	2.86	2.94	0.06	0.87	0.58	0.09

Values in a row with different letters are significantly different between treatments ($p< 0.05$).
BW, body weight (kg) per cow and kg/ha where data refer to grazing area.
[a]Milk fat plus milk protein content.
[b]Lactation curve estimated through the Wilmink (1987) model.
LR representing 6 to 7 cm was maintained all year round, and HR suggests a grazing management with a higher residual of 9 to 10 cm on average. The stocking rate is expressed as the number of cows/ha on the grazing area.

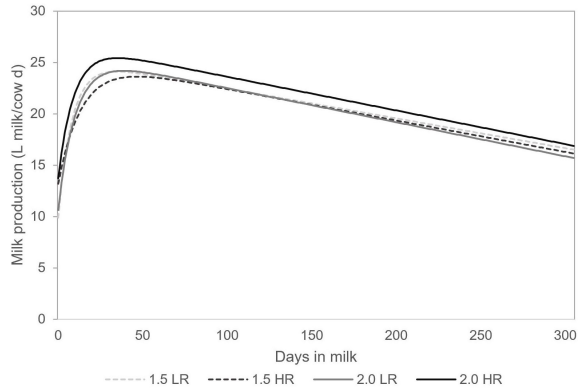


FIGURE 1
Mean lactation curves estimated with the exponential function of Wilmink (1987) (L milk/cow per day), high stocking rate low residual height (2.0 LR), high stocking rate high residual height (2.0 HR), middle stocking rate low residual height (1.5 LR), and middle stocking rate low residual height (1.5 HR).

Gareli et al. (2023) on orchard grass in the spring for dairy cows, finding greater grazing frequency, shorter resting periods, and, thus, more grazing cycles than at a higher depletion rate. In our study conditions, the effect of RH resulted in a sward height difference of 1.6 cm. The proximity of the residuals between LR and HR established that the conditions were not such to achieve a difference in forage production. Moreover, the pre-grazing conditions considered the optimal leave stage benchmark (Fulkerson and Donaghy, 2001), and residuals were not restrictive to compromise regrowth in perennial grasses (Hodgson and White, 2000).

TABLE 4 Milk yield parameters estimated with the exponential function of Wilmink (1987).

Parameter[a]	Estimate	S.E.	*t*	*p*-value
A				
Intercept	26.66	0.44	60.24	<0.001
SR (1.5)	-1.65	0.48	-3.45	0.001
RH (HR)	-0.70	0.45	-1.53	0.126
SR*RH	1.62	0.66	2.45	0.014
B				
Intercept	-14.64	1.90	-7.70	<0.001
SR (1.5)	-1.86	2.72	-0.69	0.493
RH (HR)	-1.60	2.70	-0.59	0.554
SR*RH	5.54	3.68	1.50	0.133
C				
Intercept	-0.04	0.00	-20.15	<0.001
SR (1.5)	0.01	0.00	2.36	0.018
RH (HR)	0.00	0.00	0.88	0.382
SR*RH	0.00	0.00	0.09	0.925

[a]Estimated parameters of the Wilmink (1987) function relating to the height of the lactation curve (A), the initial phase of post-calving incline to peak (B), and the subsequent post-peak decline phase (C).
The marginal effect (of each treatment) and the interactions effect between stocking rate (1.5 and 2.0 cow per ha) and residual sward height (LR or RH) on each of the parameters of the curve are shown. LR representing 6 to 7 cm was maintained all year round, and HR suggests a grazing management with a higher residual of 9 to 10 cm on average.

Grazing area FM average

In Uruguayan dairy grazing systems, the optimal average FM on grazing area to maximize the net forage accumulation on a multispecies rotation is still unknown. Beukes et al. (2018), working with *Lolium*-based pastures, reported pre-grazing values between 2,600 and 3,600 kg DM/ha and post-grazing between 1,500 and 1,700 kg DM/ha measured with a rising plate meter, representing an average FM in the grazing area of 2,350 kg DM ha. In the current study, the average FM on grazing area was lower (1,700 kg DM/ha) than the average FM stated by Beukes et al. (2018). This difference might be explained by the fact that, in our conditions, *L. multiflorum* (as annual species) occupied only 25% of the grazing area, while the remaining area was occupied by perennial grasses. Moreover, a different methodology, i.e., COMPYLD, was used to measure paddocks FM. The impact of aiming for a higher FM on the grazing area based on RH did not translate into increased forage productivity, as reported by Chapman (2016). The selected target FM in this study could have been a key aspect in duplicating the amount of forage harvested directly (7.65 ton of forage per hectare) with respect to commercial dairy systems, although it is still far lower compared to the most intensive dairy grazing system competitors (Fariña and Chilibroste, 2019).

This study has also shown that the pre- and post-grazing FM did not differ between treatments despite the differences on pre-grazing sward height (i.e., 2.0 LR higher than the rest). These findings further supported the observations by Menegazzi et al. (2021), who found that different residual sward heights resulted in different post-grazing heights, but no differences in the FM. This might have been influenced by the different vertical structures of the canopy that changed the density of the residual strata by the effect of grazing at different heights. In contrast, Gareli et al. (2023) observed differences in the pre- and post-grazing FM with different levels of RH (14 cm vs. 9 cm on orchard grass in spring), where a target of 40% of sward depletion was considered, while in our conditions sward depletion represented 63% and 58% for LR and HR, respectively. This sward depletion value was higher than the one reported by Mezzalira et al. (2014) to keep a high pasture DM intake rate. However, this does not limit our research, considering that dairy cows can graze at high levels (52%) when they are receiving high levels of supplement with concentrates (Dale et al., 2018).

Controlling RH is one of the most challenging issues in higher SR systems, where a greater amount of inputs is required (Macdonald et al., 2008) to achieve high forage harvest per hectare while maintaining moderate levels of forage intake per cow (Beukes et al., 2018). The effect of SR was not significant for FDMI, which aligned with the findings of Patton et al. (2016); however, our experiment reported an interaction between SR and RH for that variable. In this

context, a reduction in FDMI for 2.0 HR with respect to 1.5 HR could be related to the higher amounts of conserved forage offered per cow and the more restricted access to grazing. For our conditions, there were differences in total DMI, where 1.5 LR was lower than 2.0 HR, but not different from that of 1.5 HR and 2.0 LR. This opens up an interesting scenario with the possibility of continuing to increase competitiveness and at the same time maintaining a high proportion of forage in diets. However, there was a higher FDMI for the 1.5 HR treatment, which was not observed for the 2.0 HR treatment as could be expected. However, the FDMI results from the whole feeding strategies that combined different factors in time—for example, the higher amount of conserved forage offered to the 2.0 SR in contrast to 1.5 SR cows. In general, a 50% increase in the daily herbage allowance from 20 to 30 kg DM/cow was associated with an increase in FDMI of 1.3 kg/day and a 23% decrease in the utilization efficiency of available forage (Wilkinson et al., 2020). In this study, the difference in FA between 2.0 LR and 2.0 HR was only 11%, with no repercussions on FDMI. Instead, a difference of 16% in FA positively impacted a higher FDMI for 1.5HR, in contrast to that of 1.5 LR.

In grazing systems, increases in SR lead to a decline in forage allowance per cow. As a result, cows consume less forage (Macdonald et al., 2008; Baudracco et al., 2011), requiring increased provision of conserved forage to maintain optimal animal performance. This condition implied a higher amount of supplement per cow and per hectare for 2.0 LR and 2.0 HR as these treatments needed to address animal demand in contrast to 1.5 LR and 1.5 HR. This aligns with the observations of Patton et al. (2016), who demonstrated the use of supplementary feeds to preserve individual animal performance, where SR and supplementation usually increase simultaneously (Valentine et al., 2009; Fariña and Chilibroste, 2019). For our conditions, the amount of conserved forage usage for 2.0 HR was the highest, which explained for the greater time that cows were kept on the resting paddock with no access to grazing.

In this experiment, higher levels of conserved forage supplementation were associated with cows spending longer periods in resting areas, consequently reducing their time on pasture and limiting grazing opportunities. The cows in the 2.0 SR group spent less time engaged in two grazing turns compared to those in the 1.5 SR group and had more days with only one grazing turn. The increase in SR imposed greater pressure on pasture and posed a challenge in aligning animal demand with the grazing area GR to harvest the maximum amount of forage directly by cows (Roche et al., 2017). Consequently, the intensification of these pasture-based dairy systems is not feasible without considering appropriate infrastructure to provide favorable conditions for feeding supplements, address animal welfare concerns, and ensure suitable working conditions for humans. In dairy grazing systems, supplementation is a well-known alternative to increase the amount of energy consumed by cows, which often enhances individual milk yield (Patton et al., 2016). Sufficient high-quality supplements, when provided, can mitigate the negative effects of a higher SR, with outcomes such as an increase in milk yield per cow (Fariña et al., 2011) or no significant change (Baudracco et al., 2011). The findings observed in our study mirror previous studies carried out by Macdonald et al. (2008); Baudracco et al. (2011); Fariña et al. (2011), and Patton et al. (2016), which highlighted the positive effect of SR attributed to higher pasture usage and increased concentrate intake per hectare. This presents an opportunity for the intensification of dairy grazing systems to enhance farm productivity, either through SR or individual milk production (Fariña et al., 2011). The results of the current study support previous research indicating the non-existent effect of SR on individual milk production (Baudracco et al., 2011; Patton et al., 2016). However, in other studies, individual milk production was reported to be negatively (Macdonald et al., 2008) or positively affected by SR (Fariña et al., 2011; Mc Carthy et al., 2011). For the lactation curve parameters, the values differed from those reported by Baudracco et al. (2011). They found no effect of SR at the initial phase of post-calving, inclining to peak in two similar situations of 1.6 and 2.1 cows per hectare. The variation in our results could be attributed to the lower proportion of grazing in the 2.0 SR group, potentially contributing to a diet with more concentrate and less energy expenditure at the early stages of lactation. While supplementation of grazing dairy cows may be expected to increase the milk yield especially in early lactation (Bargo et al., 2003), this effect could be reflected in the lactation curves of cows during that stage. Regarding the effect of RH, this study did demonstrate an increase in individual FDMI with no significant effect on individual milk production per day, but with a positive impact on milk production per hectare with a reduction on the defoliation intensity. These results aligned with those found by Delaby and Peyraud (2003); Ganche et al. (2014), and Menegazzi et al. (2021), although these studies were not carried out at the system level.

At lower grazing intensities, cows would enhance their milk yield (Dale et al., 2018) because the lower level of depletion, in contrast to LR, would allow them to graze on mainly the upper stratum of the sward. This upper stratum typically contains a predominance of leaves and higher nutritive value (Benvenutti et al., 2016), contributing positively to the subsequent post-peak decline phase of the Wilmink (1987) model. Although these conditions might have implications for the BCS and BW of cows, in our circumstances, these variables were not affected by SR, as reported by Baudracco et al. (2011) and Patton et al. (2016), who analyzed systems with different levels of SR with supplementation throughout the lactation period. Dale et al. (2018) likewise found no variation between the low and high defoliation intensities for BCS; however, they observed a trend in BW at the end of the period for cows that grazed at a lower defoliation.

Conclusion

This study showed that a higher SR and a higher RH had no significant effect on forage production and utilization from home-grown forage. Nevertheless, distinct strategies emerged as a combination of direct and mechanical harvest between SR levels. With an increase in SR from 1.5 to 2.0 cows per hectare, direct forage consumption by cows rose and a higher supplementation (conserved forage and concentrate) was required to maintain milk and milk solids productivity. The strategic use of additional

supplements particularly in the face of restricted pasture allowance at a higher SR not only sustained the milk production per cow but also significantly increased milk production per hectare. Cows with a stocking rate of 2.0 per hectare spent less time grazing to align animal demand with GR for maximizing direct harvest. Investing in appropriate infrastructure is certainly crucial to ensuring the wellbeing of animals, optimizing labor conditions, and addressing environmental concerns. This is especially vital in light of escalating input costs and unpredictable output prices. Adopting systems that aim to harvest higher amounts of forage per hectare can contribute to the competitiveness of the Uruguayan and other dairy industries that rely on grazing systems. This underscores the importance of strategic planning, efficient resource management, and a sustainable approach to ensure the long-term viability of dairy farming in these regions.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://data.mendeley.com/datasets/hfjkkhdg94/1.

Ethics statement

The animal studies were approved by Comisión Honoraria de Experimentación Animal (CHEA). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

GO: Conceptualization, Data curation, Formal analysis, Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. NB: Formal analysis, Methodology, Software, Writing – review & editing. PC: Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This project was funded by the Agencia Nacional de Investigación e Innovación (ANII) and Red Tecnológica Sectorial de Lechería (RTS) (project number ANII-RTS_X_2014_1_3) who also funded post-graduate scholarship awarded to G. Ortega (POS_NAC_2018_1_151491).

Acknowledgments

We would like to thank all the staff of Centro Regional Sur who have supported this research from the beginning.

Conflict of interest

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

Publisher's note

References

Allen, V. G., Batello, C., Berretta, E. J., Hodgson, J., Kothmann, M., Li, X., et al. (2011). An international terminology forgrazing lands and grazing animals. *Grass Forage Sci.* 66, 2–28. doi: 10.1111/j.1365-2494.2010.00780.x2

Bargo, F., Muller, L. D., Kolver, E. S., and Delahoy, J. E. (2003). Invited review: Production and digestion of supplemented dairy cows on pasture. *J. Dairy Sci.* 86, 1–42. doi: 10.3168/jds.S0022-0302(03)73581-4

Baudracco, J., Lopez-Villalobos, N., Romero, L. A., Scandolo, D., Maciel, M., Comeron, E. A., et al. (2011). Effects of stocking rate on pasture production, milk production and reproduction of supplemented crossbred Holstein-Jersey dairy cows grazing lucerne pasture. *Anim. Feed Sci. Technol.* 168, 131–143. doi: 10.1016/j.anifeedsci.2011.03.017

Benvenutti, M. A., Pavetti, D. R., Poppi, D. P., Gordon, I. J., and Cangiano, C. A. (2016). Defoliation patterns and their implications for the management of vegetative tropical pastures to control intake and diet quality by cattle. *Grass Forage Sci.* 71, 424–436. doi: 10.1111/gfs.12186

Beukes, P. C., McCarthy, S., Wims, C. M., Gregorini, P., and Romera, A. J. (2018). Regular estimates of herbage mass can improve profitability of pasture-based dairy systems. *Anim. Prod. Sci.* 52, 1–9. doi: 10.1071/AN17166

Chapman, D. (2016). Using ecophysiology to improve farm efficiency: application in temperate dairy grazing systems. *Agriculture* 6, 17. doi: 10.2135/cropsci2006.01.0036

Chataway, R. G., Walker, R. G., and Callow, M. N. (2010). Development of profitable milk production systems for northern Australia: a field assessment of the productivity of five potential farming systems using farmlets. *Anim. Prod. Sci.* 50, 246–264. doi: 10.1071/AN09124

Chilibroste, P., and Battegazzore, G. (2019). *Proyecto Producción Competitiva. 1st ed* (Montevideo, Uruguay: Conaprole).

Chilibroste, P., Ibarra., D., Zibil., S., and Laborde, D. (2003). *Proyecto Alimentación-Reproducción Conaprole 2002: Informe final*. (Montevideo, Uruguay: Conaprole) 28 p.

Dale, A. J., Laidlaw, A. S., Gordon, A., and Ferris, C. P. (2018). The effect of grazing intensity on the performance of high-yielding dairy cows. *Grass Forage Sci.* 73, 798–810. doi: 10.1111/gfs.12350

Delaby, L., and Peyraud, J. L. (2003). The effect of two contrasting grazing managements and level of concentrate supplementation on the performance of grazing dairy cows. *Anim. Res.* 52, 437–460. doi: 10.1051/animres:2003030

Dillon, P., Roche, J. R., Shalloo, L., and Horan, B. (2005). "Optimising financial return **from** grazing **in** temperate pastures," in *Utilization of grazed grass in temperate animal systems*. Ed. J. Murphy (Cork, Ireland: International Grassland Congress).

Donaghy, D. J., and Fulkerson, W. J. (1998). Priority for allocation of water-soluble carbohydrate reserves in Lolium perenne. *Grass For. Sci.* 53, 211–218. doi: 10.1071/EA00062

Fariña, S., and Chilibroste, P. (2019). Opportunities and challenges for the growth of milk production from pasture: the case of farm systems in Uruguay. *Agric. Syst.* 176, 102631. doi: 10.1016/j.agsy.2019.05.001

Fariña, S. R., Garcia, S. C., Fulkerson, W. J., and Barchia, I. M. (2011). Pasture-based dairy farm systems increasing milk production through stocking rate or milk yield per cow: pasture and animal response. *Grass Forage Sci.* 66, 316–332. doi: 10.1111/j.1365-2494.2011.00795.x

Ferguson, J. D., Galligan, D. T., and Thornsen, N. (1994). Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* 77, 2695–2703. doi: 10.3168/jds.s0022-0302(94)77212-x

Fulkerson, W. J., and Donaghy, D. J. (2001). . Plant-soluble carbohydrate reserves and senescence-key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Aust. J. Exp. Agric.* 41, 261–275. doi: 10.1071/EA00062

Ganche, E., Delaby, L., O'Donovan, M., Boland, T. M., Galvin, N., and Kennedy, E. (2013). Post-grazing sward height imposed during the first 10 weeks of lactation: Influence on early and total lactation dairy cow production, and spring and annual sward characteristics. *Livest. Sci.* 157, 299–311. doi: 10.1016/j.livsci.2013.08.001

Ganche, E., O'Donovan, M., Pey, L., Boland, T. M., and Kennedy, E. (2014). Does post-grazing sward height influence sward characteristics, seasonal herbage dry-matter production and herbage quality? *Grass For. Sci.* 70, 130–1143. doi: 10.1111/gfs.12113

Gareli, M. S., Mendoza, A., Bello, N. M., Lattanzi, F. A., Fariña, S. R., and Savian, J. V. (2023). Effects of grazing management and concentrate supplementation on intake and milk production of dairy cows grazing orchardgrass. *Anim. Feed Sci.* 301, 115 668. doi: 10.1016/j.anifeedsci.2023.115668

Hodgson, J. H., and White, J. (2000). *New Zealand pasture and crop science* (Oxford, UK: Oxford University Press).

Macdonald, K. A., Penno, J. W., Lancaster, J. A. S., Bryant, A. M., Kidd, J. M., and Roche, J. R. (2017). Production and economic responses to intensification of pasture-based dairy production systems. *J. Dairy Sci.* 100, 1–18. doi: 10.3168/jds.2016-12497

Macdonald, K. A., Penno, J. W., Lancaster, J. A. S., and Roche, J. R. (2008). Effect of stocking rate on pasture production, milk production, and reproduction of dairy cows in pasture-based systems. *J. Dairy Sci.* 91, 2151–2163. doi: 10.3168/jds.2007-0630

Mary (2018)Horizontal feed mixer MS 85 with scale. Available at: https://mary.com.uy/producto/mixer-horizontal-m110/.

Mc Carthy, B., Delaby, L., Pierce, K. M., Journot, F., and Horan, B. (2011). Meta-analysis of the impact of stocking rate on the productivity of pasture-based milk production systems. *Anim. Consortium* 5, 784–794. doi: 10.1017/S1751731110002314

McCarthy, S., Hirst, C., Donaghy, D., Gray, D., and Wood, B. (2014). Opportunities to improve grazing management. *Proc. N. Z. Grassland Assoc.* 76, 75–80. doi: 10.33584/jnzg.2014.76.2971

McMeekan, C. P., and Walshe, M. J. (1963). Interrelationships of grazing methods and stocking rate in efficiency of pasture utilization by dairy cattle. *J. Agric. Sci.* 61, 147–166. doi: 10.1017/S0021859600005888

Menegazzi, G., Giles, P. Y., Oborsky, M., Fast, O., Mattiauda, D. A., Genro, T. C. M., et al. (2021). Effect of post-grazing sward height on ingestive behavior, dry matter intake, and milk production of Holstein dairy cows. *Front. Anim. Sci.* 2. doi: 10.3389/fanim.2021.742685

Merino, V. M., Balocchi, O. A., Rivero, M. J., and Pulido, R. G. (2019). Short-term effect of daily herbage allowance restriction on pasture condition and the performance of grazing dairy cows during autumn. *Animals* 10, 62. doi: 10.3390/ani10010062

Mezzalira, J. C., De Faccio Carvalho, P. C., Fonseca, L., Bremm, C., Cangiano, C., and Gonda, H. L. (2014). Behavioural mechanisms of intake rate by heifers grazing swards of contrasting structures. *Appl. Anim. Behav. Sci.* 153, 1–9. doi: 10.1016/j.applanim.2013.12.014

Mezzalira, J., Bremm, C., Trindade, J. K. D., Gonda, H. L., Vieira, P. C., and Carvalho, P. C. D. F. (2013). Ingestive behaviour from the feeding station topatch level in heterogeneous environments. *J. Anim. Sci. Adv.* 3, 613–623.

Patton, D., Pierce, K. M., and Horan, B. (2016). Effect of stocking rate on milk and pasture productivity and supplementary feed use for spring calving pasture fed dairy systems. *J. Dairy Sci.* 99, 1–12. doi: 10.3168/jds.2015-10305

Ramsbottom, G., Horan, B., Berry, B., and Roche, J. R. (2015). Factors associated with the financial performance of spring-calving, pasture-based dairy farms. *J. Dairy Sci.* 98, 3526–3540. doi: 10.3168/jds.2014-8516

Roche, J. R., Berry, D. P., Bryant, A. M., Burke, C. R., Butler, S. T., Dillon, P. G., et al. (2017). A 100-Year Review: A century of change in temperate grazing dairy systems. *J. Dairy Sci.* 100, 10189–10233. doi: 10.3168/jds.2017-13182

Stirling, S., Fariña, S., Pacheco, D., and Vibart, R. (2021). Whole-farm modelling of grazing dairy systems in Uruguay. *Agric. Syst.* 193, 103227. doi: 10.1016/j.agsy.2021.103227

Strucken, E. M. (2021). *Lactcurves: Lactation Curve Parameter Estimation. R package version 1.1.0. Proc. Assoc. Advmt. Anim. Breed. Genet.* 23, 139–142.

Valentine, S., Lewis, P., Cowan, R. T., and DeFaveri, J. (2009). The effects of high stocking rates on milk production from dryland and irrigated Mediterranean pastures. *Anim. Prod. Sci.* 49, 100–111. doi: 10.1071/EA07071

Wilkinson, J. M., Lee, M. R. F., Rivero, M. J., and Chamberlain, A. T. (2020). Some challenges and opportunities for grazing dairy cows on temperate pastures. *Grass Forage Sci.* 75, 1–17. doi: 10.1111/gfs.12458

Wilmink, J. B. M. (1987). Comparison of different methods of predicting 305-day milk yield using means calculated from within-herd lactation curves. *Livest. Prod. Sci.* 17, 1–17. doi: 10.1016/0301-6226(87)90049-2

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 19 April 2024
DOI 10.3389/fanim.2024.1296203

OPEN ACCESS

EDITED BY
Marcos Inacio Marcondes,
Washington State University, United States

REVIEWED BY
Vincenzo Lopreiato,
University of Messina, Italy
Zhongyue Yang,
Stanford University, United States
Juana Catarina Cariri Chagas,
Swedish University of Agricultural Sciences, Sweden

*CORRESPONDENCE
Cesar S. Pinares-Patino
troutarcoiris@gmail.com

RECEIVED 18 September 2023
ACCEPTED 05 April 2024
PUBLISHED 19 April 2024

CITATION
Mwangi PM, Eckard R, Gluecks I, Merbold L, Mulat DG, Gakige J, Marquardt S and Pinares-Patino CS (2024) Supplementation of a tropical low-quality forage with *Calliandra calothyrsus* improves sheep health and performance, and reduces methane emission.
Front. Anim. Sci. 5:1296203.
doi: 10.3389/fanim.2024.1296203

Supplementation of a tropical low-quality forage with *Calliandra calothyrsus* improves sheep health and performance, and reduces methane emission

Paul M. Mwangi[1,2], Richard Eckard[2], Ilona Gluecks[1], Lutz Merbold[3], Daniel Girma Mulat[1], Jesse Gakige[1], Svenja Marquardt[1,2] and Cesar S. Pinares-Patino[1*]

[1]Mazingira Centre, International Livestock Research Institute (ILRI), Nairobi, Kenya, [2]Faculty of Agriculture and Veterinary Sciences, University of Melbourne, Melbourne, VIC, Australia, [3]Integrative Agroecology Group, Research Division Agroecology and Environment, Agroscope, Zurich, Switzerland

Ruminant production systems in the arid and semi-arid regions of Sub-Saharan Africa confront severe challenges due to recurring droughts and the intensifying effects of climate change (CC). These systems grapple with numerous stress factors, including poor animal nutrition, water scarcity, gastrointestinal (GIT) parasite burdens, and heat stress, which contribute to below optimal animal productivity and a high environmental footprint. Addressing these issues urgently, by creating livestock systems resilient to CC that also promote better animal health, enhanced productivity, and reduced environmental impact, is paramount to safeguarding the livelihoods of the rural population. This 50-day study aimed to evaluate the effects of improved feeding and nutritional management in sheep, focusing on GIT parasite infections, feed intake and digestibility, liveweight (LW) gain, and enteric methane (CH_4) emissions. We investigated the legume forage tree, Calliandra (*Calliandra calothyrsus*), as a high-quality feed source because of its rich crude protein content and its potential as a remedy for gastrointestinal tract (GIT) parasite infections, attributed to its high condensed tannin (CT) content. Twenty-eight Dorper lambs underwent random allocation across four treatments, each consisting of seven lambs. These treatments combined either a trickle infection or no infection with *Haemonchus contortus* with a supplementation (40% of diet on a dry matter basis or lack thereof) of the basal diet (Rhodes grass hay) with dried Calliandra leaflets. The treatments were: UnHay (uninfected, fed on hay-only), InHay (infected, *fed* on hay-only), InHay+Cal (infected, fed on hay plus Calliandra), and InHay+Cal+PEG [infected, fed on hay, Calliandra, and polyethylene glycol (PEG)]. The latter was to evaluate the potential effects of Calliandra's condensed tannins. The results show that lambs in the InHay+Cal treatment exhibited a higher packed cell volume (PCV) and lower faecal egg counts (FEC) compared to the InHay group. No effects of infection on the other measured variables were observed in unsupplemented lambs. Calliandra supplementation increased total feed dry matter intake (DMI) by 20% (61.8 vs. 51.7 g DM/kg LW0.75) (InHay+Cal vs. InHay) and enhanced LW gain (7.2 g/d) in the InHay+Cal group, whereas the unsupplemented infected group (InHay) experienced LW loss (-26.6 g/d). Calliandra supplementation to infected lambs reduced daily CH_4 emission by

15% (13.9 vs. 16.2 g/d) and CH_4 yield (g/kg DMI) by 30% (18.7 vs. 26.5 g/kg DMI), compared to emissions from unsupplemented infected lambs. Nonetheless, Calliandra supplementation decreased the digestibility of crude protein and fibre and raised the faecal nitrogen (N) output to N intake (FN/NI) ratio. The effects of PEG supplementation on CT activity remained inconclusive. The study concludes that a 40% replacement of a protein-deficient basal diet with Calliandra may be excessive. However, the findings underscore the considerable advantages of integrating Calliandra into farming systems. Such nature-based solution control GIT parasite infections and their lifecycle, bolster the nutritional value of a deficient basal diet, improve animal productivity cost-effectively, and mitigate enteric methane emissions both in absolute terms and intensity.

KEYWORDS

Rhodes grass, fodder tree, gastrointestinal parasite, greenhouse gas, ruminants, condensed tannins

1 Introduction

According to the FAO database (FAO, 2018), the global population of sheep and goats is 1.18 billion and one billion, respectively. Africa has the second-highest population of small ruminants in the world at 352 million and 388 million heads of sheep and goats, respectively. These small ruminants play an important socioeconomic role as a source of nutrition, income, and other intangible benefits, such as savings, insurance against emergencies, and cultural and ceremonial purposes (Kosgey et al., 2008). They also play a complementary role to other livestock by utilizing feed resources in natural grasslands (Baker & Rege, 1994). Globally, the livestock sector greenhouse gas (GHG) emissions are equivalent to 7.1 Gt CO2-eq/year (14.5% of anthropogenic GHG emissions), of which the share of small ruminants is 6.5% (Gerber et al., 2013). Over 55% of emissions from small ruminants are attributed to enteric methane (CH_4) (Gerber et al., 2013).

The most significant production constraints facing small ruminant production systems in the tropics are gastrointestinal tract (GIT) parasite infection, shortage of feed and grazing land, and water scarcity due to recurrent droughts and climate change (CC) (Armson et al., 2021; Rahimi et al., 2021). Finding cost-effective solutions to these challenges is crucial for improving animal productivity, health, and welfare, and safeguarding the livelihoods of smallholder livestock keepers.

Feeding ruminants during dry seasons in the tropics is characterized by the provision of forages of poor feeding value (poor digestibility, low concentrations of crude protein (CP), and high concentrations of fibre and lignin). This is often below maintenance energy requirements of the animals and consequently leads to low productivity, loss of liveweight (LW), high enteric CH_4 emissions and results to high emission intensity (emissions per unit of animal product) (Kurihara et al., 1999; Chaokaur et al., 2015; Goopy et al., 2020). In the tropics, there is a diversity of forage plants, especially legumes, which contain secondary compounds such as condensed tannins (CT) (Bhatta et al., 2013). Condensed tannins can directly affect rumen microorganisms, thereby decreasing CH_4 production (Tavendale et al., 2005; Berça et al., 2023). Furthermore, consumption of CT-containing forages can lead to reduced shedding of GIT nematode (GIN) eggs, hence reducing the contamination of pastures (Min et al., 2005) as well as lower host infection through reduced parasite numbers in the GIT (Brunet et al., 2008; Oliveira et al., 2013). There is currently a challenge with GIN resistance to most commonly used deworming drugs. This widespread increase in anthelmintic resistance (Greiffer et al., 2022) has prompted the search for alternative GIN control solutions, and efforts are also supported by increased consumer demand for more sustainable livestock production with reduced use of chemicals (Waller, 1999).

Supplementing grazing ruminants with legume forage trees, such as Calliandra (*Calliandra calothyrsus*), which is high in CP and CT, during the dry seasons may provide various benefits to livestock production systems, including reducing LW loss, providing nutrients to enhance animal resilience to GIN, and reducing CH_4 emissions (Kaitho et al., 1996; Min et al., 2005; Piñeiro-Vázquez et al., 2018). Calliandra is a multipurpose tree legume native to humid and sub-humid regions of Central America and Mexico. It was introduced to the Central Highlands of Kenya in the 1980s and has since been widely promoted and adopted as a supplement for ruminants on low-quality forages (Wambugu et al., 2001). Various studies conducted in Sub-Saharan Africa (SSA) have shown that supplementation with tropical tannin-rich forage legumes increases the productivity of animals fed on poor-quality diets (Kinuthia et al., 2007; Yisehak et al., 2014). Forage trees containing CT have also demonstrated anti-methanogenic activity when fed to ruminants (Tiemann et al., 2008; Piñeiro-Vázquez et al., 2018). However, to our knowledge, no research in SSA has evaluated the effects of these forages on the

health of animals in combination with enteric methane emissions. In this context, our study assesses the potential of Calliandra as a supplement to sheep consuming poor-quality Rhodes grass (*Chloris gayana*) hay evaluating its effects on voluntary feed intake, LW gain (LWG), nitrogen and energy partitioning, GIN infection (*Haemonchus contortus*) response, and enteric CH_4 emissions. We hypothesized that supplementation of poor-quality Rhodes grass hay with Calliandra would enhance sheep productivity (LWG) and health while reducing enteric CH_4 emissions.

2 Materials and methods

This study was conducted at the Mazingira Centre, International Livestock Research Institute (ILRI), Nairobi, Kenya. The experimental protocol was reviewed and approved by the ILRI Institutional Animal Care and Use Committee (IACUC; Reference number 2022-03).

2.1 Animal management and experimental design

Dorper lambs aged 10–11 months old, mixed sex, and 28.1 ± 2.7 kg liveweight (LW) were selected from a cohort of lambs born at the ILRI Kapiti Research Station & Wildlife Conservancy, located 60 km South-East of Nairobi. In this conservancy, sheep flocks are kept on a grassland interspersed with Acacia trees and shrubs. These lambs were then transported to the ILRI farm in Nairobi where they underwent a 3-week quarantine period. During this period, they were kept as a single group and fed Rhodes grass (*Chloris gayana)* hay before starting the experiment. At the beginning of the quarantine period, each lamb was given anthelmintic treatment using 10% albendazole (albafas) at a dose of 8 mg/kg LW. Subsequently, faecal examinations were conducted regularly to ensure that they attained a worm-free status and remained worm-free.

At the end of the quarantine period, the animals were acclimatized to their experimental diets for a period of seven days. Thereafter, the experiment was started lasting 50 days (Days 1–50). For the initial 40 days (Days 140), the lambs were housed in individual pens within an animal shed each equipped with feed and water bins. On Day 41, lambs were moved to individual metabolic crates and kept for seven days during which total faeces and urine were collected over the last five days (Days 43–47). Afterwards, the lambs were transferred to respiration chambers to measure CH_4 emission over the last three days of the experiment (Days 48–50).

The experiment used a completely randomized design. Twenty-eight Dorper lambs were subdivided in a balanced manner (LW and sex) into four experimental groups (seven animals each; three castrated males and four females). The lambs were then randomly allocated to four experimental treatments that combined infection status (infected or uninfected with *Haemonchus contortus*) and Calliandra supplementation (supplemented or unsupplemented), and fed a basal diet of poor-quality Rhodes grass hay. The experimental treatments were uninfected fed on hay only (UnHay), infected fed on hay only (InHay), infected fed on hay plus Calliandra supplement (InHay+Cal), and infected fed on hay plus Calliandra supplement plus polyethylene glycol (PEG) supplement (InHay+Cal+PEG). InHay+Cal+PEG treatment was used to evaluate the anticipated role of CT in Calliandra on feed intake, digestibility, N metabolism, and CH_4 emissions, as PEG binds to tannins to form stable complexes (Makkar et al., 1995). The level of Calliandra supplementation was set to replace 40% of the voluntary feed intake (dry matter basis) of the Rhodes grass hay, whereas the level of PEG supplementation was set to neutralize the total concentration of CT in the diet (PEG : CT ratio of 1:1), based on results of preliminary laboratory analyses.

Given the availability of only three animal respiration chambers, the experimental animals were subdivided into ten batches (Eight batches of three animals each and two batches of two animals each). The batches entered the experiments in a staggered manner, every three days. Batches were balanced as much as possible for sex and experimental treatment, each batch included only one lamb from either of the four treatment groups.

2.2 Feeds and feeding

The Rhodes grass hay had 92.2% dry matter (DM) and on DM basis it contained 90.36% organic matter (OM), 73.0% neutral detergent fibre (NDF), and 5.6% crude protein (CP) (Table 1). Calliandra leaflets had a DM content of 91.8%, 94.1% OM, 17.4% NDF, 21.1% CP, and 9.2% total condensed tannins (CT) (Table 1). Calliandra leaves were harvested from a single farm in Kakamega County (northwest of Nairobi). Branches were cut and allowed to dry in the field over PVC sheets until some primary leaflets started self-detaching from the leaf rachis. Primary leaflets (pinnae) were then manually harvested. The harvested material was transported in jute sacks and stored under protection from rain and sun until the start of the experiment. Thus, the Calliandra material used in this study was composed of secondary leaflets in the pinnae.

TABLE 1 Mean (± standard deviation) chemical composition (dry matter (DM) basis) of Rhodes grass (*Chloris gayana*) hay and air-dried Calliandra (*Calliandra calothyrsus*) leaves.

Nutrient	Rhodes Hay (*n*=7)	Calliandra leaves (*n*=7)
Dry matter (%)	92.2 ± 0.51	91.8 ± 0.57
Organic matter (%)	90.3 ± 1.30	94.1 ± 0.16
Neutral detergent fibre (%)	73.0 ± 1.69	17.4 ± 0.69
Acid detergent fibre (%)	40.0 ± 1.48	11.7 ± 0.59
Crude protein (%)	5.6 ± 0.63	21.1 ± 0.31
Gross Energy (MJ/kg DM)	17.4 ± 0.23	20.5 ± 0.53
Condensed tannins (%)	n.d.	9.2 ± 0.97

n.d., not determined.

During the seven-day acclimatization period, all animals were fed on chaffed Rhodes grass hay *ad libitum*. During the first five days of acclimatization, lambs in the supplemented experimental treatments (InHay+Cal and InHay+Cal+PEG) were gradually offered Calliandra. By the end of the acclimatization period, the supplemented animals had adjusted to their full allowance of Calliandra, with no refusals. Voluntary feed intake (VFI) of Rhodes grass was determined during the acclimatization period. The average VFI of the hay was determined on a metabolic LW basis ($LW^{0.75}$). The unsupplemented animals were fed hay at 1.1 VFI. Calliandra supplementation was set to replace 40% of the VFI of hay (DM basis); hence, the hay offered to Calliandra-supplemented animals was set at 0.7 VFI of hay. This feeding level remained consistent throughout the study.

Lambs in the InHay+Cal+PEG treatment were offered the Calliandra supplement sprayed with PEG. The amount of PEG used was based on preliminary sampling and analysis of Calliandra samples that had a CT concentration of 28.2 g CT/kg DM. The preliminary sampling was done 12 weeks prior to collection of the Calliandra forage used in the experiment (Both the preliminary and experimental samples were collected from the same farms). A solution of PEG was prepared daily using 250 g of PEG 6000 dissolved in 1250 ml distilled water and depending on the intake of Calliandra in the ration, a fixed volume of the solution was sprayed onto the Calliandra forage twice a day. The average daily dose of PEG solution was 50 ml (i.e., 10 g PEG/animal), providing a PEG : CT ratio of 1:1, a level slightly higher than 1:1 required to neutralize CT activity. Further analysis of CT in the Calliandra fed during the experiment (using specific standards) was carried out at the end of the study, which showed a higher concentration of total CT (92.1 g/kg DM), resulting in a PEG : CT ratio of 1:2.8.

The daily rations of hay and Calliandra were divided into two equal portions and fed at 0830 and 1630 h. The lambs were offered water *ad libitum* using a plastic bucket, and a mineral block was provided free of choice.

2.3 Experimental larvae preparation and infection

Larvae were prepared by infecting three adult donor sheep of the Dorper breed with a single dose of 5,000 *H. contortus* L_3 larvae suspended in distilled water, prior to deworming them by oral dosing with 10% albendazole (albafas) at a dosage of 8 mg/kg LW. After a 21-day prepatent period, following L_3 larvae dosing, the total faecal output was collected from each donor sheep. Larval production was performed according to the process described by Mwangi et al. (2023). In brief, the jars containing the faeces-vermiculite mix were incubated at 27°C (Lovibond, Germany). Hatched larvae were washed off the sides of the jars into cell culture flasks, and each flask was filled up to 60 ml (distilled water containing larvae) and stored at 4°C until they were administered to the lambs in the infection experimental treatments. The viability of the larvae was confirmed under a dissection microscope (Zeiss, Model Axiocam ERc 5s, Germany).

The larvae doses used for the experiment were calculated as described by Mwangi et al. (2023). Starting from Day 1 of the study, lambs in the infection experimental treatments (InHay, InHay+Cal and InHay+Cal+PEG) were dosed with 1000 *H. contortus* L_3 stage larvae over five consecutive days, leading to a total dose of 5,000 larvae per animal. The uninfected lambs were sham-infected with clean water as a control. The donor lambs were not included in the experiment.

2.4 Measurements and sample collection

2.4.1 Parasitological examination and haematology

A faecal sample for parasitological examination was collected per animal on Day 1 of the experiment, and weekly thereafter. The sample was collected directly from the rectum and placed in a plastic sampling bottle. Faecal egg counting was conducted using a modified McMaster technique (Whitlock, 1948).

At the times of faecal sampling, blood samples were collected via jugular venipuncture into EDTA sampling bottles for hematological examination. Packed cell volume (PCV, %) was determined using a hematology analyzer (Nihon Kodehn, MEK 6550K, Japan).

2.4.2 Feed intake

The lambs were fed individually throughout the study. A sample of the feeds on offer (hay and Calliandra) were collected daily during meal weighing. These samples were stored at -20°C, weekly, and subsequently oven-dried for DM determination. Feed refusals were collected daily from each animal before the morning feeding and treated similarly to the feeds on offer, pooled weekly per animal and then oven-dried.

Feed intake by individual animals (feed on offer minus refusals, for each feed item) was calculated on DM basis for the pen period (Days 1–40), during their time in metabolic crates (Days 43–47) and respiration chambers (Days 48–50). Lambs supplemented with Calliandra showed no refusal of Calliandra forage.

2.4.3 Liveweight and liveweight gain

Liveweight (LW) measurement of each lamb was recorded before morning feeding on Day 1, and weekly thereafter until the end of the experimental period (Day 50) using an outdoor automatic digital weighing scale (Gallagher, Model 3B4092, Hamilton, New Zealand).

2.4.4 Total faeces and urine outputs (balance period)

A total collection of daily outputs of faeces and urine was conducted over Days 43–47, while the lambs were kept in individual metabolic crates. Collections were not carried out over the first two days in metabolic crates (Days 41 and 42 due to disruption in feed intake caused by animal movement from pens to crates). The metabolic crates allowed for separate collection of faeces and urine for each animal. Faeces passed through the crate floor to a mesh below, while urine passed through this mesh and flowed into a

Perspex tray, eventually being collected in a bottle containing 70 ml of 10% HCl. This setup ensured the urine pH remained below 3 at the conclusion of the 24-hour collection period and prevented ammonia volatilization. The urine tray was sprayed with fresh water five times daily during daylight to prevent nitrogen loss from urine droplets. Roughly 0.5 L of water was used for this, and its addition did not influence urine nitrogen and energy calculations.

Every morning before feeding, the daily faecal and urine outputs were collected. Faecal outputs from each lamb were weighed and recorded, a 10% sub-sample was stored at -20°C in a zip lock bag. Simultaneously, the day's total urine (plus water) output was sieved and its volume recorded, and a 10% sub-sample was stored at -20°C in an airtight bottle. The daily faecal and urine samples were then pooled by individual animals for future processing for determination of DM (faeces) and subsequent chemical analyses.

2.4.5 Methane emissions from enteric fermentation

Enteric CH_4 emission from each lamb was measured over three consecutive days (Days 48 to 50) using three open-circuit respiration chambers (No Pollution Industrial Systems Limited, UK) as described by Mwangi et al. (2023). During this measurement period, each lamb spent one day in each of the three chambers. On each day, the CH_4 measurement was conducted over 23.5 hours (starting at 0830 h and finishing to 0800 h following day), with chambers opened twice a day for 15 min each for feeding and watering (morning and afternoon) and cleaning (before morning feeding). Emissions calculations were extrapolated to 24-h periods.

During measurement, the internal temperature of the chambers was maintained at 22°C with a relative humidity of 47%. The ventilation rate of the chambers was set to 8 l/s. A cavity ringdown laser absorption spectrometer (Picarro G2508 analyzer, Santa Clara, USA) was used to measure the CH_4 concentrations (in ppm) at intervals of approximately five minutes (including the incoming background gas sample). CH_4 emissions were calculated using the difference between inlet and outlet gas concentrations multiplied by volumetric air flow and corrected to standard temperature and pressure (25°C and 101,300 Pa).

A whole-system recovery of known volumes of pure CH_4 into the respiration chamber system was carried out before the start of the methane measuring period and at the end of the measurement period using a gas phase titration unit (Environics 4020, Environics Inc., Tolland, USA). The CH_4 recovery rate was 98–106%.

2.4.6 Rumen sampling and pH measurement

Once the lambs exited from the respiration chambers (0900 h, before feeding), rumen samples were collected from each sheep using an orogastric tube. With the animal properly restrained and the neck extended forward, a PVC tube speculum was placed in the mouth. A flexible orogastric tube was gently passed through the speculum to the base of the tongue. While the animals were allowed to swallow, the tube was slowly pushed through the oesophagus and into the rumen. Sample extraction was performed once the tube was in the rumen using a pump connected to the tube. The first 10–20 ml of rumen sample collected was discarded, and a second sample (~40 ml) was collected, and pH was measured immediately using a pH and conductivity meter (Jenway 3540 model, UK).

2.5 Laboratory analyses

Samples of feeds on offer (pooled on a weekly basis) and within-animal pooled samples of feed refusals and faeces were oven-dried (Genlab oven, Model SDO/425/DIG, UK) at 50°C for 72 h. The dried samples were ground to pass a 5 mm sieve using a Wiley mill (Model MF 10B, IKA Werke, Willmington, N.C., USA) and then passed a 1 mm sieve using a hammer mill (MF 10 basic, IKA, Werke GmbH & CO. KG, Staufen, Germany). The total DM was determined by drying the samples at 105°C in an oven (Genlab oven, model SDO/425/TDIG, UK) for 24 h while ash was determined by combustion in a muffle furnace (Nabertherm GmbH, Germany) at 550°C for six hours according to the methods of the Association of Official Analytical Chemists (AOAC, 1990 methods no. 924.05). Feed, refusals, and faecal samples were analysed for NDF and ADF using the methods of Van Soest et al. (1991) with an Ankom 200 fibre analyzer (model A2001, USA). The total nitrogen (N) content in urine samples was determined using the micro-Kjeldahl procedure (AOAC, 1990, method no. 988.05). The total N content in feed, refusals, and faecal samples was determined using an organic elemental analyzer (Vario Max, model vario Solid Sampler, Germany). Total condensed tannins (CT) in Calliandra were preliminarily analysed in the Competence Division Method Development and Analytics Laboratory of Agroscope (Posieux, Switzerland) using the modified butanol-HCI procedure described by Terrill et al. (1992), using cyanidin to set the standard calibration curve. Subsequent analyses of CT in Calliandra, conducted at the end of the study, were performed at the Texas A&M Agrilife Center (Stephenville, TX) using the simplified butanol-HCl method and specific plant standards, as described by Wolfe et al. (2008).

Gross energy (GE) content of feed on offer, feed refusal, and faeces was determined according to the methods of Harris (1970) using a bomb calorimeter (Parr 6200, model A1290DDEE, UK). The gross energy contents of the urine samples were determined by a method described by Zhao et al. (2016), where the urine was soaked in a filter paper with a predetermined GE, the soaked filter paper was dried (50° C, 2 h), weighed, and its GE determined in a bomb calorimeter. To calculate the GE of urine, the GE value of the filter paper was subtracted from the GE value of the urine-soaked filter paper.

2.6 Calculations

Daily feed intake of DM and its constituents (OM, N, NDF, ADF, GE on a DM basis) by individual animals was calculated from the amounts on offer and refusals. Feed intake was calculated for the periods when the animals were in the individual pens, metabolic crates, and respiration chambers. Daily feed intake was expressed both daily (g/d) and as g/kg metabolic LW (g/kg LW0.75).

The average daily liveweight gain (LWG, g/d) was calculated both for the pen period (Days 1–40) and entire experimental period (Days 1–50).

Apparent total tract digestibility (%) of feed DM and its constituents (OM, N, NDF, ADF, GE; DM basis) was calculated from the ingested and excreted amounts in faeces of each component during the balance period (Days 43–47). For the same period, nitrogen partitioning into faeces, urine, and retained was calculated. Partitioning was expressed as % of N intake.

Daily gross energy intake (GEI) during the balance period was calculated using the DMI and GE concentrations. The partitioning of GEI into faeces, urine, and CH_4 was calculated, as well as digestible energy (DE) and metabolisable energy (ME). Digestible energy (DE) was calculated by subtracting faecal energy (FE) from GEI (DE=(GEI-FE)), while ME was calculated by subtracting FE, urine energy (UE) and energy lost in CH_4 from the GEI (ME=GEI-(FE+UE+CH_4E). The methane energy for the balance period was calculated using the CH_4 energy conversion factor (Ym, MJ CH_4/MJ GEI) determined during the chamber period.

Given that each animal rotated throughout the three respiration chambers (one day per chamber), the average daily CH_4 emissions from each animal were calculated by averaging the emissions over the three days. Calculations of the daily emissions in each chamber accounted for the ventilation rate and net concentrations at each data point, gas conditions, and CH_4 recovery rate. Daily CH_4 emissions were expressed on an absolute basis (g/d), CH_4 yield [g/kg DMI, g/kg organic matter (OM) intake, OMI; g/kg digestible DMI (DDMI) and g/kg digestible OMI (DOMI)], and Ym (CH_4 energy/GEI).

2.7 Data analysis

Data were analysed using a linear mixed-effects model in R statistical software (version 4.1.2; R Development Core Team, USA). The data sets were first tested for normality by producing residual vs fitted plots, normal quantile-quantile plots, and density plots of the residuals in R., Visual inspection of the plots was done to confirm the normal distribution of data. The model for each variable was fitted using the "lmerTest" package in R. The treatment was set as the fixed effect, while the sex of the lambs and batch number were set as random effects. ANOVA type 3 was used to test the significance of the fixed factors. The least square means were calculated using the "lsmeans" package and Tukey's built-in "Multicompview" package in R was used to separate the means per treatment. The significance level was set at P<0.05.

3 Results

3.1 Packed cell volume and faecal egg count

The packed cell volumes (PCV) on Day 1 were comparable across treatments (P>0.05), a trend that persisted until Day 21 post-infection (Table 2). However, by Day 50, the PCV of infected, unsupplemented lambs (InHay) was significantly lower than that of lambs in other treatments (P<0.05). At this stage, there was no difference in PCV between Calliandra supplemented lambs, regardless of PEG supplementation (InHay+Cal and InHay+Cal+PEG) (Table 2). The faecal egg counts (FEC) on Day 1 confirmed that all lambs were worm-free (Table 2). By Day 21 post-infection, there was no significant difference in FEC among the infected lambs (P>0.05). Yet, on Day 50, FEC in InHay lambs was significantly higher than in Calliandra-supplemented lambs (P<0.05) (Table 2). Throughout the study, uninfected lambs remained worm-free.

3.2 Feed intake, liveweight and liveweight gain

Experimental treatments significantly influenced feed DMI (g/d and g/kg $LW^{0.75}$) (Table 3). Calliandra-supplemented lambs had a significantly higher DMI than unsupplemented lambs (P<0.05), but PEG supplementation did not alter DMI (P>0.05). Moreover, infection status did not influence feed intake in unsupplemented lambs (Table 3). There was no significant difference in lamb LW across treatments on days 1, 40, and 50 (P>0.05) (Table 3). However, treatments significantly affected LWG during both the pen period (Days 1–40) and the whole experimental period (days 1–50) (Table 3). Notably, Calliandra-supplemented lambs exhibited positive LWG, while unsupplemented lambs experienced weight

TABLE 2 Mean (± standard error of means, sem) packed cell volume (PCV, %) and faecal egg counts (FEC, eggs/g) on Days 1, 21 and 50 of the study for lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
PCV Day 1 (%)	32.8	29.5	33.5	36.6	1.86	0.071
PCV Day 21 (%)	33.8	29.2	30.4	31.0	1.75	0.171
PCV Day 50 (%)	29.7[a]	20.5[b]	25.8[a]	27.1[a]	1.84	0.002
FEC Day 1 (eggs/g)	0	0	0	0	_	_
FEC Day 21 (eggs/g)	0	827	1040	655	300	0.249
FEC Day 50 (eggs/g)	0	8550[a]	1284[b]	3302[b]	1082	<0.001

[a,b]Mean values within a row with different superscript letters differ significantly (P<0·05).

TABLE 3 Mean (± standard error of means, sem) intakes of dry matter (DM) in Rhodes grass hay, Calliandra and total intake (g/d and per unit of metabolic weight, $LW^{0.75}$) over the entire experiment (50 days) by lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
Dry matter intake						
Rhodes grass (g/d)	626[a]	644[a]	439[b]	425[b]	21.3	<0.001
Calliandra (g/d)	0	0	314	307	6.59	<0.001
Total (g/d)	626[a]	644[a]	75[b]	73[b]	24.1	0.001
Total (g/kg $LW^{0.75}$)	51.6[a]	51.7[a]	61.8[b]	61.1[b]	1.09	<0.001
Liveweight						
LW Day 1 (kg)	27.9	28.8	28.1	27.5	1.12	0.843
LW Day 50 (kg)	27.1	27.5	28.4	28.0	1.04	0.807
LWG 1–50 (g/d)	-16.68[a]	-26.60[a]	7.16[b]	11.30[b]	9.65	<0.001

[a,b]Mean values within a row with different superscript letters differ significantly (P<0·05).

loss. Supplementation with PEG did not influence the LWG of Calliandra-supplemented lambs. PEG supplementation had no effect on LWG in Calliandra-supplemented lambs (InHay+Cal +PEG vs. InHay+Cal) (P>0.05). Furthermore, infection status did not affect LWG in unsupplemented lambs (P>0.05) (Table 3).

3.3 Nutrient intakes, apparent feed digestibility, nitrogen and energy partitioning (balance period)

Calliandra-supplemented lambs (InHay+Cal and InHay +Cal+PEG) had higher (P<0.05) intakes of DM, OM, and CP than the unsupplemented lambs (UnHay and InHay) (Table 4). Unsupplemented lambs had higher (P<0.05) NDF intake than Calliandra-supplemented lambs, while there was no significant treatment effect on ADF intake (Table 4). Similarly, PEG supplementation had no effect on DMI or its constituents in Calliandra-supplemented lambs. Infection had no effect on the feed intake of Calliandra-unsupplemented lambs (UnHay and InHay) (Table 4).

The apparent total tract digestibility of DM, OM, CP, NDF, and ADF differed significantly (P<0.05) among the experimental treatments (Table 5). Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG) had similar digestibility. However, lambs not receiving PEG (InHay+Cal) had significantly lower crude protein (CP) digestibility (P<0.05) than those that did receive PEG (InHay+Cal+PEG). While the digestibility of DM, OM, NDF, and ADF was numerically and slightly higher in PEG-supplemented lambs, it wasn't statistically significant (P>0.05) compared to lambs without PEG. Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG) had significantly lower (P<0.05) feed digestibility than the unsupplemented ones (UnHay and InHay). However, there were no discernable differences (P>0.05) in feed digestibility between the uninfected and infected lambs that were solely on a hay diet (UnHay vs. InHay) (Table 5).

The experimental treatments exhibited significant differences (P<0.05) in nitrogen (N) intake and its partitioning (g/d) into faeces

TABLE 4 Mean (± standard error of means, sem) dry matter intake (DMI), organic matter (OMI), crude protein (CPI), neutral detergent fibre (NDFI) and acid detergent fibre (ADFI) by lambs over the balance period fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
Nutrient intake						
DMI (g/d)	571[b]	594[b]	752[a]	724[a]	25.7	<0.001
DMI (g/kg $LW^{0.75}$)	47.2[b]	47.5[b]	61.6[a]	60.4[a]	2.01	<0.001
OMI (g/d)	512[b]	531[b]	689[a]	664[a]	23.1	<0.001
CPI (g/d)	35.3[b]	38.2[b]	91.1[a]	89.0[a]	2.32	<0.001
NDFI (g/d)	413[a]	423[a]	374[b]	356[b]	16.7	0.020
ADFI (g/d)	220	224	210	199	9.06	0.196

[a,b]Mean values within a row with different superscript letters differ significantly (P<0·05).

TABLE 5 Mean (± standard error of means, sem) digestibilities (%) of dry matter (DMD), organic matter (OMD), crude protein (CPD), neutral detergent fibre (NDFD), acid detergent fibre (ADFD) and gross energy (GED) by lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
DMD	52.8^{ab}	53.7^{a}	49.4^{b}	50.7^{ab}	1.04	0.009
OMD	56.8^{ab}	57.6^{a}	52.3^{c}	53.4^{bc}	0.98	<0.001
CPD	42.4^{ab}	44.8^{a}	35.3^{b}	44.1^{a}	2.84	0.043
NDFD	65.3^{a}	66.2^{a}	55.4^{b}	58.7^{b}	1.00	<0.001
ADFD	63.3^{a}	63.8^{a}	49.6^{b}	53.9^{b}	1.24	<0.001

^{a,b,c}Mean values within a row with different superscript letters differ significantly (P<0·05). Digestibilities were measured during the balance period (Days 43–47).

(FN), urine (UN), and retained (RN). Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG) had similar (P>0.05) daily intake and partitioning of N. However, lambs not given PEG had higher (P<0.05) faecal partitioning than those given PEG (InHay+Cal vs. InHay+Cal+PEG) (Table 6). As expected, lambs without Calliandra supplementation (UnHay and InHay) had lower (P<0.05) daily N intake, faecal partitioning, and retained N compared to Calliandra-supplemented lambs (InHay+Cal vs. InHay+Cal+PEG) (Table 6). There was no significant difference (P>0.05) in daily N partitioning into urine between the unsupplemented lambs (UnHay and InHay) and the Calliandra-supplemented lambs without PEG (InHay+Cal). However, lambs given both Calliandra and PEG (InHay+Cal+PEG) had the most pronounced (P<0.05) daily partitioning of N into urine. The infection status did not influence (P>0.05) the daily N intake or partitioning in unsupplemented lambs (UnHay vs. InHay) (Table 6).

The ratio of faecal N to N intake (FN/NI) was the highest (P<0.05) in lambs that received Calliandra supplementation without PEG (InHay+Cal), with no differences (P>0.05) between the other treatments (UnHay vs. InHay vs. InHay+Cal+PEG) (Table 6). In contrast, the ratio of urinary N to N intake (UN/NI) did not differ (P>0.05) between the Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG). However, these lambs had lower (P<0.05) UN/NI ratios than the unsupplemented lambs (UnHay and InHay). Infection status did not impact the FN/NI or UN/NI ratios of unsupplemented lambs (P > 0.05) (Table 6). The retained N to N intake ratio (RN/NI) did not significantly vary (P>0.05) across experimental treatments. However, lambs in the combined Calliandra and PEG treatment (InHay+Cal+PEG) showed a higher RN/NI, particularly when compared to unsupplemented lambs (P=0.12) (Table 6).

Experimental treatments differed in the daily intake of GE (GEI) and its daily partitioning (megajoules/d, MJ/d) into faecal energy (FE), urinary energy (UE), CH_4 energy (CH_4 E), digestible energy (DE), and metabolisable energy (ME). Overall, both GEI and its partitioning were higher (P<0.05) in Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG) than in those without supplementation (UnHay and InHay) (Table 6). PEG supplementation did not affect (P>0.05) the daily GEI or its partitioning in Calliandra-supplemented lambs (InHay+Cal+PEG vs. InHay+Cal). Similarly, the infection status did not affect (P>0.05) the daily GEI and its partitioning in unsupplemented lambs (UnHay and InHay) (Table 6). The ratio of digestible energy (DE) to GE (DE/GEI) differed among the experimental treatments. However, the ME/GEI ratio did not differ (P>0.05) between the experimental treatments (Table 6).

3.4 Enteric methane emission

Daily CH_4 emissions CH_4 yield, and CH_4 conversion factor (Ym) differed significantly among treatments (Table 7). Calliandra-supplemented lambs emitted less CH_4 compared to their unsupplemented counterparts (Table 7). PEG supplementation had no discernible effect on these measures. Infection had no effect (P>0.05) on CH_4 emissions in unsupplemented lambs (Table 7).

3.5 Rumen pH

Rumen pH did not differ significantly (P>0.05) across treatments. The average pH for the respective treatments were: UnHay (7.06), InHay (7.06), InHay+Cal (7.15), and InHay+Cal+PEG (7.12).

4 Discussion

4.1 Effects of *Haemonchus contortus* infection on animal health, feed intake and digestibility, nutrient partitioning, liveweight gain and CH_4 emission

This study found reduced *Haemonchus contortus* infection with Calliandra supplementation. However, there were no effects of infection on feed intake and digestibility, nutrient partitioning, liveweight gain, or CH_4 emission. These findings confirmed previous observations in lambs infected with *H. contortus* (Mwangi et al., 2023). However, Mwangi et al. (2023) found that LW loss by infected lambs was significantly higher and twice as high than the LW loss in uninfected lambs, similarly in the current study, the LW loss in the infected lambs fed solely on hay was 37% higher

10.3389/fanim.2024.1296203

TABLE 6 Mean (± standard error of means, sem) intakes of nitrogen (N) and gross energy (GEI) and their partitioning by lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
Nitrogen (N) partitioning						
N intake (NI, g/d)	5.65^{a}	6.12^{a}	14.58^{b}	14.25^{b}	0.37	<0.001
Faecal N (FN, g/d)	3.16^{a}	3.37^{a}	9.42^{c}	7.95^{b}	0.23	<0.001
Urine N (UN, g/d)	1.69^{b}	1.81^{b}	2.34^{ab}	2.79^{a}	0.23	0.005
FN/NI	0.58^{ab}	0.55^{b}	0.65^{a}	0.56^{b}	0.03	0.043
UN/NI	0.31^{a}	0.30^{a}	0.16^{b}	0.20^{b}	0.03	<0.001
Retained N (RN)						
g/d	0.79^{b}	0.94^{b}	2.75^{a}	3.48^{a}	0.43	<0.001
RN/NI	0.11	0.15	0.19	0.24	0.06	0.122
Energy partitioning						
Intake (GEI, MJ/d)	9.95^{b}	10.31^{b}	14.0^{a}	13.5^{a}	0.45	<0.001
Faecal (FE, MJ/d)	4.61^{b}	4.73^{b}	7.18^{a}	6.75^{a}	0.20	<0.001
Urine (UE, MJ/d)	0.46^{ab}	0.30^{b}	0.55^{a}	0.61^{a}	0.09	0.042
Methane (CH_4 E, MJ/d)	0.83^{ab}	0.89^{a}	0.77^{ab}	0.74^{b}	0.03	0.011
Digestible (DE, MJ/d)	5.34^{b}	5.57^{b}	6.82^{a}	6.76^{a}	0.30	<0.001
Metabolizable (ME, MJ/d)	4.04^{c}	4.36^{bc}	5.53^{a}	5.41^{ab}	0.34	0.003
DE/GEI	0.536^{ab}	0.539^{a}	0.488^{c}	0.500^{cb}	0.01	0.001
MEI/GEI	0.40	0.42	0.39	0.40	0.02	0.461

^{a,b,c}Mean values within a row with different superscript letters differ significantly (P<0·05).
Partitioning was measured during the balance period (Days 43–47).

than that of their uninfected counterparts, however this difference was not statistically significant.

A decrease in PCV and increase in FEC are reliable indicators of GIN infection (Xiang et al., 2021). In this study, parasite infection in the infected treatment groups was successful. Our results also indicate the effects of infection on PCV and FEC were evident from 3-week post-infection.

Haemonchus contortus infection results in the destruction of the abomasal mucosa and increased mucus production (Ramos-Bruno et al., 2021), consequently compromising feed intake and digestibility, nutrient absorption, and feed conversion efficiency (Kyriazakis, 2014; Hoste et al., 2016; Sahoo and Khan, 2016). A recent study conducted by Xiang et al. (2021) concluded that lambs infected with *H. contortus* (10,000 L_3 larvae) had decreased feed

TABLE 7 Mean (± standard error of means, sem) daily methane emission (g/d), methane yield [expressed per kg of intake of dry matter (DMI), organic matter (OMI), digestible DMI (DDMI), digestible organic matter intake (DOMI)] and methane conversion factor (Ym, MJ CH_4/MJ GEI) by lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
g/d	15.1^{ab}	16.2^{a}	13.9^{ab}	13.5^{b}	0.60	0.011
g/kg DMI	25.6^{a}	26.5^{a}	18.7^{b}	18.6^{b}	0.69	<0.001
g/kg OMI	29.0^{a}	29.9^{a}	21.0^{b}	20.9^{b}	0.83	<0.001
g/kg DDMI	46.8^{a}	48.0^{a}	38.2^{b}	36.8^{b}	1.54	<0.001
g/kg DOMI	50.1^{a}	51.0^{a}	41.5^{b}	40.3^{b}	1.82	<0.001
Ym (MJ CH_4/MJ GEI)	0.082^{a}	0.085^{a}	0.057^{b}	0.057^{b}	0.002	<0.001

^{a,b}Mean values within a row with different superscript letters differ significantly (P<0·05).
Methane emission measurements conducted over three days (Days 48–50).

intake and nutrient digestibility (especially of protein and lipids), reduced LWG, disturbed protein absorption, altered plasma amino acid profiles, and changes in gastrointestinal microbial community composition. In contrast, a meta-analysis conducted by Méndez-Ortíz et al. (2019) on the effects of gastrointestinal nematodes on feed intake and LWG by lambs revealed that 41% and 27% of the studies did not observe a negative effect of GIN on feed intake and LWG, respectively. In the present study, however, there was no effect of *H. contortus* infection on feed intake, feed digestibility, nitrogen retention, or diet metabolisability and the rumen pH was not altered. In line with the above, various authors (Sykes and Coop, 1977; Kyriazakis et al., 1996; Hoste et al., 2016) have suggested that the effects of *H. contortus* on feed intake and animal performance are much less pronounced and transient than those of infection by other gastrointestinal nematodes.

It is plausible to assess the nutritional cost of infection in terms of nitrogen and energy metabolism (Retama-Flores et al., 2012; Méndez-Ortíz et al., 2019; Ramos-Bruno et al., 2021). For example, Ramos-Bruno et al. (2021) suggested that the nutritional cost of infection depends on the animal's ability to withstand the parasitic impact (resilience) or the ability to mount an immune response (resistance). In line with the latter, Blackburn et al. (2013) suggested that the host immune response rather than direct parasite damage is the major cause of loss in productivity. In the present study, infection did not affect feed digestibility, N retention, or diet metabolisability. Therefore, there was no significant impact of infection on animal performance. The LW loss by infected and uninfected lambs could be explained by the poor quality of the Rhodes grass hay.

GIN infection in small ruminants substantially alters the anatomy, function, microbial structure, and composition of the rumen and abomasum (Li et al., 2016; El-Ashram et al., 2017; Corrêa et al., 2020, 2021; Xiang et al., 2021), resulting in decreased daily emissions of CH_4 (arising from reduced feed intake) but increased CH_4 yields (emissions per gram of intake). In fact, studies involving parasite-naïve sheep or mixed GIN infection (Fox et al., 2018; Lima et al., 2019; Corrêa et al., 2021; Fernandes et al., 2022) showed depressed feed intake and high CH_4 yields associated with parasite infection. The lambs in this study, sourced from a flock kept on rangelands, had previous exposure to *H. contortus* and other GIN. Thus, the lack of GIN infection effect on feed intake, digestibility, and CH_4 emissions in the current study supports the hypothesis of Mwangi et al. (2023) that prior exposure of lambs to GIN confers them with a degree of resilience to the negative effects of infection on feed intake and digestibility (and consequently CH_4 emission) and animal performance.

4.1.1 Effects of Calliandra supplementation in alleviating *H. contortus* infection

Consumption of tannin-rich forages has been associated with the effects of these compounds on different stages of the GIN life cycle. *In vitro* studies involving condensed tannin (CT) extracts (including those of Calliandra) have reported that CT is effective in inhibiting the egg, larvae, and adult stages of *H. contortus* and disrupts its life cycle (Wabo et al., 2011; Hoste et al., 2012; Mustabi et al., 2019). Similarly, *in vivo* studies (Cresswell, 2007; Manolaraki et al., 2010) have reported decreased egg production when CT-containing forages were fed. For example, Cresswell (2007) fed fresh Calliandra forage (114 g total CT/kg DM) to lambs infected with 3,200 *H. contortus* L_3 larvae, which showed 24–68% lower egg production, compared with control animals. Reduced egg production has been attributed to the reduced fertility of female worms (Manolaraki et al., 2010; Martínez-Ortíz-de-Montellano et al., 2010). Reduced establishment rates of the infective stage of larvae have also been reported when CT-containing diets are fed to goats (Paolini et al., 2003; Brunet et al., 2008). Furthermore, tannin-rich fodder may also disrupt GIN egg development becoming infective L_3 larvae, thereby disrupting the life cycle of nematodes and reducing pasture contamination (Molan et al., 2000; Min et al., 2005). In the present study, the FEC in Calliandra-supplemented lambs was 46–63% lower than that in unsupplemented lambs, confirming the anthelmintic effect of CT in Calliandra.

In the present study, *H. contortus* infected lambs fed only hay had significantly lower PCV than their counterparts supplemented with Calliandra (InHay vs. InHay+Cal). This can be partially attributed to the improved N nutrition provided by Calliandra. Studies involving sheep infected with *H. contortus* and supplementation of a basal diet (low in CP) with higher protein sources (Abbott et al., 1988; Wallace et al., 1996; Bricarello et al., 2005) have reported improved animal health (e.g., higher PCV and total plasma proteins). However, in this study, Calliandra supplementation, which contained a high concentration of CT, likely turned the CP at least partially unavailable for the host. Although the amount of retained N (RN) was higher in supplemented lambs, RN/NI did not differ between the supplemented and unsupplemented lambs (InHay+Cal vs InHay).

4.1.2 Effects of Calliandra supplementation on feed intake and liveweight gain

The total DMI of lambs supplemented with Calliandra was significantly higher (by 17–20%) than that of their unsupplemented counterparts (InHay+Cal vs. InHay or UnHay). However, care must be exercised when interpreting this difference. Unsupplemented lambs ate more of the basal diet (hay) than their Calliandra-supplemented counterparts, that is, the intake of Calliandra effectively substituted the intake of hay. The astringent properties of CT reduce feed intake and animal performance when the CT content in the diet is >50 g/kg DM (Barry and McNabb, 1999; Naumann et al., 2017). In the present study, the concentration of total CT in the InHay+Cal diet was approximately 39 g/kg DMI, with no apparent impact on feed intake. However, literature shows both negative effects of CT on feed intake (and digestibility) at concentrations <50 g CT/kg DM, and no impact at >50 g CT/kg DM. Thus, it is likely (Méndez-Ortiz et al., 2018) that the impact of CT on feed intake depends more on the structure of CT rather than its concentration. The astringency arising from CT-protein complexes is responsible for low palatability; the greater the protein bound by CT, the greater the astringency and the lower the palatability (Naumann et al., 2017). The level of astringency depends on the ability of CT to bind to proteins (McMahon et al.,

2000; Waghorn, 2008; Naumann et al., 2017). A reduced rate of fibre digestion associated with CT activity slows the clearance of feed residues from the rumen, thereby reducing voluntary feed intake (Waghorn, 2008).

The improved total feed intake found in this study confirms reports from other studies where low-quality forages were supplemented with tannin-rich forages (Yisehak et al., 2014; Korir et al., 2016; Gaviria-Uribe et al., 2020). For example, Yisehak et al. (2014) reported improved feed intake by sheep when *Albizia gummifera*, a leguminous fodder tree, was supplemented at 30% of the basal diet consisting of forage from natural mixed species. Korir et al. (2016), in their study with cattle fed on wheat straw, reported an improved feed intake when a straw basal diet in cattle was supplemented with Calliandra dry leaves (20 g/kg LW). The increase in feed intake observed as a result of legume forage supplementation is also in agreement with other studies where ruminants fed on a low-protein diet were supplemented with non-forage protein sources (for example, McGuire et al., 2013). The increase in feed intake when sources of protein are supplemented is attributed to increased ruminal microbial activity and increased rumen turnover (Köster et al., 1996). The higher fibre content of poor-quality diets reduces voluntary feed intake (Minson, 1990; Bosa et al., 2012). Hence, it is likely that the increased intake observed when higher-quality forages are supplemented results from an increased rate of passage (Barahona et al., 1997). In this study, the basal diet contained 73.0 and 5.6% NDF and CP, respectively, whereas the corresponding contents of Calliandra were 17.4 and 21.1%, respectively.

In the present study, Calliandra-supplemented lambs had a higher LWG than their unsupplemented counterparts (InHay+Cal vs. InHay or UnHay), which is consistent with the findings of other studies. For example, Yisehak et al. (2014) reported a LWG of 14 g/d when Bonga lambs were supplemented with *Albizia gummifera*, while their unsupplemented counterparts fed on hay alone gained 3 g/d. In another study, Nherera et al. (1998) reported 44 g/d LWG in goats fed on maize stover and supplemented with Calliandra which was higher than goats supplemented with Leucaena. Similarly, Palmer and Ibrahim (1996) reported that sheep supplemented with Calliandra at inclusion of 27% on DM basis had higher LWG than their counterparts fed on a tropical grass alone (25 vs. -27 g/d). In Kenya, Kinuthia et al. (2007) observed that weaner goats supplemented with 200 g air-dried Calliandra leaves over seven months had higher LWG than those fed Rhodes hay alone (57 vs. 17 g/d). The superior LWG of Calliandra-supplemented lambs observed in the present study could be attributed to improved feed intake and nutrition (increased N retention and metabolisable energy).

4.1.3 Effect of Calliandra supplementation on nutrient intakes, apparent feed digestibility, and nitrogen and energy partitioning

During the balance period, Calliandra-supplemented lambs had significantly higher daily intakes of DM, OM, and CP than their unsupplemented counterparts. The reasons for these higher intakes are discussed in the preceding subsection. The unchanged intake of NDF and ADF with supplementation can be attributed to the low level of fiber in the Calliandra. In this study, the Calliandra consisted not of whole leaves, but of their primary leaflets, which had low NDF content (Table 1).

In this study, lambs supplemented with Calliandra had 9% lower OM digestibility than their unsupplemented counterparts (InHay+Cal vs. InHay). However, the decrease in CP and ADF digestibilities were much higher (22%). The latter is in close agreement with the findings of Tiemann et al. (2008), in which lambs fed on a tropical grass and supplemented with Calliandra (CT=175 g/kg DM) at 30% of the daily diet (DM basis) had depression in CP and ADF digestibilities of the order of 38%, compared to those receiving no supplement. Similarly, Fassler and Lascano Aguilar (1995) and Hove et al. (2001) reported a much greater effect on ADF digestion than NDF digestion when CT-rich tropical legumes were supplemented to small ruminants.

In the current study, the only measured aspect of CT was its concentration, and the estimated total concentration of CT in the diet of supplemented lambs (InHay+Cal) was ~39 g/kg DM, which is far below the suggested limit of tolerance of 50 g/kg DM) (Barry & McNabb, 1999). However, as stated earlier, the anti-nutritional effects of CT cannot be ascribed to its concentration alone (Waghorn, 2008; Naumann et al., 2017; Besharati et al., 2022). Waghorn (2008) concluded that the effects of CT on ruminal proteolysis, crude protein digestibility, OM digestibility, and N balance are well understood, and that the degree to which CT interferes with these processes is a function of the astringency, concentration, and chemical and physical properties of CT. The negative effects of CT on CP digestibility and, to a minor degree, on fibre digestibility are common features reported in the literature (e.g., Jayanegara et al., 2012; Cabral Filho et al., 2013; Méndez-Ortiz et al., 2021). Besharati et al. (2022) concluded that CT mainly affects CP digestibility by creating hydrogen bonds that are stable in the pH range of 3.5–8.0, whereas Naumann et al. (2017) concluded that at a high tannin: protein ratio, the protein becomes coated with CT, leading to insoluble precipitates, which is especially true in CT-rich Calliandra (Rira et al., 2022). High concentrations of dietary CT increase dietary faecal nitrogen excretion; however, tannin consumption may also increase endogenous N loss (Besharati et al., 2022). Rira et al. (2022) reported that tannins interfere with the microbial colonisation of plant material, especially by fibrolytic bacteria, thereby affecting fibre digestion. McMahon et al. (2000) from a review of *in vitro* studies concluded that CT could inhibit cellulose digesting ruminal bacteria and fungi. Furthermore, the strong chelating properties of CT may reduce the availability of metal ions necessary for microbial metabolism (McMahon et al., 2000).

Compared to unsupplemented lambs (InHay+Cal vs. InHay), Calliandra supplementation significantly increased faecal N partitioning (FN/NI) and reduced urinary N (UN/NI), whereas retained N (RN/NI) remained unaltered. This is in close agreement with previous reports (McSweeney et al., 2001; Tiemann et al., 2008; Besharati et al., 2022). For example, Tiemann et al. (2008) with sheep supplemented with Calliandra, found increased FN/NI and lowered UN/NI, whereas RN/NI was

negative. Similar findings were also observed when *Gymnopodium floribundum*, a Polygonaceae plant species, was supplemented to sheep in a diet providing 86 g CT/kg DM (Méndez-Ortiz et al., 2021). The increased faecal N partitioning is in line with the depressed digestibility of CP, whereas decreased urinary N excretion likely reflects the effect of CT on ruminal proteolysis by complexing proteins, making them less available to proteolytic microorganisms (Mueller-Harvey, 2006). The latter suggests that CT may have an additional role in reducing urinary sources of NH_3 and N_2O (Śliwiński et al., 2004; Waghorn, 2008). In the present study, despite the higher N loss in faeces, the retained N (g/d) of Calliandra-supplemented lambs was significantly higher than that of the unsupplemented lambs.

In this study, Calliandra-supplemented lambs had higher daily energy intake (GEI), daily faecal energy (FE), and urinary energy (UE) outputs than their unsupplemented counterparts (InHay+Cal vs. InHay). The DE/GEI ratio was lower for the supplemented lambs, whereas the ME/GEI ratio did not differ between these groups of lambs. The former indicates the effects of compromised feed digestibility discussed earlier. Our findings are in partial agreement with those of Tiemann et al. (2008), who observed a 12% decrease in DE/GE and ME/GE ratios in Calliandra-supplemented lambs compared with the control (grass only). Similarly, Méndez-Ortiz et al. (2021) reported depressed DE/GE (by 26%) and ME/GE (by 29%) ratios in lambs supplemented with *Gymnopodium floribundum* compared to unsupplemented lambs. The differences between these studies may be due to the nature and concentration of CT (Waghorn, 2008). In our study, the digestible (DE) and metabolisable (ME) energies available for supplemented lambs were higher than those for their unsupplemented counterparts.

4.1.4 Effect of Calliandra supplementation on enteric methane emission

In this study, daily CH_4 emissions (g/d), CH_4 yield (per unit of intake), and Ym were decreased by Calliandra supplementation (InHay+Cal vs. InHay). Our findings confirm those from other *in vitro* and *in vivo* studies that reported significant reductions in CH_4 emissions when CT-containing forages were fed (Pinares-Patiño et al., 2003; Puchala et al., 2005; Tiemann et al., 2008; Jayanegara et al., 2012; Rira et al., 2022). Forages rich in CT can reduce daily CH_4 emissions. For example, Pinares-Patiño et al. (2003) and Piñeiro-Vázquez et al. (2018), working with sheep and heifers, respectively, reported 62–65% reduction in CH_4 yield (emissions per unit of feed intake) with diets comprising >80% CT-rich legumes. Tiemann et al. (2008) reported a 17% decrease when lambs were supplemented with Calliandra at a rate of 30% daily DMI. The negative effects of CT on CH_4 emissions can be associated with the reduced digestion of CP and fibre, hence reducing the availability of reducing equivalents for methanogenesis, as well as direct anti-methanogenic action (McMahon et al., 2000; Tavendale et al., 2005; Rira et al., 2022). In fact, Rira et al. (2022), from *in situ* studies, reported that archaea diversity was reduced in high CT-containing *Calliandra calothyrsus*, and concurrently, *in vitro* studies revealed that CH_4 production was low. Furthermore, Kelln et al. (2020) indicated that CT (Catechins) act as H_2 sinks, reducing the availability of H_2 to archaea.

4.3 Effect of PEG treatment in reducing condensed tannins activity in Calliandra supplemented lambs

Polyethylene glycol (PEG) is a non-ionic detergent with high affinity for CT (Bento et al., 2005). Because of PEG's ability to bind CT, the addition of PEG to diets can reduce the effect of CT and has led to its use in testing the effects of CT on feed intake, rumen function, and metabolism of N and energy (de Frutos Fernández et al., 2004), and methane emissions (Wang et al., 2018). The ability of PEG to bind CT has been suggested to improve the feeding value of CT-rich plant species, especially in the tropics (Waghorn, 2008). In the current study, the PEG dose was calculated based on a preliminary analysis of the CT concentration in the Calliandra forage material collected prior to collection of the actual experimental Calliandra forage, which showed a total CT of 28.2 g/kg DM. However, analysis of samples of the Calliandra forage used in the experiment (carried out at the end of the study) in a different laboratory yielded a higher concentration of total CT (92.1 g/kg DM). The reason for this large difference in the analytical results may be due to differences in CT concentration between the samples or due to the different analytical procedures used. Studies in Sub-Saharan Africa have reported total CT concentrations in Calliandra in the range of 10–200 g/kg DM (Dzowela et al., 1995; Hove et al., 2001). The large range can be attributed to various factors, including the environment where the sample was collected, sample drying, and method of analysis (Wolfe et al., 2008). Based on the lack of differences in the effects of InHay+Cal and InHay+Cal+PEG treatments on infection response, DMI, LWG, feed digestibility, N and gross energy partitioning, and CH_4 emission, we are confident that the initial analyses of CT concentrations underestimated the real concentrations, resulting in undercalculation of PEG dosage required to neutralize CT activities. Other studies consider Calliandra to be a high CT-rich legume (Perez-Maldonado and Norton, 1996; Rakhmani et al., 2005; Cresswell, 2007; Tiemann et al., 2008; Rira et al., 2022).

In this study, underestimating CT concentration in Calliandra led to a lower PEG dosage in the InHay+Cal+PEG treatment. The intended ratio of PEG: CT was 1.1, while an actual PEG : CT ratio of 1:2.8 was achieved. Consequently, the PEG level used was insufficient to fully counteract the CT properties. In effect, the mean values of PCV and FEC, feed intake, LWG, nutrient intake in feed, feed digestibility, N partitioning to urine and retained N, energy partitioning, and CH_4 emission did not differ between Calliandra-supplemented treatments with and without PEG supplementation (InHay+Cal vs. InHay+Cal+PEG). On the other hand, CP digestibility and partitioning of N intake to faecal N were higher and lower, respectively, in PEG-supplemented lambs than in lambs receiving no PEG supplement (InHay+Cal+PEG vs. InHay +Cal). However, the extent to which PEG supplementation reduced the protein-binding property of CT remains uncertain.

5 Conclusion and recommendations

In the present study, supplementation of a poor-quality basal diet with Calliandra resulted in reduced GIN infection, improved feed intake, improved liveweight gain and reduced CH_4 emission from sheep. However, these effects were partially offset by reduced crude protein and fibre digestibility. Small-holder livestock keepers in SSA may not be able to achieve high (40%) supplementation levels of forage from fodder trees because of competing interests from other food crops. Hence, lower supplementation levels may be optimal and more achievable in local farming situations. Calliandra crops, when well-managed, could potentially provide year-round green forage that helps control GIT parasite infection, improves the feeding value of the basal diet, hence lowering the cost of animal production. Furthermore, incorporating Calliandra can lead to a notable reduction in enteric CH_4 emissions, both in absolute (g/animal/day) and yield (g/kg DMI) basis.

Data availability statement

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

Ethics statement

The animal study was approved by International Livestock Research Institute's Institutional Animal Care and Use Committee (IACUC, Reference number 2022-03). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

PM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. RE: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. IG: Conceptualization, Methodology, Supervision, Writing – review & editing. LM: Funding acquisition, Writing – review & editing. DM: Investigation, Writing – review & editing. JG: Investigation, Writing – review & editing, Data curation. SM: Conceptualization, Methodology, Supervision, Writing – review & editing. CP: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The study was funded by the International Development Research Centre (IDRC) -Canada, Project 109211 -Livestock Keeping in a Changing Climate. Additional financial support for CGIAR authors came through the CGIAR Research Initiatives, 'Livestock and Climate' and 'Mitigate+: Low-Emission Food Systems' which are supported by contributions to the CGIAR Trust Fund (https://www.cgiar.org/funders/). Lutz Merbold acknowledges funding received from the European Union's Horizon Europe Programme (grant agreement number 101058525) for the project "Knowledge and climate services from an African observation and Data Research Infrastructure (KADI)."

Acknowledgments

The authors acknowledge the Tick Unit, Animal and Human health, ILRI for facilitating faecal egg counts and packed cell volume examination in their facility. We also thank CLINGLOBAL South Africa for providing the *H. contortus* L_3 used for infection of experimental animals. We appreciate Erick Kiprotich and Nelson Saya for assisting with animal handling and care, sample collection and preparation, and respiration chamber management. We also appreciate Dr. Alice Onyango, Rodgers Rogito, Francis Njenga, and Collins Oduor for carrying out chemical analysis of the samples. The authors are thankful to Jane Poole and Nicholas Ndiwa for their guidance on experimental design and statistical analyses. We also acknowledge Professor Graham Hepworth (The University of Melbourne) for his guidance on the experimental design and statistical analysis. The authors also acknowledge Silvia Ampuero (Agroscope, Zurich, Switzerland) for the preliminary analyses of condensed tannins in Calliandra and Nichole Cherry, James P. Muir and Luis O. Tedeshi (Texas A&M AgriLife Research, TX) for the final analyses of condensed tannins in Calliandra. We sincerely appreciate Daniel Korir for offering data management and analysis support to the experimental team.

Conflict of interest

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

Publisher's note

References

Śliwiński, B. J., Kreuzer, M., Sutter, F., Machmüller, A., and Wettstein, H. R. (2004). Performance, body nitrogen conversion and nitrogen emission from manure of dairy cows fed diets supplemented with different plant extracts. *J. Anim. Feed Sci.* 13, 73–91. doi: 10.22358/jafs/67390/2004

Abbott, E. M., Parkins, J. J., and Holmes, P. H. (1988). Influence of dietary protein on the pathophysiology of haemonchosis in lambs given continuous infections. *Res. Veterinary Sci.* 45, 41–49. doi: 10.1016/S0034-5288(18)30892-0

AOAC (1990). Official methods of analysis of the Association of Official Analytical Chemists *(Issue BOOK)* (Washington DC: Association of official analytical chemists).

Armson, B., Ekiri, A. B., Alafiatayo, R., and Cook, A. J. (2021). Small ruminant production in Tanzania, Uganda, and Ethiopia: A systematic review of constraints and potential solutions. *Veterinary Sci.* 8, 1–13. doi: 10.3390/vetsci8010005

Baker, R. L., and Rege, J. E. O. (1994). "Genetic resistance to diseases and other stresses in improvement of ruminant livestock in the tropics," in *7th world congress on genetics applied to livestock production* (Guelph Canada: International Committee for World Congresses on Genetics Applied to Livestock Production).

Barahona, R., Lascano, C. E., Cochran, R., Morrill, J., and Titgemeyer, E. C. (1997). Intake, digestion, and nitrogen utilization by sheep fed tropical legumes with contrasting tannin concentration and astringency. *J. Anim. Sci.* 75, 1633–1640. doi: 10.2527/1997.7561633x

Barry, T. N., and McNabb, W. C. (1999). The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *Br. J. Nutr.* 81, 263–272. doi: 10.1017/S0007114599000501

Bento, M. H. L., Acamovic, T., and Makkar, H. P. S. (2005). The influence of tannin, pectin and polyethylene glycol on attachment of 15N-labelled rumen microorganisms to cellulose. *Anim. Feed Sci. Technol.* 122, 41–57. doi: 10.1016/j.anifeedsci.2005.04.010

Berça, A. S., Tedeschi, L. O., da Silva Cardoso, A., and Reis, R. A. (2023). Meta-analysis of the relationship between dietary condensed tannins and methane emissions by cattle. *Anim. Feed Sci. Technol.* 298, 115564. doi: 10.1016/j.anifeedsci.2022.115564

Besharati, M., Maggiolino, A., Palangi, V., Kaya, A., Jabbar, M., Eseceli, H., et al. (2022). Tannin in ruminant nutrition. *Molecules* 27, 8273. doi: 10.3390/molecules27238273

Bhatta, R., Enishi, O., Yabumoto, Y., Nonaka, I., Takusari, N., Higuchi, K., et al. (2013). Methane reduction and energy partitioning in goats fed two concentrations of tannin from Mimosa spp. *J. Agric. Sci.* 151, 119–128. doi: 10.1017/S0021859612000299

Blackburn, P. J., Carmichael, I. H., Walkden-Brown, S. W., and Greenslade, S. (2013). "Cost of immune response to Trichostrongylus vitrinus infection in meat sheep," in *Proceedings of the Australian Sheep Veterinarians 2013 Conference*, Albany (Sydney, Australia: Sheep Veterinary Association). 41–46.

Bosa, R., Faturi, C., Vasconcelos, H. G. R., Cardoso, A. M., Ramos, A. F. O., and de Azevedo, J. C. (2012). Intake and apparent digestibility with different inclusion levels of coconut meal for sheep feeding/Consumo e digestibilidade aparente de dietas com diferentes niveis de inclusao de torta de coco para alimentacao de ovinos. *Acta Scientiarum. Anim. Sci.* 34, 57–63. doi: 10.4025/actascianimsci.v34i1.11936

Bricarello, P. A., Amarante, A. F. T., Rocha, R. A., Cabral Filho, S. L., Huntley, J. F., Houdijk, J. G. M., et al. (2005). Influence of dietary protein supply on resistance to experimental infections with Haemonchus contortus in Ile de France and Santa Ines lambs. *Veterinary Parasitol.* 134, 99–109. doi: 10.1016/j.vetpar.2005.05.068

Brunet, S., de Montellano, C., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., Aguilar-Caballero, A. J., Capetillo-Leal, C., et al. (2008). Effect of the consumption of Lysiloma latisiliquum on the larval establishment of gastrointestinal nematodes in goats. *VETERINARY Parasitol.* 157, 81–88. doi: 10.1016/j.vetpar.2008.07.013

Cabral Filho, S. L. S., Abdalla, A. L., Bueno, I. C., da, S., Gobbo, S. P., and Oliveira, A. A. M. (2013). Effect of sorghum tannins in sheep fed with high-concentrate diets. *Arquivo Brasileiro Medicina Veterinária e Zootecnia* 65, 1759–1766. doi: 10.1590/S0102-09352013000600025

Chaokaur, A., Nishida, T., Phaowphaisal, I., and Sommart, K. (2015). Effects of feeding level on methane emissions and energy utilization of Brahman cattle in the tropics. *Agricul. Ecosyst. Environ.* 199, 225–230. doi: 10.1016/j.agee.2014.09.014

Corrêa, P. S., Mendes, L. W., Lemos, L. N., Crouzoulon, P., Niderkorn, V., Hoste, H., et al. (2020). Tannin supplementation modulates the composition and function of ruminal microbiome in lambs infected with gastrointestinal nematodes. *FEMS Microbiol. Ecol.* 96, fiaa024. doi: 10.1093/femsec/fiaa024

Corrêa, P. S., Mendes, L. W., Lemos, L. N., Sampaio, A. C. K., Issakowicz, J., McManus, C. M., et al. (2021). The effect of Haemonchus contortus and Trichostrongylus colubriforms infection on the ruminal microbiome of lambs. *Exp. Parasitol.* 231, 108175. doi: 10.1016/j.exppara.2021.108175

Cresswell, K. J. (2007). Anthelmintic effects of tropical shrub legumes in ruminant animals (Australia: James Cook University). Available at: https://doi.org/http://eprints.jcu.edu.au/2086.

de Frutos Fernández, P., Mantecón, Á.R., Angulo, G. H., and García, F. J. G. (2004). Tannins and ruminant nutrition. *Spanish J. Agric. Res.* 2, 191–202. doi: 10.5424/sjar/2004022-73

Dzowela, B. H., Hove, L., Topps, J. H., and Mafongoya, P. L. (1995). Nutritional and anti-nutritional characters and rumen degradability of dry matter and nitrogen for some multipurpose tree species with potential for agroforestry in Zimbabwe. *Anim. Feed Sci. Technol.* 55, 207–214. doi: 10.1016/0377-8401(95)00803-U

El-Ashram, S., Al Nasr, I., Abouhajer, F., El-Kemary, M., Huang, G., Dinçel, G., et al. (2017). Microbial community and ovine host response varies with early and late stages of Haemonchus contortus infection. *Veterinary Res. Commun.* 41, 263–277. doi: 10.1007/s11259-017-9698-5

FAO (2018). "Food and agriculture organisation of the united nations, faostat. Stat," in *Database*, vol. 16. (Rome, Italy: FAO). Available at: http://www.fao.org/faostat/en/#data/QC.

Fassler, O. M., and Lascano Aguilar, C. E. (1995). The effect of mixtures of sun-dried tropical shrub legumes on intake and nitrogen balance by sheep. *Trop. Grasslands.* 29, 92–96. Available at: https://hdl.handle.net/10568/44164.

Fernandes, M. A., Lima, P., de, M. T., do Amarante, A. F. T., Abdalla, A. L., and Louvandini, H. (2022). Hematological, biochemical alterations and methane production in sheep submitted to mixed infection of Haemonchus contortus and Trichostrongylus colubriformis. *Small Ruminant Res.* 216, 106798. doi: 10.1016/j.smallrumres.2022.106798

Fox, N. J., Smith, L. A., Houdijk, J. G. M., Athanasiadou, S., and Hutchings, M. R. (2018). Ubiquitous parasites drive a 33% increase in methane yield from livestock. *Int. J. Parasitol.* 48, 1017–1021. doi: 10.1016/j.ijpara.2018.06.001

Gaviria-Uribe, X., Bolivar, D. M., Rosenstock, T. S., Molina-Botero, I. C., Chirinda, N., Barahona, R., et al. (2020). Nutritional quality, voluntary intake and enteric methane emissions of diets based on novel cayman grass and its associations with two leucaena shrub legumes. *Front. Veterinary Sci.* 7 (October), 1–12. doi: 10.3389/fvets.2020.579189

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., et al. (2013). *Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities* (Rome: Food and Agriculture Organization of the United Nations (FAO).

Goopy, J. P., Korir, D., Pelster, D., Ali, A. I. M., Wassie, S. E., Schlecht, E., et al. (2020). Severe below-maintenance feed intake increases methane yield from enteric fermentation in cattle. *Br. J. Nutr.* 123, 1239–1246. doi: 10.1017/S0007114519003350

Greiffer, L., Liebau, E., Herrmann, F. C., and Spiegler, V. (2022). Condensed tannins act as anthelmintics by increasing the rigidity of the nematode cuticle. *Sci. Rep.* 12, 18850. doi: 10.1038/s41598-022-23566-2

Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., et al. (2012). Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. *Veterinary Parasitol.* 186, 18–27. doi: 10.1016/j.vetpar.2011.11.042

Hoste, H., Torres-Acosta, J. F. J., Quijada, J., Chan-Perez, I., Dakheel, M. M., Kommuru, D. S., et al. (2016). Interactions between nutrition and infections with Haemonchus contortus and related gastrointestinal nematodes in small ruminants. *Adv. Parasitol.* 93, 239–351. doi: 10.1016/bs.apar.2016.02.025

Hove, L., Topps, J. H., Sibanda, S., and Ndlovu, L. R. (2001). Nutrient intake and utilisation by goats fed dried leaves of the shrub legumes Acacia angustissima, Calliandra calothyrsus and Leucaena leucocephala as supplements to native pasture hay. *Anim. Feed Sci. Technol.* 91, 95–106. doi: 10.1016/S0377-8401(01)00233-4

Jayanegara, A., Leiber, F., and Kreuzer, M. (2012). Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from in *vivo* and in *vitro* experiments. *J. Anim. Physiol. Anim. Nutr.* 96, 365–375. doi: 10.1111/j.1439-0396.2011.01172.x

Kaitho, R. J., Umunna, N. N., Nsahlai, I. V., Tamminga, S., Van Bruchem, J., Hanson, J., et al. (1996). Palatability of multipurpose tree species: effect of species and length of study on intake and relative palatability by sheep. *Agroforestry Systems.* 33, 249–261. doi: 10.1007/BF00055426

Kelln, B. M., Penner, G. B., Acharya, S. N., McAllister, T. A., and Lardner, H. A. (2020). Impact of condensed tannin-containing legumes on ruminal fermentation, nutrition, and performance in ruminants: a review. *Can. J. Anim. Sci.* 101, 210–223. doi: 10.1139/cjas-2020-0096

Kinuthia, M. N., Wanyoike, M. M., Gachuiri, C. K., and Wakhungu, J. W. (2007). Effect of supplementing weaner goats with graded levels of Calliandra calothyrsus and Lucerne (Medicago sativa) on feed intake and weight gain. *Livestock Res. Rural Dev.* 19, 1–9.

Korir, D., Goopy, J. P., Gachuiri, C., and Butterbach-Bahl, K. (2016). Supplementation with Calliandra calothyrsus improves nitrogen retention in cattle fed low-protein diets. *Anim. Production Sci.* 56, 619–626. doi: 10.1071/AN15569

Kosgey, I. S., Rowlands, G. J., van Arendonk, J. A. M., and Baker, R. L. (2008). Small ruminant production in smallholder and pastoral/extensive farming systems in Kenya. *Small Ruminant Res.* 77, 11–24. doi: 10.1016/j.smallrumres.2008.02.005

Köster, H. H., Cochran, R. C., Titgemeyer, E. C., Vanzant, E. S., Abdelgadir, I., and St-Jean, G. (1996). Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows. *J. Anim. Sci.* 74, 2473–2481. doi: 10.2527/1996.74102473x

Kurihara, M., Magner, T., Hunter, R. A., and McCrabb, G. J. (1999). Methane production and energy partition of cattle in the tropics. *Br. J. Nutr.* 81, 227–234. doi: 10.1017/S0007114599000422

Kyriazakis, I. (2014). Pathogen-induced anorexia: a herbivore strategy or an unavoidable consequence of infection? *Anim. Production Sci.* 54, 1190–1197. doi: 10.1071/AN14431

Kyriazakis, I., Anderson, D. H., Oldham, J. D., Coop, R. L., and Jackson, F. (1996). Long-term subclinical infection with Trichostrongylus colubriformis: effects on food intake, diet selection and performance of growing lambs. *Veterinary Parasitol.* 61, 297–313. doi: 10.1016/0304-4017(95)00824-1

Li, R. W., Li, W., Sun, J., Yu, P., Baldwin, R. L., and Urban, J. F. (2016). The effect of helminth infection on the microbial composition and structure of the caprine abomasal microbiome. *Sci. Rep.* 6, 1–10. doi: 10.1038/srep20606

Lima, P., de, M. T., Crouzoulon, P., Sanches, T. P., Zabré, G., Kabore, A., et al. (2019). Effects of Acacia mearnsii supplementation on nutrition, parasitological, blood parameters and methane emissions in Santa Inês sheep infected with Trichostrongylus colubriformis and Haemonchus contortus. *Exp. Parasitol.* 207, 107777. doi: 10.1016/j.exppara.2019.107777

Manolaraki, F., Sotiraki, S., Stefanakis, A., Skampardonis, V., Volanis, M., and Hoste, H. (2010). Anthelmintic activity of some Mediterranean browse plants against parasitic nematodes. *Parasitology* 137, 685–696. doi: 10.1017/S0031182009991399

Martínez-Ortíz-de-Montellano, C., Vargas-Magaña, J. J., Canul-Ku, H. L., Miranda-Soberanis, R., Capetillo-Leal, C., Sandoval-Castro, C. A., et al. (2010). Effect of a tropical tannin-rich plant Lysiloma latisiliquum on adult populations of Haemonchus contortus in sheep. *Veterinary Parasitol.* 172, 283–290. doi: 10.1016/j.vetpar.2010.04.040

McGuire, D. L., Bohnert, D. W., Schauer, C. S., Falck, S. J., and Cooke, R. F. (2013). Daily and alternate day supplementation of urea or soybean meal to ruminants consuming low-quality cool-season forage: I—Effects on efficiency of nitrogen use and nutrient digestion. *Livestock Sci.* 155, 205–213. doi: 10.1016/j.livsci.2013.05.015

McMahon, L. R., McAllister, T. A., Berg, B. P., Majak, W., Acharya, S. N., Popp, J. D., et al. (2000). A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. *Can. J. Plant Sci.* 80, 469–485. doi: 10.4141/P99-050

McSweeney, C. S., Palmer, B., McNeill, D. M., and Krause, D. O. (2001). Microbial interactions with tannins: nutritional consequences for ruminants. *Anim. Feed Sci. Technol.* 91, 83–93. doi: 10.1016/S0377-8401(01)00232-2

Méndez-Ortiz, F. A., Sandoval-Castro, C. A., Sarmiento-Franco, L. A., Ventura-Cordero, J., González-Pech, P. G., Vargas-Magaña, J. J., et al. (2021). Impact of dietary condensed tannins and haemonchus contortus infection in growing sheep: effects on nutrient intake, digestibility, and the retention of energy and nitrogen. *Acta Parasitologica*. 67 (1), 196–206. doi: 10.1007/s11686-021-00441-0

Méndez-Ortíz, F. A., Sandoval-Castro, C. A., Vargas-Magaña, J. J., Sarmiento-Franco, L., Torres-Acosta, J. F. J., and Ventura-Cordero, J. (2019). Impact of gastrointestinal parasitism on dry matter intake and live weight gain of lambs: A meta-analysis to estimate the metabolic cost of gastrointestinal nematodes. *Veterinary Parasitol.* 265, 1–6. doi: 10.1016/j.vetpar.2018.11.008

Méndez-Ortiz, F. A., Sandoval-Castro, C. A., Ventura-Cordero, J., Sarmiento-Franco, L. A., and Torres-Acosta, J. F. J. (2018). Condensed tannin intake and sheep performance: A meta-analysis on voluntary intake and live weight change. *Anim. Feed Sci. Technol.* 245, 67–76. doi: 10.1016/j.anifeedsci.2018.09.001

Min, B. R., Hart, S. P., Miller, D., Tomita, G. M., Loetz, E., and Sahlu, T. (2005). The effect of grazing forage containing condensed tannins on gastro-intestinal parasite infection and milk composition in Angora does. *VETERINARY Parasitol.* 130, 105–113. doi: 10.1016/j.vetpar.2005.03.011

Minson, D. J. (1990). *Intake of grazed forage In 'Forage in ruminant nutrition'*. (Ed. D. J. Minson) pp. 60–84. London, Uk: Academic Press Inc.–Harcourt Brace Jovanovich Publishers.

Molan, A. L., Waghorn, G. C., Min, B. R., and McNabb, W. C. (2000). The effect of condensed tannins from seven herbages on Trichostrongylus colubriformis larval migration in *vitro*. *Folia Parasitologica* 47, 39–44. doi: 10.14411/fp.2000.007

Mueller-Harvey, I. (2006). Unravelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agriculture*. 86 (13), 2010–2037. doi: 10.1002/jsfa.2577

Mustabi, J., Prahesti, K. I., and Nurpaidah. (2019). Efficacy of calliandra (Calliandra calothyrsus) leaf extract on Haemonchus contortus mortality in vitro. *IOP Conference Series: Earth and Environmental Science*. 343 (1). doi: 10.1088/1755-1315/343/1/012032

Mwangi, P. M., Eckard, R., Gluecks, I., Merbold, L., Mulat, D. G., Gakige, J., et al. (2023). "Impact of Haemonchus contortus infection on feed intake, digestion, liveweight gain, and enteric methane emission from Red Maasai and Dorper sheep," in *Frontiers in animal science*, vol. Vol. 4. . doi: 10.3389/fanim.2023.1212194

Naumann, H. D., Tedeschi, L. O., Zeller, W. E., and Huntley, N. F. (2017). The role of condensed tannins in ruminant animal production: advances, limitations and future directions. *Rev. Bras. Zootecnia* 46, 929–949. doi: 10.1590/s1806-92902017001200009

Nherera, F. V., Ndlovu, L. R., and Dzowela, B. H. (1998). Utilisation of Leucaena diversifolia, Leucaena esculenta, Leucaena pallida and Calliandra calothyrsus as nitrogen supplements for growing goats fed maize stover. *Anim. Feed Sci. Technol.* 74, 15–28. doi: 10.1016/S0377-8401(98)00164-3

Oliveira, L. M. B., Macedo, I. T. F., Vieira, L. S., Camurça-Vasconcelos, A. L. F., Tomé, A. R., Sampaio, R. A., et al. (2013). Effects of Mimosa tenuiflora on larval establishment of Haemonchus contortus in sheep. *Veterinary Parasitol.* 196, 341–346. doi: 10.1016/j.vetpar.2013.04.014

Palmer, B., and Ibrahim, T. M. (1996). "Callandra calothyrsus forage for the tropics-a current assessment," in *International workshop on the genus calliandra* (Bogor, Indonesia: Winrock International). Available at: http://hdl.handle.net/102.100.100/227088?index=1.

Paolini, V., Bergeaud, J.-P., Grisez, C., Prevot, F., Dorchies, P., and Hoste, H. (2003). Effects of condensed tannins on goats experimentally infected with Haemonchus contortus. *Veterinary Parasitol.* 113, 253–261. doi: 10.1016/S0304-4017(03)00064-5

Perez-Maldonado, R. A., and Norton, B. W. (1996). The effects of condensed tannins from Desmodium intortum and Calliandra calothyrsus on protein and carbohydrate digestion in sheep and goats. *Br. J. Nutr.* 76, 515–533. doi: 10.1079/BJN19960060

Pinares-Patiño, C. S., Ulyatt, M. J., Waghorn, G. C., Lassey, K. R., Barry, T. N., Holmes, C. W., et al. (2003). Methane emission by alpaca and sheep fed on lucerne hay or grazed on pastures of perennial ryegrass/white clover or birdsfoot trefoil. *J. Agric. Sci.* 140, 215–226. doi: 10.1017/S002185960300306X

Piñeiro-Vázquez, A. T., Canul-Solis, J. R., Jiménez-Ferrer, G. O., Alayón-Gamboa, J. A., Chay-Canul, A. J., Ayala-Burgos, A. J., et al. (2018). Effect of condensed tannins from Leucaena leucocephala on rumen fermentation, methane production and population of rumen protozoa in heifers fed low-quality forage. *Asian-Australasian J. Anim. Sci.* 31, 1738. doi: 10.5713/ajas.17.0192

Puchala, R., Min, B. R., Goetsch, A. L., and Sahlu, T. (2005). The effect of a condensed tannin-containing forage on methane emission by goats. *J. Anim. Sci.* 83, 182–186. doi: 10.2527/2005.831182x

Rahimi, J., Mutua, J. Y., Notenbaert, A. M. O., Marshall, K., and Butterbach-Bahl, K. (2021). Heat stress will detrimentally impact future livestock production in East Africa. *Nat. Food* 2, 88–96. doi: 10.1038/s43016-021-00226-8

Rakhmani, S., Brooker, J. D., Jones, G. P., and Palmer, B. (2005). Composition of condensed tannins from Calliandra calothyrsus and correlation with in sacco digestibility. *Anim. Feed Sci. Technol.* 121, 109–124. doi: 10.1016/j.anifeedsci.2005.02.010

Ramos-Bruno, E., Sandoval-Castro, C. A., Torres-Acosta, J. F. J., Sarmiento-Franco, L. A., Torres-Fajardo, R., Chan-Pérez, J. I., et al. (2021). Nitrogen retention in hair sheep lambs with a gradient of Haemonchus contortus infection. *Veterinary Parasitol.* 296, 109488. doi: 10.1016/j.vetpar.2021.109488

Retama-Flores, C., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., Aguilar-Caballero, A. J., Cámara-Sarmiento, R., and Canul-Ku, H. L. (2012). Maize supplementation of Pelibuey sheep in a silvopastoral system: fodder selection, nutrient intake and resilience against gastrointestinal nematodes. *Animal* 6, 145–153. doi: 10.1017/S1751731111001339

Rira, M., Morgavi, D. P., Popova, M., Maxin, G., and Doreau, M. (2022). Microbial colonisation of tannin-rich tropical plants: Interplay between degradability, methane production and tannin disappearance in the rumen. *Animal* 16, 100589. doi: 10.1016/j.animal.2022.100589

Sahoo, A., and Khan, F. (2016). Nutritional and biological control synergism against gastrointestinal nematodes in small ruminants. *J. Vet. Sci. Anim. Husb* 4, 104. doi: 10.15744/2348-9790.4.104

Sykes, A. R., and Coop, R. L. (1977). Intake and utilization of food by growing sheep with abomasal damage caused by daily dosing with Ostertagia circumcincta larvae. *J. Agric. Sci.* 88, 671–677. doi: 10.1017/S0021859600037369

Tavendale, M. H., Meagher, L. P., Pacheco, D., Walker, N., Attwood, G. T., and Sivakumaran, S. (2005). Methane production from in *vitro* rumen incubations with Lotus pedunculatus and Medicago sativa, and effects of extractable condensed tannin fractions on methanogenesis. *Anim. Feed Sci. Technol.* 123, 403–419. doi: 10.1016/j.anifeedsci.2005.04.037

Terrill, T. H., Rowan, A. M., Douglas, G. B., and Barry, T. N. (1992). Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *J. Sci. Food Agric.* 58, 321–329. doi: 10.1002/jsfa.2740580306

Tiemann, T. T., Lascano, C. E., Wettstein, H.-R., Mayer, A. C., Kreuzer, M., and Hess, H. D. (2008). Effect of the tropical tannin-rich shrub legumes Calliandra calothyrsus and Flemingia macrophylla on methane emission and nitrogen and energy balance in growing lambs. *Animal* 2, 790–799. doi: 10.1017/S1751731108001791

Wabo, P. J., Yondo, J., Fossi, T. O., Marie, C. K., Bilong, B. C. F., and Mpoame, M. (2011). The in *vitro* effects of Chenopodium ambrosioides (Chenopodiaceae) extracts on the parasitic nematode Heligmosomoides bakeri (Nematoda, Heligmosomatidae). *J. Pharmacog. Phytother.* 3, 56–62.

Waghorn, G. (2008). Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. *Anim. Feed Sci. Technol.* 147, 116–139. doi: 10.1016/j.anifeedsci.2007.09.013

Wallace, D. S., Bairden, K., Duncan, J. L., Fishwick, G., Holmes, P. H., McKellar, Q. A., et al. (1996). Influence of soyabean meal supplementation on the resistance of Scottish blackface lambs to haemonchosis. *Res. IN VETERINARY Sci.* 60, 138–143. doi: 10.1016/S0034-5288(96)90008-9

Waller, P. J. (1999). International approaches to the concept of integrated control of nematode parasites of livestock. *Int. J. Parasitol.* 29, 155–164. doi: 10.1016/S0020-7519(98)00178-7

Wambugu, C., Franzel, S., Tuwei, P., and Karanja, G. (2001). Scaling up the use of fodder shrubs in central Kenya. *Dev. Pract.* 11, 487–494. doi: 10.1080/09614520120066765

Wang, S., Terranova, M., Kreuzer, M., Marquardt, S., Eggerschwiler, L., and Schwarm, A. (2018). Supplementation of pelleted hazel (Corylus avellana) leaves

decreases methane and urinary nitrogen emissions by sheep at unchanged forage intake. *Scientific Reports.* 8 (1), 1–10. doi: 10.1038/s41598-018-23572-3

Wolfe, R. M., Terrill, T. H., and Muir, J. P. (2008). Drying method and origin of standard affect condensed tannin (CT) concentrations in perennial herbaceous legumes using simplified butanol-HCl CT analysis. *J. Sci. Food Agric.* 88, 1060–1067. doi: 10.1002/jsfa.3188

Xiang, H., Fang, Y., Tan, Z., and Zhong, R. (2021). Haemonchus contortus infection alters gastrointestinal microbial community composition, protein digestion and amino acid allocations in lambs. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.797746

Yisehak, K., Biruk, K., Abegaze, B., and Janssens, G. P. J. (2014). Growth of sheep fed tannin-rich Albizia gummifera with or without polyethylene glycol. *Trop. Anim. Health Production* 46, 1113–1118. doi: 10.1007/s11250-014-0605-6

Zhao, J., Ma, X., Jin, Y., Su, R., Liu, W., Ren, Y., et al. (2016). Energy requirements for the maintenance and growth of Dorper-Jinzhong crossbred ram lambs. *Ital. J. Anim. Sci.* 15, 94–102. doi: 10.1080/1828051X.2016.1147336

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 25 April 2024
DOI 10.3389/fanim.2024.1329061

OPEN ACCESS

EDITED BY
Juana Catarina Cariri Chagas,
Swedish University of Agricultural Sciences, Sweden

REVIEWED BY
Anderson Corassa,
Federal University of Mato Grosso, Brazil
Leticia Soares,
Natural Resources Institute Finland (Luke), Finland

*CORRESPONDENCE
Melissa Izabel Hannas
melissa.hannas@ufv.br

RECEIVED 27 October 2023
ACCEPTED 10 April 2024
PUBLISHED 25 April 2024

CITATION
da Motta SAB, Barcellos J, Lourenço ACM, Ramos BT, Lima IL, Mike BP, Rennó LN and Hannas MI (2024) Determination of apparent and standardized ileal digestibility of amino acids in corn HP-DDG fed to growing pigs. *Front. Anim. Sci.* 5:1329061. doi: 10.3389/fanim.2024.1329061

Determination of apparent and standardized ileal digestibility of amino acids in corn HP-DDG fed to growing pigs

Stephane Alverina Briguente da Motta[1], Joyce Barcellos[1], Antônio Carlos Macedo Lourenço[1], Bruno Teixeira Ramos[1], Ideraldo Luiz Lima[2], Brian Patrick Mike[3], Luciana Navajas Rennó[1] and Melissa Izabel Hannas[1]*

[1]Department of Animal Science, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil, [2]IL Lima Animal Nutrition Consulting, Vila Velha, Espírito Santo, Brazil, [3]FS Bioenergy, Lucas do Rio Verde, Mato Grosso, Brazil

The corn-based ethanol production industry provides co-products with potential value as animal feed. However, the nutritional value of these co-products should be adequately determined for their sustainable implementation in swine diets. Therefore, a study was conducted to determine the concentration of amino acids (AA), apparent ileal digestible amino acids (AID), standardized ileal digestible amino acids (SID), and crude protein (CP) in corn distillers dried grains with high protein content (corn HP-DDG) for pigs. Six growing pigs (initial body weight: 46.30 ± 2.14 kg) were surgically fitted with a T-cannula at the distal ileum and allotted to a duplicate 2 × 3 incomplete Latin Square Design. Diets containing corn HP-DDG as the only AA source and a nitrogen-free diet (NFD) were formulated. Corn HP-DDG was used as a test ingredient to replace 40% of the starch in NFD, and titanium dioxide (0.5%) was added as an indigestible marker to both diets. Pigs were fed between 08:00 and 18:00 h during five days of adaptation and a sequence of two days of ileal digesta collection. On an as-fed basis, the chemical composition of corn HP-DDG was 40.41% CP, 1.39% lysine, 1.57% methionine + cysteine, 1.61% threonine, 0.23% tryptophan, and 2.15% valine. The AID and SID values of corn HP-DDG were 74.04% and 80.87% for CP; 76.32% and 79.15% for lysine; 84.75% and 86.52% for methionine + cysteine; 71.97% and 78.30% for threonine; 83.86% and 92.44% for tryptophan; and 76.34% and 80.47% for valine, respectively. In conclusion, the SID CP and AA in corn HP-DDG were within the previously published values, and the determined SID coefficients should be used to formulate accurate diets for pigs.

KEYWORDS

HP-DDG, amino acids digestibility, corn, co-products, swine, pigs

1 Introduction

For decades, the modern swine industry has operated at a narrow net profit margin as pig production costs have frequently increased to a greater than the price of live pigs/pork meat. Consequently, the pork meat industry has put efforts into developing innovative solutions to improve the economic efficiency of intensive pig rearing without compromising pork meat quality, animal welfare, and sustainability. The utilization of alternative feed ingredients in practical feeds, such as co-products from other industries (e.g., food, alcohol production, etc.), contributes to providing nutrients and for more ecologically responsible practices in pork production.

Among the several co-products that could be used in pig feeds, those from the ethanol industry are especially notable. The fermentation of corn starch produces a variety of co-products, such as dry distiller grains (DDG), DDG with solubles (DDGS), and high-protein DDG (HP-DDG), which differ from each other with regards to the concentrations of protein, fiber, fat, and minerals (Stein and Shurson, 2009; Woyengo et al., 2014). Including the referred co-products in swine diets has been shown to support adequate performance and reduce food costs (Zijlstra and Beltranena, 2022). Nonetheless, there is variation in the nutritional composition of these co-products due to the nature of the raw materials and technologies utilized during their manufacturing processes (Liu, 2011; Rho et al., 2017).

Recently, fiber separation technology (FST; ICM, Inc., Colwich, Kansas, USA) has been implemented in some ethanol production facilities in Brazil, creating a specific corn co-product. FST removes fiber from the grain before corn starch fermentation into ethanol (Paula et al., 2021), increasing the amount of fermentable starch in the fermenters and thus enhancing ethanol production capacity and yield. This approach allows the production of high-protein corn DDG (HP-DDG), which has a crude protein content of approximately 40% (Shurson, 2018; Paula et al., 2021). Additionally, it increases the lipid content, and reduces neutral detergent fiber content (Paula et al., 2021).

Knowledge of the concentration and digestibility of amino acids in ingredients is essential to optimize the economic and environmental efficiency with which nitrogen is manipulated in pig feeds. Based on the innovative nature of the co-products from the ethanol industry investigated in the current study and the advantages of their utilization in practical pig feeding, we believe that it is opportune to investigate their nutritional value for pigs. Such data, whether compiled with published literature, could contribute to updates of existing feeding tables, such as the National Research Council (2012) and Rostagno et al. (2017). Therefore, the objective of this study was to determine the values of apparent ileal digestible amino acids (AID) and standardized ileal digestible amino acids (SID) and digestible crude protein (CP) from corn HP-DDG from Brazil, produced using FST technology in growing pigs.

2 Materials and methods

All procedures adopted in this study were approved by the Ethics Committee for the Use of Production Animals at the Federal University of Viçosa (protocol number 59/2023) and followed the National Council for Experimentation Animal Control norms.

2.1 Animal housing, diets, and experimental design

The experiment was conducted at the Unit of Teaching, Research, and Extension in Pig Improvement of the Department of Animal Science at the Universidade Federal de Viçosa. This study aimed to determine the AID and SID of CP and amino acids in corn HP-DDG using the ileal digestibility technique, with the determination of basal endogenous losses.

Before the experiment, six growing barrows were surgically fitted with a simple T-cannula at the distal ileum 20 cm from the ileocecal valve, using a technique adapted from Donkoh et al. (1994). The animals were fasted for 12 h before the procedure to minimize digesta contamination during the operation. Subsequently, the animals were anesthetized, placed in the left lateral decubitus position, and extensively shaved to ensure asepsis at the surgical site. A vertical 5 cm–6 cm incision was made in the flank region, positioned 3 cm–4 cm from the caudal vertebra to the last rib. The small intestine was then excised and manipulated. An incision of 2 cm–2.5 cm was made along the antimesenteric side of the small intestine, 10 cm–15 cm from the cranial segment to the ileocecal junction. A suture was placed around the incision and a cannula was inserted and fixed to the intestine. A 1-cm diameter skin incision was made from 3 cm to 4 cm dorsal to the initial incision. Subsequently, all the tissues were sutured.

Pigs were individually housed in 2.3 m × 2.16 m × 0.95 m (length × width × height) pens equipped with a trough feeder and a nipple waterer without any bedding material. The room was washed and disinfected using quicklime, water, and a disinfectant solution.

During the five days following the surgical procedure, the animals received therapeutic antibiotics as a prophylactic measure. During the postoperative period, feed was gradually reintroduced to the animals and provided *ad libitum*. The transition diet was formulated using corn and soybean meal to meet all nutritional requirements (Table 1) as described by Rostagno et al. (2017). The skin suture was removed 15 days after the surgery.

The ileal digestibility test with a simple T-cannula was performed 15 days after the surgery, considering the recovery of all animals. Six barrows (initial body weight: 46.30 kg ± 2.14 kg) were allotted to a duplicate 2 × 3 incomplete Latin Square Design, with two diets and two 7-d periods in each square, totaling six observations per treatment. In the second period, the animals were changed to exclude the effects of the individual on the measured

10.3389/fanim.2024.1329061

TABLE 1 Calculated composition and nutrients in transition diet.

Ingredients	%
Corn, 7.88% CP	61.85
Soybean meal, 45% CP	31.73
Soybean oil	1.47
Vitamin Supplement[1]	0.30
Mineral Supplement premix[2]	0.25
Antioxidant[3]	0.01
Dicalcium phosphate	1.91
Limestone	0.86
Salt	0.48
Lysine-HCL	0.45
DL-Methionine	0.18
L-Threonine	0.21
L-Tryptophan	0.03
L-valine	0.07
Inert	0.20
Total	100.00
Nutrients	
ME, kcal/kg	3,256.50
Crude Protein, %	20.03
SID Lysine, %	1.26
SID Methionine + Cysteine, %	0.76
SID Threonine, %	0.86
SID Tryptophan, %	0.25
Available Phosphorus, %	0.45
Calcium, %	0.91
Sodium, %	0.20

[1]Provided the following quantities per kilogram of complete diet: Se as sodium selenite and selenium yeast, 75.0 mg/kg; vitamin A as retinyl acetate, 2,000 IU/kg; vitamin D3 as cholecalciferol, 375,000 IU/kg; vitamin E as DL-alpha tocopherol, 6,250 UI/kg; vitamin K3 as menadione nicotinamide bisulfate 750.0 mg/kg; thiamin as thiamine mononitrate, 500.0 mg/kg; riboflavin, 1,500 mg/kg; pyridoxine as pyridoxine hydrochloride, 500.0 mg/kg; vitamin B12, 7,500.00 mcg/kg; folic acid, 250.0 mg/kg; pantothenic acid as D-calcium pantothenate, 5,000.00 mg/kg; niacin, 8,750.00 mg/kg; biotin, 37.50 mg/kg.
[2]Provided the following quantities per kilogram of complete diet: Fe, 15.00 g/kg as iron sulfate, Cu, 40 g/kg as copper sulfate, Mn, 13g/kg as manganese monoxide; Zn, 25 g/kg as zinc sulfate; I, 350.00 mg/kg as calcium iodate.
[3]Bannox antioxidant.

food digestibility. The corn HP-DDG source was obtained from FS Bioenergy (Lucas do Rio Verde, Mato Grosso, Brazil), and was characterized as a co-product of corn obtained from ethanol production using FTS technology, with an average composition of 43.85% crude protein, 11.17% ether extract, 7.09% crude fiber, and 2.17% mineral matter concentration (Table 2). A nitrogen-free diet (NFD) was formulated to measure the basal endogenous losses of CP and AA. The test diet was prepared by replacing 40% cornstarch with corn HP-DDG as the sole AA source. Vitamins and minerals were included in all diets to meet or exceed the estimated requirements according to Rostagno et al. (2017). Titanium dioxide (TiO2) was included in both diets at 0.5%, as an indigestible marker (Table 3). Chemical and analysis of the composition of experimental diets are presented in Table 4.

2.2 Experimental procedure

Pigs were fed twice daily (at 0700 and 1700 h) based on their metabolic weight ($BW^{0.75}$, kg), with the feed mixed with water in a 1:1 ratio to avoid waste and facilitate intake. Throughout the experiment, pigs had free access to water. The first five days of each period were considered an adaptation period to the diet. On days 6 and 7 of each period, ileal digesta samples were collected over 10 h.

The ileal digesta was collected using plastic bags (5 cm × 20 cm) attached directly to the cannula using a cable tie. Digesta flowing into the bags was collected and immediately stored under −20°C to prevent bacterial degradation of amino acids. New bags were then attached to the cannula to continue collection.

During the 5-d period between ileal digesta collections, the animals were fed a corn and soybean meal-based diet (Table 1) to meet the requirements describe d by Rostagno et al. (2017). Water was provided *ad libitum* throughout the study.

During the second collection period, one animal subjected to DIP treatment presented with rectal prolapse and was removed from the evaluation by a specialist.

2.3 Processing of collected material and analysis

At the end of the experimental period, ileal digesta samples were thawed, mixed, homogenized, and lyophilized by the experimental unit and period. A subsample was collected for analysis, with six repetitions per treatment.

Samples of the diets, corn HP-DDG, and ileal digesta were analyzed to determine dry matter (DM), nitrogen (N), TiO2, and AAs. The methodologies used were as follows: the INCT-CA G-003/1 method for DM content, the Kjeldahl method for N content, and the INCT-CA method M-007/1 for TiO2 concentration, as described by Detmann et al. (2012). The analyses were conducted at the Laboratory of Animal Nutrition of the Department of Animal Science, Federal University of Viçosa, Mato Grosso, Brazil. Additionally, high-pressure liquid chromatography (HPLC) for AA content was conducted at the CBO Laboratory (Valinhos, São Paulo, Brazil), according to the methods described by White et al. (1986), Hagen et al. (1989), and Lucas and Sotelo (1980).

The factor 6.25 was used to calculate crude protein based on the N content.

10.3389/fanim.2024.1329061

2.4 Calculations

The AID and SID of CP and AA of corn HP-DDG were calculated for the diet containing corn HP-DDG using a direct procedure. The values calculated for this diet represented the values of the test ingredients. The following formulas were used to determine the AID and SID coefficients (Sakomura and Rostagno, 2016):

FI1 = Test diet indigestibility factor

$$FI1 = \frac{\%\ TiO2\ \ test\ \ diet}{\%\ TiO2\ \ test\ \ digesta}$$

Apparent ileal digestibility coefficient of crude protein (AID CP):

$$AID\ \ CP(\%) = \frac{[(\%\ \ CP\ \ in\ \ diet - (\%\ \ CP\ \ in\ \ digesta\ \ \times FI1))]}{\%\ \ CP\ \ in\ \ diet} \times 100$$

FI2 = NFD indigestibility factor

Standardized ileal digestibility coefficient of crude protein (SID CP)

$$SID\ \ CP(\%) = \frac{[(\%\ \ CP\ \ in\ \ diet - (\%\ \ CP\ \ in\ \ digesta\ \ \times FI1) - (\%\ \ CP\ \ in\ \ digesta\ \ \times\ FI2))]}{\%\ \ CP\ \ in\ \ diet} \times 100$$

E1 = Digesta of test diet

Apparent ileal digestibility coefficient of amino acids (AID AA)

$$AID\ \ AA(\%) = \frac{[(mg\ \ AA/g\ \ diet - (mg\ \ AA/g\ \ E1 \times FI1)]}{mg\ \ AA/g\ \ in\ \ diet} \times 100$$

E2 = Digesta of NFD

Standardized ileal digestibility coefficient of amino acids (SID AA)

$$SID\ \ AA(\%) = \frac{[(mg\ \ AA/g\ \ diet - (mg\ \ AA/g\ \ E1\ \ \times FI1) - (mg\ \ AA/g\ \ E2\ \ \times FI2))]}{mg\ \ AA\ \ in\ \ diet} \times 100$$

For the determination of the basal ileal endogenous losses of amino acid, the following formula was used (Adeola et al., 2016):

Basal ileal endogenous losses of amino acid.

$$Basal\ \ Ileal\ \ AAend = mg\ \ AA/g\ \ diet\ \ x\ \ (\%\ \ TiO2\ \ NDF\ \ diet/\ \%\ \ TiO2\ \ digesta)$$

The apparent ileal digestible and standardized ileal digestible crude protein and amino acids in corn HP-DDG were calculated by multiplying each AID and SID coefficient by the CP and AA content in the test ingredient.

2.5 Data analysis

Digestibility coefficients were established from the mean values obtained from the repetitions and the standard deviation of the means was subsequently calculated. Means that deviated from the treatment mean by more than 1.5 SDs were considered outliers.

Means obtained for AID and SID coefficients for lysine, methionine, tryptophan, arginine, glycine, phenylalanine plus tyrosine, and proline, respectively, from the same replicate in the treatment with corn HP-DDG were identified as outliers and removed.

3 Results and discussion

Co-products manufactured by the ethanol industry have been used extensively in animal production. Despite their advantages, the published literature regarding the nutritional value of such ingredients is not consistent, which restricts their utilization in pig feed. Such a gap in knowledge, for example, reflects the absence of information regarding the nutritional value of ethanol co-products and the limits of their utilization in pig feeds in the "Brazilian Tables for Poultry and Swine" (Rostagno et al., 2017), which has been the main reference for poultry and swine nutrition in Brazil. Consequently, researchers frequently use alternative references, such as the National Research Council (2012), which might not precisely reflect Brazilian conditions. The last revised edition of Nutrient Requirements of Swine (National Research Council, 2012) describes the crude protein (CP) content of corn HP-DDG manufactured using a processing method with FST technology as 49.7%. In the current study, the CP content in the ethanol industry co-product under study was equivalent to 44.4% and 40.4% on a dry matter and as-fed basis, respectively (Table 2). Other studies involving corn HP-DDG manufactured in Brazil reported similar protein values on a dry matter basis ranging from 46.5% to 45.1% CP (Palowski et al., 2021; Paula et al., 2021; Dias et al., 2023). Overall, our results suggest lower values for certain amino acids in corn HP-DDG compared to National Research Council (2012) on an as-fed basis, specifically, methionine (0.74% vs. 0.80%), threonine (1.61% vs. 1.90%), tryptophan (0.23% vs. 0.38%), and valine (2.15% vs. 2.19%). The only exception was lysine, which was slightly higher than NRC values (1.39% vs. 1.34%). The underlying reasons for the referred variations in protein and amino acid content might be the different processing methods utilized during the manufacturing of the ethanol co-products (temperature, cooking time, stem, etc.). Such variations reinforce the need to characterize the nutritional value of ethanol co-products manufactured according to the FST technique implemented by the Brazilian ethanol industry.

Basal endogenous losses, expressed in grams per kilogram of Dry Matter Intake (DMI), were determined in pigs fed a nitrogen-free diet (NDF). The essential amino acids lost were lysine (0.212 g/kg DMI), methionine (0.094 g/kg DMI), threonine (0.538 g/kg DMI), tryptophan (0.335 g/kg DMI), valine (0.418 g/kg DMI), isoleucine (0.286 g/kg DMI), leucine (0.509 g/kg DMI), histidine (0.175 g/kg DMI), and phenylalanine (0.280 g/kg DMI). For non-essential amino acids, arginine (0.409 g/kg DMI), alanine (0.569 g/kg DMI), cysteine (0.049 g/kg DMI), tyrosine (0.212 g/kg DMI),

10.3389/fanim.2024.1329061

TABLE 2 Analyzed nutrient composition of ingredients (as-fed basis).

Item	Corn HP-DDG[1]
Dry Matter, %	90.84
Crude Protein, %	40.41
Indispensable AA, %	
Lysine	1.39
Methionine	0.74
Threonine	1.61
Tryptophan	0.23
Arginine	1.78
Valine	2.10
Isoleucine	1.55
Leucine	4.94
Histidine	1.20
Phenylalanine	2.16
Dispensable AA, %	
Alanine	3.07
Cysteine	0.83
Tyrosine	1.73
Glycine	1.59
Serine	1.90
Proline	3.63
Hydroxyproline	0.02
Glutamic Acid	7.42
Aspartic Acid	2.77
Sum of Amino Acids %	40.65

[1]Corn HP-DDG (corn distillers dried grains with high protein, F S Bioenergia, Lucas do Rio Verde—MT, Brazil).

TABLE 3 Composition of diets used in the experiment (as-fed basis).

Ingredients, %	Nitrogen Free Diet	Corn HP-DDG
Corn HP-DDG	–	40.00
Soybean oil	4.00	–
Sugar	20.00	20.00
Corn starch	67.33	31.33
Dicalcium phosphate	1.96	1.96
Limestone	0.76	0.76
Vitamin Supplement	0.30	0.30
Mineral Supplement	0.25	0.25
Salt	0.40	0.40
Potassium carbonate	0.40	0.40
Magnesium Oxide	0.10	0.10
Cellulose	4.00	4.00
Titanium Dioxide	0.50	0.50
Total	100	100

[1]Provided the following quantities per kilogram of complete diet: Se as sodium selenite and selenium yeast, 75.0 mg/kg; vitamin A as retinyl acetate, 2,000 IU/kg; vitamin D3 as cholecalciferol, 375,000 IU/kg; vitamin E as DL-alpha tocopheryl, 6,250 UI/kg; vitamin K3 as menadione nicotinamide bisulfate 750.0 mg/kg; thiamin as thiamine mononitrate, 500.0 mg/kg; riboflavin, 1,500 mg/kg; pyridoxine as pyridoxine hydrochloride, 500.0 mg/kg; vitamin B12, 7500.00 mcg/kg; folic acid, 250.0 mg/kg; pantothenic acid as D-calcium pantothenate, 5,000.00 mg/kg; niacin, 8,750.00 mg/kg; biotin, 37.50 mg/kg.
[2]Provided the following quantities per kilogram of complete diet: Fe, 15.00 g/kg as iron sulfate, Cu, 40 g/kg as copper sulfate, Mn, 13 g/kg as manganese monoxide; Zn, 25 g/kg as zinc sulfate; I, 350.00 mg/kg as calcium iodate.

glycine (1.273 g/kg DMI), serine (0.555 g/kg DMI), proline (3.187 g/kg DMI), glutamic acid (0.695 g/kg DMI), and aspartic acid (0.366 g/kg DMI) were used. Using NFD, we noticed that the endogenous losses of proline and glycine were considerably high. Such outcomes are supported by previous research findings where NFD was used to estimate the loss of proteinaceous compounds in pigs (Kim et al., 2009; Urriola et al., 2009; Zhai and Adeola, 2011). The basal losses of essential amino acids observed herein were also similar to the mean values described by Adeola et al. (2016) in the literature review.

We also determined the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) coefficients of CP and amino acids in corn high-protein dried distillers' grains (HP-DDG) in pigs. Our findings revealed AID and SID values of 74% and 80%, respectively (Table 5). These values are superior to the previously reported AID and SID values. Paula et al. (2021); Yang et al. (2021), and National Research Council (2012) described the AID and SID CP coefficients in HP-DDG in pigs as 63% and 67%, 64% and 77%, and 70% and 76%, respectively. Our coefficients are similar to those determined by Widmer et al. (2007). The authors reported AID and SID CP coefficients of HP-DDG in pigs of 72% and 80%, respectively. The wide variation in digestibility values (Table 5) has been previously highlighted by Stein (2008) who emphasized the influence of different manufacturing methods utilized by the ethanol industry.

The AID and SID amino acids (AAs) observed in this study were higher than those reported previously. The AID and SID values for the essential AAs were higher than those recently determined by Yang et al. (2021) and Paula et al. (2021). Our results are similar to those described by National Research Council (2012), except for lysine and tryptophan, whose AID values were equivalent to 65% and 69%, respectively, and the SID values were 82% and 69%, respectively. Comparing our findings with those of Widmer et al. (2007), we observed similar coefficients, except for lysine, tryptophan, and arginine, whose AID coefficients were 57%, 71%, and 75%, and SID coefficients were 64%, 81%, and 83%, respectively. The lower digestibility of such AAs may be associated with heat damage during the distillation process in DDG production. Lysine is particularly sensitive to temperature due to the Maillard reaction, in which

10.3389/fanim.2024.1329061

TABLE 4 Chemical and analysis of the composition of experimental diets (as-fed).

Ingredients	Nitrogen Free Diet	Corn HP-DDG
Dry Matter, %	91.54	91.89
Crude Protein, %	ND[1]	19.62
Indispensable AA, %		
Lysine	ND[1]	0.69
Methionine	ND[1]	0.37
Threonine	ND[1]	0.78
Tryptophan	0.04	0.36
Arginine	ND[1]	0.86
Valine	0.01	1.00
Isoleucine	ND[1]	0.74
Leucine	0.01	2.39
Histidine	ND[1]	0.57
Phenylalanine	ND[1]	1.04
Dispensable AA, %		
Alanine	0.01	1.48
Cystine	ND[1]	0.38
Tyrosine	ND[1]	0.77
Glycine	ND[1]	0.77
Serine	ND[1]	0.94
Proline	0.01	1.71
Hydroxyproline	ND[1]	0.01
Glutamic Acid	0.03	3.58
Aspartic Acid	0.04	1.35
Sum of Amino Acids	0.22	19.79

[1]ND, not detect.

its amino group reacts with reducing sugars in the presence of heat (Erbersdobler and Hupe, 1991; Paula et al., 2021). Our estimates for nonessential AA digestibility coefficients showed the same pattern as those found for essential AAs, which were higher than those reported in the literature. Notably, the physicochemical properties of ingredients are also determinants of AA digestibility, as highlighted by Paula et al. (2021), who found high SID AA values in corn DDG with high protein content and lower neutral detergent fiber.

The apparent and standardized ileal digestibility coefficients of crude protein (CP) and amino acids (AA) in corn high-protein dried distillers' grains (HP-DDG) are presented in Table 6. Except for lysine, whose AID and SID coefficients were greater, and histidine and phenylalanine, whose digestibility was similar, the SID values found herein for all other AAs were lower than those reported by Paula et al. (2021), who investigated corn HP-DDG produced using FST technology. Glycine, another exception, had a similar AID but lower SID coefficient compared with the Paula et al. (2021) estimates. The differences between our findings and those in the referred literature might be explained by the lower basal endogenous loss of glycine observed in our study.

The average AID and SID coefficients for essential AAs were 80.6% and 84.3%, respectively, whereas those for non-essential AAs were 76.1% and 82.3%, respectively. Our findings regarding the chemical composition and digestibility of AAs in Brazilian corn high-protein dried distillers' grains (HP-DDG) in pigs contribute to increasing the accuracy with which this ingredient is manipulated in feeds by the swine production industry. Pig nutritionists can design feeding programs with higher economic and environmental efficiency. The chemical composition and ileal digestibility coefficients of Brazilian corn high-protein dried distillers' grains (HP-DDG) presented herein whether compiled with published literature can be used for new revised editions of the Brazilian Tables for Poultry and Swine. Even though our outcomes are relevant and meaningful for swine nutrition, much

TABLE 5 Apparent ileal digestibility and standardized ileal digestibility coefficients of crude protein and amino acids in Corn HP-DDG.

	AID, %	SID[1%]	SD[2]	Review[3]
Crude Protein	74.04	80.87	6.31	70–84
Indispensable AA				
Lysine %	76.32	79.15	3.90	61–85
Methionine %	87.21	89.57	3.58	81–89
Met + Cys %	84.75	86.52	5.14	70–84
Threonine %	71.97	78.30	8.43	67–83
Tryptophan %	83.86	92.44	2.44	75–90
Arginine %	82.05	86.40	4.09	81–92
Valine %	76.64	80.47	6.58	74–85
Isoleucine %	77.08	80.61	6.56	74–87
Leucine %	83.91	85.87	5.18	84–90

(Continued)

10.3389/fanim.2024.1329061

TABLE 5 Continued

	AID, %	SID[1%]	SD[2]	Review[3]
Indispensable AA				
Histidine %	80.29	83.10	5.37	74–87
Phenylalanine %	82.34	84.82	4.82	81–89
Dispensable AA				
Alanine %	80.29	83.83	5.67	78–86
Cysteine %	83.69	84.88	5.63	70–84
Tyrosine %	81.93	84.45	5.19	67–83
Phe + Tyr %	83.66	86.16	3.77	
Glycine %	60.09	75.25	8.57	56–81
Serine %	77.68	83.12	5.97	77–86
Gly + Ser %	68.00	77.83	8.64	
Proline %	63.88	81.02	7.45	73–100
Glutamic Acid %	83.17	84.96	5.38	82–89
Aspartic Acid %	78.73	81.22	6.32	67–82

[1]Values for SID were calculated by correcting the AID value for basal endogenous losses. Basal endogenous losses were determined from pigs fed the Nitrogen Free Diet (g/kg of DMI): Lysine, 0.212; Methionine, 0.094; Threonine, 0.538; Tryptophan, 0.335; Arginine, 0.409; Valine, 0.418; Isoleucine, 0.286; Leucine, 0.509; Histidine, 0.175; Phenylalanine, 0.280; Alanine, 0.569; Cysteine, 0.049; Tyrosine, 0.212; Glycine, 1.273; Serine, 0.555; Proline, 3.187; Glutamic Acid, 0.695; Aspartic Acid, 0.366.
[2]SD, Mean standard deviation.
[3]Lower and higher values are cited in Section 3. Feeding applications of corn fermented protein coproducts in swine diets by Stein (2008).

TABLE 6 Apparent ileal digestible and standardized ileal digestible crude protein and amino acids in corn HP-DDG (as-fed basis).

	Corn HP-DDG	
	AID[1]	SID[2]
Crude Protein %	29.92	32.68
Indispensable AA		
Lysine %	1.06	1.10
Methionine %	0.64	0.66
Met + Cys %	1.33	1.35
Threonine %	1.15	1.26
Tryptophan %	0.19	0.21
Arginine %	1.46	1.53
Valine %	1.60	1.69
Isoleucine %	1.19	1.24
Leucine %	4.14	4.24
Histidine %	0.96	0.99
Phenylalanine %	1.77	1.83
Dispensable AA		
Alanine %	2.46	2.57
Cysteine %	0.69	0.70
Tyrosine %	1.41	1.46
Phe + Tyr %	3.25	3.35
Glycine %	0.95	1.19
Serine %	1.47	1.57
Gly + Ser %	2.37	2.71
Proline %	2.31	2.94
Glutamic Acid %	6.17	6.30
Aspartic Acid %	2.18	2.25

(Continued)

TABLE 6 Continued

[1]AID, Apparent ileal digestible amino acids, as-fed.
[2]SID, Standardized ileal digestible amino acid, as-fed.

work must still be done towards characterizing ethanol industry co-products and clarifying the extent to which new processing techniques employed by the ethanol industry can affect the availability of nutrients in co-products.

Data availability statement

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

Ethics statement

The animal study was approved by the Ethics Committee for the Use of Production Animals at Universidade Federal de Viçosa under protocol number 59/2023. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SM: Writing – review & editing, Formal Analysis, Investigation, Writing – original draft. JB: Conceptualization, Data curation, Investigation, Supervision, Writing – original draft. AL: Formal Analysis, Investigation, Writing – original draft. BR: Investigation, Writing – original draft. IL: Conceptualization, Methodology, Resources, Visualization, Writing – review & editing. BM: Conceptualization, Resources, Visualization, Writing – review & editing. LR: Conceptualization, Methodology, Writing – review & editing. MH: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [n&z.ousco; 88887.510984/2020-00, n&z.ousco; 88887.844747/2023-0]; Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) [n&z.ousco; 6.36/2021]; Instituto de Ciência e Tecnologia de Ciência Animal (INCT-CA) [n&z.ousco; 465377/2014-9], and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [n&z.ousco; 465377/2014-9].

Acknowledgments

We thank FS Bioenergia for donating the co-product and providing financial support. We also thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and National Institute of Animal Science and Technology (INCT-CA) for the support.

Conflict of interest

IL is a consultant for FS Bioenergia. BM is a manager at FS Bionergia.

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

This research is partially financially supported by FS Bionergia.

Publisher's note

References

Adeola, O., Xue, P. C., Cowieson, A. J., and Ajuwon, K. M. (2016). Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. *Anim. Feed. Sci. Technol.* 221, 274–283. doi: 10.1016/j.anifeedsci.2016.06.004

Detmann, E., Souza, M. A., Valadares Filho, S. C., de Queiroz, A. C., Berchielli, T. T., de OS Saliba, E., et al. (2012). Métodos para Análises de Alimentos. *Suprema*. Visconde do Rio Branco, 214 p.

Dias, K. M., Oliveira, C. H., Calderano, A. A., Bernardes, R. D., Ribeiro, A. M., Lima, I. L., et al. (2023). Research Note: Nitrogen-corrected apparent metabolizable energy and standardized ileal amino acid digestibility determination of high-protein dried distiller's grains and corn bran with solubles for broilers. *Poultry. Sci.* 102, 102757. doi: 10.1016/j.psj.2023.102757

Donkoh, A., Moughan, P. J., and Smith, W. C. (1994). Comparison of the slaughter method and simple T-piece cannulation of the terminal ileum for determining ileal amino acid digestibility in meat and bone meal for the growing pig. *Anim. Feed. Sci. Technol.* 49, 43–56. doi: 10.1016/0377-8401(94)90080-9

Erbersdobler, H. F., and Hupe, A. (1991). Determination of lysine damage and calculation of lysine bio-availability in several processed foods. *Z. für. Ernährungswissenschaft.* 30, 46–49. doi: 10.1007/bf01910731

Hagen, S. R., Frost, B., and Augustin, J. (1989). Precolumn phenylisothiocyanate derivatization and liquid chromatography of amino acids in food. *J. Assoc. Off. Analytical. Chem.* 72, 912–916. doi: 10.1093/jaoac/72.6.912

Kim, B. G., Petersen, G. I., Hinson, R. B., Allee, G. L., and Stein, H. H. (2009). Amino acid digestibility and energy concentration in a novel source of high-protein distillers dried grains and their effects on growth performance of pigs. *J. Anim. Sci.* 87, 4013–4021. doi: 10.2527/jas.2009-2060

Liu, K. (2011). Chemical composition of distillers grains, a review. *J. Agric. Food Chem.* 59, 1508–1526. doi: 10.1021/jf103512z

Lucas, B., and Sotelo, A. (1980). Effect of different alkalies, temperature, and hydrolysis times on tryptophan determination of pure proteins and foods. *Analytical. Biochem.* 109, 192–197. doi: 10.1016/0003-2697(80)90028-7

National Research Council and Southern, L. L, Adeola, O., de Lange, C. F. (2012). *Nutrient requirements of swine* (Washington DC: National Academies Press).

Palowski, A., Yang, Z., Jang, J., Dado, T., Urriola, P. E., and Shurson, G. C. (2021). Determination of in *vitro* dry matter, protein, and fiber digestibility and fermentability of novel corn coproducts for swine and ruminants. *Trans. Anim. Sci.* 5, txab055. doi: 10.1093/tas/txab055

Paula, V. R., Milani, N. C., Azevedo, C. P., Sedano, A. A., Souza, L. J., Mike, B. P., et al. (2021). Comparison of digestible and metabolizable energy and digestible phosphorus and amino acid content of corn ethanol coproducts from Brazil and the United States produced using fiber separation technology for swine. *J. Anim. Sci.* 99, skab126. doi: 10.1093/jas/skab126

Rho, Y., Zhu, C., Kiarie, E., and de Lange, C. M. (2017). Standardized ileal digestible amino acids and digestible energy contents in high-protein distiller's dried grains with solubles fed to growing pigs. *J. Anim. Sci.* 95, 3591–3597. doi: 10.2527/jas.2017.1553

Rostagno, H. S., Albino, L. F. T., Hannas, M. I., Donzele, J. L., Sakomura, N. K., Perazzo, F. G., et al. (2017). *Brazilian tables for poultry and swine: feed composition and nutritional requirements for poultry and swine* (Viçosa: Departamento de Zootecnia UFV).

Sakomura, N. K., and Rostagno, H. S. (2016). *Research methods in monogastric nutrition. 2nd ed* (Jaboticabal, Brazil: Funep), 262 p.

Shurson, G. J. (2018). "DDGS user handbook: precision DDGS nutrition. Chapter 5," in *Emerging Technologies in Ethanol Production and Nutritional Composition of the High Protein Corn Co-Products Produced*, *4th ed* (U.S. Grains Council, Washington, DC), 22–28.

Stein, H. H. (2008). "Chapter 4 in Using Distillers Grains in the U.S. and International Livestock and Poultry Industries," in *Use of Distillers Co-products in Diets Fed to Swine*. Eds. B. A. Babcock, D. J. Hayes and J. D. Lawrence (Midwest Agribusiness Trade Research and Information Center at the Center for Agricultural and Rural Development, Iowa State University, Ames, IA).

Stein, H. H., and Shurson, G. C. (2009). Board-invited review: The use and application of distillers dried grains with solubles in swine diets. *J. Anim. Sci.* 87, 1292–1303. doi: 10.2527/jas.2008-1290

Urriola, P. E., Hoehler, D., Pedersen, C., Stein, H., and Shurson, G. (2009). Amino acid digestibility of distillers dried grains with solubles, produced from sorghum, a sorghum-corn blend, and a corn fed to growing pigs. *J. Anim. Sci.* 87, 2574–2580. doi: 10.2527/jas.2008-1436

White, J. A., Hart, R. J., and Fry, J. C. (1986). An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. *J. Autom. Chem.* 8, 170–177. doi: 10.1155/S1463924686000330

Widmer, M. R., McGinnis, L. M., and Stein, H. H. (2007). Energy, phosphorus, and amino acid digestibility of high-protein distillers dried grains and corn germ fed to growing pigs. *J. Anim. Sci.* 85, 2994–3003. doi: 10.2527/jas.2006-840

Woyengo, T. A., Beltranena, E., and Zijlstra, R. T. (2014). Nonruminant nutrition symposium: Controlling feed cost by including alternative ingredients into pig diets: A review. *J. Anim. Sci.* 92, 1293–1305. doi: 10.2527/jas.2013-7169

Yang, Z., Palowski, A., Jang, J. C., Urriola, P. E., and Shurson, G. C. (2021). Determination, comparison, and prediction of digestible energy, metabolizable energy, and standardized ileal digestibility of amino acids in novel maize co-products and conventional distillers dried grains with solubles for swine. *Anim. Feed. Sci. Technol.* 282, 115149. doi: 10.1016/j.anifeedsci.2021.115149

Zhai, H., and Adeola, O. (2011). Apparent and standardized ileal digestibilities of amino acids for pigs fed corn- and soybean meal-based diets at varying crude protein levels. *J. Anim. Sci.* 89, 3626–3633. doi: 10.2527/jas.2010-3732

Zijlstra, R. T., and Beltranena, E. (2022). Feeding coproducts to pigs reduces costs and reach sustainable food production. *Anim. Front.* 12, 18–22. doi: 10.1093/af/vfac067

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 27 August 2024
DOI 10.3389/fanim.2024.1415622

OPEN ACCESS

EDITED BY
James Levi Klotz,
United States Department of Agriculture, United States

REVIEWED BY
Alfredo DiCostanzo,
University of Nebraska-Lincoln, United States
Matt Akins,
Agricultural Research Service (USDA), United States

*CORRESPONDENCE
Michael Undi
michael.undi@ndsu.edu

RECEIVED 10 April 2024
ACCEPTED 08 August 2024
PUBLISHED 27 August 2024

CITATION
Undi M, Sedivec K and Bachler J (2024) Assessing supplementing strategies for beef cattle in a bale grazing system using grass hay during variable winter conditions.
Front. Anim. Sci. 5:1415622.
doi: 10.3389/fanim.2024.1415622

Assessing supplementing strategies for beef cattle in a bale grazing system using grass hay during variable winter conditions

Michael Undi[1]*, Kevin Sedivec[1,2] and Jessalyn Bachler[3]

[1]Central Grasslands Research Extension Center, North Dakota State University, Streeter, ND, United States, [2]School of Natural Resource Sciences, Range Science Program, North Dakota State University, Fargo, ND, United States, [3]Business and Trades Technology Department, Williston State College, Williston, ND, United States

Introduction: Beef cattle in the Northern Great Plains of the United States of America are normally kept in open dry lot pens in winter. Practices such as bale grazing, swath grazing, stockpiling, and corn residue grazing, can be used to extend the grazing season and minimize dry lot use. Extending the grazing season has several advantages over dry lot use but arguably the most important benefit is cost savings due to lower labor and input costs. Strategies selected to supplement cattle in extended grazing systems should maintain cost savings while providing required nutrients to cattle. This study was conducted to evaluate supplementing strategies for beef cattle in a bale grazing system using grass hay during variable winter conditions. The study was conducted across variable winter conditions that are encountered in winters in the US Northern Great Plains.

Methods: The study extended over four years. Each year, non-lactating pregnant beef cows (n = 64, year 1; n = 80, year 2, 3, 4) were divided into eight groups of similar average body weight and randomly assigned to one of four bale grazing treatments as follows: a) bale grazing grass hay, b) bale grazing grass hay treated with a liquid supplement, c) bale grazing grass hay and alfalfa hay, and d) bale grazing grass hay and plus 1.8 kg corn DDGS/head/day. Animal performance was assessed from two-day body weights and body condition scores taken at the start and end of the study. Data analysis considered the fixed effects of treatment, year, and treatment x year interaction.

Results: Final BW tended (P = 0.09) to be greatest following corn DDGS supplementation and lowest when grass hay was offered. The treatment strategy x year interaction (P = 0.026) for ADG showed that corn DDGS supplementation resulted in positive ADG across the years regardless of environmental conditions. Liquid or alfalfa hay supplementation resulted in positive ADG when environmental conditions were favorable. Final BCS (P = 0.005) and BCS change (P = 0.004) were greater following corn DDGS supplementation, intermediate following alfalfa hay or liquid supplementation and lowest when grass hay was fed. Supplementation costs ranged from $1.33 to $1.90/head/day, the highest cost occurred with corn DDGS supplementation

mainly due to cost of corn DDGS and labor required to deliver corn DDGS to cattle on pasture.

Discussion: Alfalfa hay or molasses-based liquids increased diet CP content but did not supply adequate energy in severely cold winters. Despite relatively higher supplement costs, high energy supplements such as corn DDGS may be required in severely cold winters where cattle require extra energy. Supplement selection should consider supplement effectiveness to meet animal nutrient requirements particularly in adverse winter conditions such as those encountered in the US Northern Great Plains.

KEYWORDS

bale grazing, grass hay, supplementation, alfalfa hay, corn DDGS, liquid supplement

1 Introduction

Cattle in the US Northern Great Plains are normally kept in open dry lot pens in winter (Asem-Hiablie et al., 2016), a practice associated with high winter feed costs. Alternatively, dry lot use can be minimized by extending the grazing season through strategies such as bale grazing, swath grazing, stockpiling, and grazing corn residue. Bale grazing is a practice of placing hay bales in a grid pattern on hayfields or pastures for grazing in the fall and winter (McGeough et al., 2018). Benefits of extending the grazing season include returning nutrients directly onto land to optimize nutrient capture by growing plants, minimizing nutrient loss through runoff or leaching (Jungnitsch et al., 2011; Bernier et al., 2014), and reducing farm greenhouse gas emissions (Alemu et al., 2016). More importantly, lower labor and input costs associated with extended grazing can decrease production costs and potentially enhance profitability of livestock production.

Forages utilized for bale grazing are predominantly perennial in nature, mainly grasses and grass-legume mixtures although straw may also be utilized (McGeough et al., 2018). In situations where cattle are offered low-quality grass hay or straw, supplementation may be required to meet cattle nutrient requirements. Supplementation becomes especially critical during harsh winter conditions such as those encountered in the Northern Plains. Supplementation is expensive due to costs associated with cost of supplement, labor, and equipment associated with supplement delivery (Cappellozza et al., 2013). Since extended grazing systems are predicated on lower winter feed costs relative to dry lot feeding, supplementation strategies selected for these systems should maintain cost savings while providing targeted amount of required nutrients. Cost savings can be maintained through strategies that either reduce frequency of supplement delivery to cattle on pasture or eliminate pasture visits for supplementation purposes altogether.

For supplements such as corn DDGS, which have to be delivered to cattle on pasture, supplementation costs can be minimized by decreasing labor and equipment inputs through reducing frequency of supplementation (Wickersham et al., 2008). However, less frequent supplement delivery can only be justified if grazing animals continue to consume forage and maintain good nutrient status (Schauer et al., 2005). Also, less frequent supplement delivery can reduce competition for supplement when greater quantities are provided in a single setting (DelCurto et al., 2000). Previous studies (Schauer et al., 2005; Loy et al., 2007; Cappellozza et al., 2013) have shown that less frequent supplement delivery does not negatively impact animal performance and can decrease costs associated with supplementation. Pasture visits for supplementation purposes may be eliminated by supplying high-quality forage to complement low-quality forage or treating low-quality forage with molasses-based liquid supplements. Alfalfa hay (DelCurto et al., 1990; Vanzant and Cochran, 1994; Horney et al., 1996; Weder et al., 1999) and good-quality grass hay (Horney et al., 1996; Villalobos et al., 1997) have been utilized to complement cattle grazing low-quality pastures. Liquid supplements poured directly onto hay can reduce hay waste, improve hay storage, and improve nutrient content of low-quality forage (Walker et al., 2013; Warner et al., 2015). Currently, there is limited information on strategies for supplementing cattle in extended grazing systems. This study was conducted to evaluate supplementing strategies for beef cattle in a bale grazing system using grass hay during variable winter conditions. The study was conducted across variable winter conditions that are encountered in winters in the US Northern Great Plains.

2 Materials and methods

2.1 Ethics statement

Animal handling and care procedures were approved by the North Dakota State University (NDSU) Animal Care and Use Committee.

2.2 Study site

This study was conducted at the North Dakota State University, Central Grasslands Research Extension Center, located in Stutsman and Kidder counties northwest of Streeter, North Dakota, USA (46° 45′N, 99°28′W). Soils at the site are Lihen-Telfer loamy fine sands (57.6%), Krem-Flaxton complex (28.4%), Williams-Bowbells loams (9.3%), and Hecla-Ulen loamy fine sands (5.6%; U.S. Department of Agriculture, Natural Resources Conservation Service (NRCS), 2021). The soils are classified as moderately to well-drained loamy soils with a slope of 0 to 6% (U.S. Department of Agriculture, Natural Resources Conservation Service (NRCS), 2021).

2.3 Hay treatments

A 10.5-ha field was divided into 8, 1.3-ha paddocks separated with three-strand, high-tensile wire electric fencing. A 1000-L tire tank was installed between two paddocks to supply fresh water. Two 7m x 2.5m portable windbreaks, made of solid metal sheets, were placed in a V-shape in each paddock and tied together to protect livestock from the northwestern wind. Round hay bales were placed into paddocks in a 2 x 2 grid pattern with approximately 15m between bales in the fall of each year. The bales were placed in an upright position and net wrap was removed prior to feeding. Every forth bale was pre-weighed before placement in paddocks. Grass hay bales were obtained from a field of mixed cool-season grasses that had not been harvested for several years. Predominant grasses in the field were intermediate wheatgrass (*Thinopyrum intermedium* L.) and tall wheatgrass (*Thinopyrum ponticum* L.). Hay used in all treatments was obtained from the same field which made hay quality comparable across treatments. Each year, hay bales were subsampled and analyzed for nutrient composition prior to bale grazing.

Four bale grazing treatments (two paddocks/treatment) were set up as follows: a) bale grazing grass hay, bale grazing grass hay treated with a liquid supplement, c) bale grazing grass hay and alfalfa hay, and d) bale grazing grass hay plus corn DDGS. Liquid supplementation involved pouring a molasses-based liquid supplement, Range-40 (QLF Inc., Dodgeville, WI), onto grass hay bales at a rate of approximately 10% of average bale weight. With an average bale weight of 543kg, approximately 54kg of liquid supplement was added to grass hay bales. The required rate of dispensing liquid supplement onto bales was estimated from time required to dispense a known amount of liquid supplement into a container of known volume. Timing of liquid supplement dispensation was checked regularly to ensure that the rate remained similar among bales. Poured bales were allowed to sit upright until liquid supplement had seeped through the bale and then were flipped on their side. Supplementation with alfalfa hay was accomplished by providing one bale of alfalfa hay for every three bales of grass hay fed during each feeding cycle. Corn DDGS was delivered twice weekly to cattle on pasture and fed in bunks to provide 1.8 kg corn DDGS/head/day.

2.4 Animal study

The study extended over four years. Each year, non-lactating pregnant Angus-cross beef cows (year 1, n = 64, BW = 592 ± 64 kg, parity = 3.1 ± 0.87; year 2, n = 80, 617 ± 59 kg, parity = 3.4 ± 1.13; year 3, n = 80, 635 ± 45 kg, parity = 3.4 ± 1.24; year 4, n = 80, 615 ± 30 kg, parity = 3.7 ± 0.99) were divided into eight groups of similar average body weight and randomly assigned to treatments. Bale grazing generally commenced in mid-November of each year until January except in year 2, when grazing started earlier. Duration of grazing was 70d, 66d, 67d, and 65d for year 1, 2, 3, and 4, respectively. Cows had access to four hay bales at a time and access to a new set of four bales was controlled using a portable electric wire. Cows were moved to a new set of bales when it was visually estimated that at least 20% of the original hay weight from each bale was remaining. Cows had *ad libitum* access to fresh water, commercial mineral supplement (Purina Animal Nutrition, Shoreview, MN), and a salt block (NaCl, 98% minimum guaranteed analysis, American Stockman). Cow performance was assessed from two-day body weights (BW) and body condition scores (BCS) taken at the start and end of the study. Body condition scores were assigned by two trained technicians working independently using a 9-point system (1 = emaciated, 9 = obese; Rasby et al., 2014). Body weight data were adjusted for fetal tissue weight before statistical analysis. Fetal tissue weight was predicted from actual calf birth weight (CBW) and day of pregnancy (t) at time of weighing (NASEM, 2016) as follows:

$$Fetal\ weight\ (kg) = (CBW \times 0.01828) \times e^{[(0.02 \times t) - (0.0000143 \times t \times t)]}$$

Dry matter intake (DMI) was estimated from a modification of the forage disappearance technique described by Kelln et al. (2011) which calculates DMI from the weight difference between fed forage and residual forage accounting for number of days on feed. Use of the forage disappearance technique to estimate DMI depends on successful separation of soil and fecal matter from residual forage. In this study, it was impossible to effectively separate soil and fecal matter from residual forage thus, a 20% hay wastage value (Jaderborg et al., 2021) was adopted to estimate residual forage. Crude protein (CP) and metabolizable energy (ME) intakes were calculated from forage DMI considering diet CP and ME content, respectively.

2.5 Feed sampling and analysis

Grass hay, liquid-treated grass hay, and alfalfa hay bales were core-sampled (four cores per bale) using a hand-held electric Penn State core sampler. Samples were dried in a forced-air oven at 60°C for at least 48 h and then ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Samples were submitted to Dairyland Laboratories (Dairyland Laboratories Inc., St. Cloud, MN) for chemical analysis. Samples were further dried at 100°C for 24 h to determine total dry matter (DM; Association of Official Analytical Chemists (AOAC), 2005; method 930.15). Crude protein (CP) was determined using a LECO 628 analyzer (LECO

Corporation, St. Joseph, MI; AOAC Method 990.03). Neutral detergent fiber (NDF; AOAC; method 2002.4) and acid detergent fiber (ADF; AOAC; method 973.18) were determined using an ANKOM Fiber Analyzer (ANKOM Technology Corporation, Macedon, NY). Concentrations of Ca, P, K, Mg, and S were determined by inductively-coupled plasma emission spectroscopy (AOAC; method no. 985.01).

2.6 Statistical analysis

Animal performance data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc, 2008) with paddock as the experimental unit. The fixed effects in the model were treatment, year, and treatment × year interaction. The effects of cow within treatment were considered random. Least square means were calculated and, where appropriate, differences among treatment means were tested using the Bonferroni test at a significance level of $P \leq 0.05$. Average daily gain (ADG) was calculated as the difference between initial weight and end weight divided by the number of bale grazing days. Change in BCS was calculated as the difference between initial BCS and final BCS.

2.7 Nutrient requirements

Nutrient requirements of beef cattle determined under standardized conditions are applied indiscriminately to an infinite combination of animal, management and environmental conditions (Fox et al., 1988). The requirements are typically estimated based on the assumption that animals are fed under ideal conditions with little or no environmental stress, and therefore should perform to their genetic potential (Abney and Galyean, 2006). Bale grazing cattle in the US Northern Great Plains are exposed to cold temperatures, low wind chills, freezing rain and snow. To evaluate supplementation strategies under conditions encountered in the US Northern Great Plains, the NASEM (2016) Beef Cattle Nutrient Requirements Model (BCNRM) was used to predict metabolizable energy (ME) and metabolizable protein (MP) requirements and supply for cows kept in environmental temperatures encountered in this study. Monthly average temperatures encountered in this study are shown in Table 1 (North Dakota Agricultural Weather Network, 2021). The first year was marked by below-average temperatures particularly in December and January. Temperatures in year 2 were milder relative to all other years because grazing commenced earlier. The third and fourth years were similar and colder relative to year 2 but warmer than year 1. The 2016 NASEM model was used to compare energy and protein balance in cows kept in various supplementation strategies over the years. Energy and protein balance were calculated as the difference between dietary energy or protein supply and energy or protein requirement. The 2016 NASEM model was also used to compare adequacy of supplementation strategies across the years. This was accomplished by adopting year 3 liquid supplementation as the baseline and comparing this baseline to supplementation strategies in year 1, 2, and 4, designated as severe, mild, and moderate, respectively. For this purpose, relative energy intake (%) was calculated by comparing ME intake from each supplementation strategy in years 1, 2, and 3 to ME intake from liquid-supplement hay in year 3, the baseline year.

2.8 Supplementation costs

Prices of corn DDGS, liquid supplement, grass and alfalfa hay were obtained from a local farm input supplier (Farmers Coop Elevator Company, Streeter, N.D.) The price grass and alfalfa hay of $60 and $80/MT, respectively, included the cost of purchasing and hauling the hay to the bale grazing site. Corn DDGS and liquid supplement were priced at $255 and $319/MT, respectively. Labor ($19/h) included time for placing portable windbreaks at the start of the study, moving portable electric wire to allow access to new feed, twice weekly visits to feed corn DDGS, and hauling water to the site.

3 Results

The supplements fed in this study are shown in Table 2. Alfalfa hay and corn DDGS contained 17.7 and 31.3% CP and 8.9 and 10.3 MJ/kg ME. The liquid supplement contained 40% CP and 9.8 MJ/kg ME (Table 2). Grass hay fed over four years averaged 79 g/kg CP with an ME concentration of 7.3 MJ/kg (Table 3). Addition of a liquid supplement to grass hay increased ME and CP by 8% and 14%, respectively (Table 3). Supplementation with alfalfa hay increased ME and CP concentration by 5% and 38%, respectively. The highest increase occurred with corn DDGS supplementation, increasing ME and CP concentration by 11% and 44%, respectively (Table 3).

Dry matter intake was greater ($P \leq 0.001$) following corn DDGS supplementation (Table 4) because DDGS-supplemented cows received 1.8 kg corn DDGS/head/day in addition to grass hay. As

TABLE 1 Average monthly temperatures (°C) during bale grazing.

Year	October	November	December	January	Mean
1[1]	–	-1.6	-14.0	-20.9	-12.2
2	2.3	-2.8	-11.7	–	-4.0
3	–	-6.0	-6.3	-7.9	-6.7
4	–	-2.9	-9.5	-14.3	-8.9

[1]Year 1 = Nov. 4, 2016 to Jan. 12, 2017, year 2 = Oct. 24 to Dec. 28, 2017, year 3 = Nov. 5, 2018 to Jan. 10, 2019, year 4 = Nov. 14, 2019 to Jan. 17, 2020.

TABLE 2 Chemical composition (%DM) of supplements fed to cattle grazing grass hay over four years.

	Alfalfa hay	Corn DDGS	Range 40[1]
CP, g/kg	177 ± 6.4	315 ± 1.9	400
ME, MJ/kg	8.9 ± 1.33	10.3 ± 2.56	9.8
NDF, g/kg	517 ± 44.6	324 ± 24.7	–
ADF, g/kg	383 ± 32.6	147 ± 34.9	–
Ca, g/kg	17.6 ± 2.0	0.6 ± 0.1	4
P, g/kg	2.0 ± 0.4	10.4 ± 0.6	12.6
Mg, g/kg	3.9 ± 0.1	4.1 ± 0.2	2.3
K, g/kg	22.9 ± 4.3	7.1 ± 0.5	21

[1]Minimum manufacturer guaranteed analysis.

a percent of BW, DMI was greater ($P \leq 0.001$) following corn DDGS supplementation relative to other treatments. Crude protein intake was influenced by treatment and year ($P \leq 0.001$; Table 4) and was greatest following corn DDGS supplementation and lowest when only grass hay was fed. Energy intake was influenced by treatment and year ($P \leq 0.001$; Table 4) and was consistently greater following corn DDGS supplementation relative to alfalfa hay, liquid supplement or grass hay (Figure 1).

By design, there was no difference ($P = 0.92$) among treatments in initial cow BW but there were differences ($P \leq 0.001$) among the years (Table 4). Final BW tended ($P = 0.09$) to be greatest following corn DDGS supplementation and lowest when only grass hay was offered (Table 4). Average daily gain was influenced by treatment and year ($P = 0.026$; Table 4). Corn DDGS supplementation resulted in positive ADG across the years regardless of environmental conditions (Figure 2). In year 2, supplementation strategies improved ADG above bale grazing grass hay only. As well, cows gained weight in year 2 probably due to more favorable environmental conditions (Figure 2). In year 3 and 4, ADG were minimal following bale grazing grass hay, liquid-supplemented hay or alfalfa hay (Figure 2). There was no difference ($P = 0.97$) among treatments in initial BCS but there were differences ($P \leq 0.001$) among years (Table 4). Final BCS was greater ($P = 0.005$) following corn DDGS supplementation, intermediate following alfalfa hay or liquid supplementation and lowest in cows fed grass hay only (Table 4). Final BCS differed ($P \leq 0.001$) on a yearly basis. Change in BCS was greater ($P = 0.004$) following corn DDGS supplementation, alfalfa hay or liquid supplementation and lowest when cows were fed only grass hay.

TABLE 3 Chemical composition (mean ± SD; % DM) of grass hay, or grass hay supplemented with a liquid, alfalfa hay, or corn DDGS.

	HAY[1]	H-LQS	H-ALF[2]	H-DDG[2]
CP, g/kg	79 ± 5.1	90 ± 4.4	109 ± 8.2	114 ± 5.6
ME, MJ/kg	7.3 ± 1.24	7.9± 4.37	7.7 ± 1.22	8.2 ± 1.13
NDF, g/kg	661 ± 6.9	654 ± 8.1	624 ± 15.9	607 ± 4.3
ADF, g/kg	473 ± 9.6	478 ± 30.9	451 ± 14.7	425 ± 11.6
Ca, g/kg	6.1 ± 0.4	5.4 ± 0.5	8.9 ± 0.3	5.3 ± 0.4
P, g/kg	1.1 ± 0.4	1.6 ± 0.2	1.3 ± 0.4	2.4 ± 0.4
Mg, g/kg	1.8 ± 0.2	1.6 ± 0.1	2.3 ± 0.2	2.2 ± 0.2
K, g/kg	9.2 ± 3.2	9.1 ± 0.3	12.4 ± 3.7	9.7 ± 3.2

[1]HAY, grass hay; H-LQS, liquid-treated grass hay; H-ALF, grass hay plus alfalfa hay; H-DDG, grass hay plus corn DDGS.
[2]Calculated diet composition based on the proportions of alfalfa (H-ALF) and corn DDGS (H-DDG) offered and hay refusal rate of 20%.

4 Discussion

Grass hay provided adequate MP to meet protein requirements of non-lactating beef cows in the second trimester of pregnancy in all environmental conditions encountered in the study. Positive MP balance across four years of the study (Figure 3) suggest that there was no apparent need for protein supplementation. Grass hay, however, did not provide adequate energy to meet daily ME requirements for non-lactating beef cows in the second trimester of pregnancy in environmental conditions encountered in this study. Based on the 2016 NASEM model, feeding grass hay only resulted in negative energy balance across all study years (Figure 4), suggesting a need for supplementing cattle even in mild weather conditions as occurred in year 2. This is further supported by weight loss by cows bale grazing grass hay in all environmental conditions encountered in this study.

Molasses-based liquid supplements supply rumen degradable protein which provides rumen microbes with a source of nitrogen for the synthesis of microbial protein (Manoukian et al., 2021). When applied to hay, liquid supplements serve several functions including reducing hay waste (Walker et al., 2013), improving hay storage (Warner et al., 2015), and improving hay nutrient content (Walker et al., 2013; Warner et al., 2015). Liquid treatment can improve nutrient composition of hay such that additional supplementation of protein or energy may not be required (Warner et al., 2015). In this study, liquid supplementation increased diet CP content above feeding grass hay only. Based on approximately 10% application rate of liquid supplement, CP content of liquid-treated was expected to be higher than the 9% reported in this study (Table 3). It is speculated that, although care was taken to ensure uniform treatment of bales, this may not have occurred consistently every year. Secondly, distribution of liquid within hay bales may not have been as even as expected, leaving pockets on untreated hay. This would be particularly important in hay that was treated in cooler October temperatures. Liquid supplementation supplied MP on excess of cow requirements in all environmental conditions encountered (Figure 3). Simulation using the 2016 NASEM model showed that liquid supplementation provided adequate energy to meet requirements of dry cows in a year which averaged -7°C (year 3) but provided energy that exceeded requirements in a mild year 2 (Figure 3). However, liquid supplementation did not supply adequate energy in moderate years (year 4) or years with severe temperatures as shown in year 1 (Figure 5). Therefore, liquid supplementation may be an option for bale grazing cattle on low-quality grass hay in mild winters but cannot be the sole supplement as winters become severe.

Supplementation with alfalfa hay supplied MP on excess of cow requirements in all environmental conditions encountered (Figure 3).

TABLE 4 Cow performance following bale-grazing of grass hay, or grass hay supplemented with a liquid, alfalfa hay, or corn DDGS.

	Treatment (T)				SE	Year (Y)				SE	P-value		
	HAY[1]	H-LQS	H-ALF	H-DDG		1[2]	2	3	4		T	Y	T x Y
DMI, kg/d	11.9^{b}	12.0^{b}	12.2^{b}	13.6^{a}	0.14	12.1^{c}	12.5^{ab}	12.7^{a}	12.4^{b}	0.12	<0.001	<0.001	0.82
DMI, % BW	1.96^{b}	1.96^{b}	1.97^{b}	2.23^{a}	0.01	2.05^{a}	2.02^{b}	2.01^{b}	2.02^{b}	0.01	<0.001	<0.001	0.50
CP intake, kg/d	0.93^{d}	1.07^{c}	1.34^{b}	1.47^{a}	0.01	1.15^{c}	1.17^{c}	1.21^{b}	1.31^{a}	0.01	<0.001	<0.001	<0.001
ME intake, MJ/d	87.7^{c}	93.2^{b}	93.8^{b}	99.7^{a}	1.06	90.6^{b}	91.3^{b}	97.8^{a}	94.7^{ab}	0.85	<0.001	<0.001	<0.001
Initial BW, kg	614	615	618	612	9.3	591^{c}	618^{b}	635^{a}	616^{ab}	7.9	0.92	<0.001	0.96
Final BW, kg	606	618	622	631	9.9	570^{b}	647^{a}	641^{a}	622^{a}	8.7	0.09	<0.001	0.87
ADG, kg/d	-0.11^{c}	0.05^{b}	0.06^{b}	0.30^{a}	0.05	-0.29^{c}	0.43^{a}	0.07^{b}	0.08^{b}	0.05	<0.001	<0.001	0.026
Initial BCS	5.8	5.8	5.8	5.8	0.05	5.5^{c}	5.4^{d}	6.5^{a}	5.8^{b}	0.05	0.97	<0.001	0.69
Final BCS	5.7^{b}	5.8^{ab}	5.8^{ab}	5.9^{a}	0.04	5.4^{c}	5.6^{b}	6.9^{a}	5.3^{c}	0.04	0.005	<0.001	0.75
BCS change	-0.08^{b}	0.04^{a}	0.03^{ab}	0.07^{a}	0.04	-0.13^{c}	0.22^{b}	0.39^{a}	-0.42^{c}	0.04	0.004	<0.001	0.23

[1]HAY, grass hay; H-LQS, liquid-treated grass hay; H-ALF, grass hay plus alfalfa hay; H-DDG, grass hay plus corn DDGS.
[a-c]Means with a different letter within row for treatment (T) or within row for year (Y) differ significantly ($P \leq 0.05$).
[1]Bale grazing duration: 70 days (Nov. 4, 2016 to Jan. 12, 2017) in year 1, 66 days (Oct. 24 to Dec. 28, 2017) in year 2, 67 days (Nov. 5, 2018 to Jan. 10, 2019) in year 3, and 65 days (Nov. 14, 2019 to Jan. 17, 2020) in year 4.

Supplementation with alfalfa hay improved cow daily gains during mild winters but did not provide adequate energy to meet requirements in cold winters such as occurred in year 1. The 2016 NASEM model showed that alfalfa hay supplied adequate energy only during mild years but not in years with moderate or severe environmental temperatures (Figure 4). In mild environmental temperatures (year 2), alfalfa hay supplementation was nearly as effective as liquid supplementation in providing energy to meet cow energy requirements (Figure 5). Although alfalfa hay can effectively meet CP requirements in rations with low-quality roughages, alfalfa hay does not have the caloric density of oilseed meals or other by-product feeds to meet energy needs (DelCurto et al., 2000). In fact, the energy density of alfalfa is similar to that of high-quality grass hay. Thus, if cows are energy-deficient and marginal in body condition, supplements with higher energy density may be more appropriate (DelCurto et al., 2000). Previous studies (Vanzant and Cochran, 1994; Horney et al., 1996; Weder et al., 1999) have reported improved animal performance when cattle grazing low-quality forage were offered higher-quality alfalfa hay. In practical terms, alfalfa hay as a supplement for beef cattle bale grazing grass hay may be an option in mild winters. In cold winters, higher energy supplements such as corn DDGS and other grains may be required to meet nutritional requirements.

Supplementation with corn DDGS resulted in the highest increase in diet CP and supplied the highest of amount of MP to cows across the years (Figure 3). The high dietary energy resulting from supplementation with DDGS resulted with a diet that

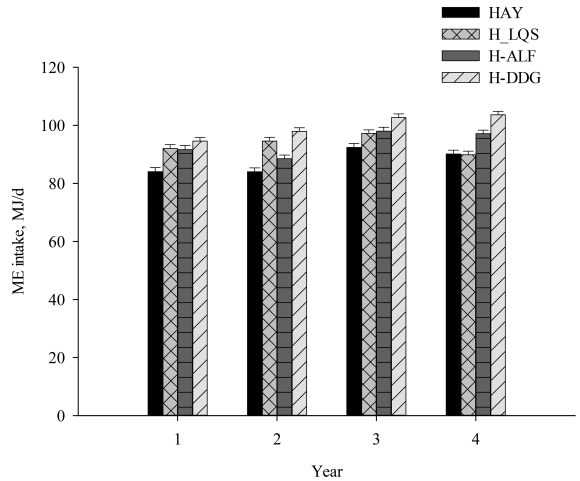


FIGURE 1
Energy (MJ/day ME) intake by cows bale grazing grass hay (HAY) or liquid-treated grass hay (H-LQS), grass hay plus alfalfa hay (H-ALF), or grass hay plus corn DDGS (H-DDG).

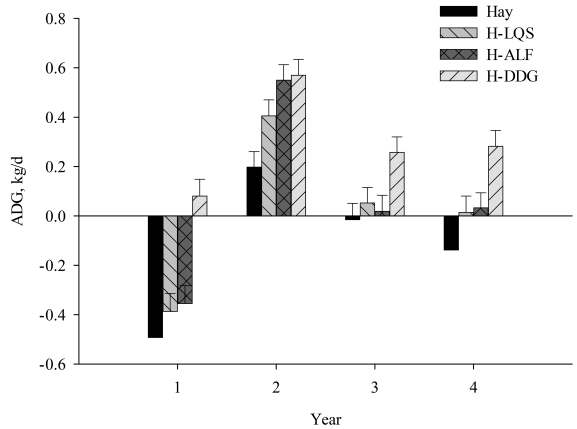


FIGURE 2
Average daily gains of cows bale grazing grass hay (HAY) or liquid-treated grass hay (H-LQS), grass hay plus alfalfa hay (H-ALF), or grass hay plus corn DDGS (H-DDG).

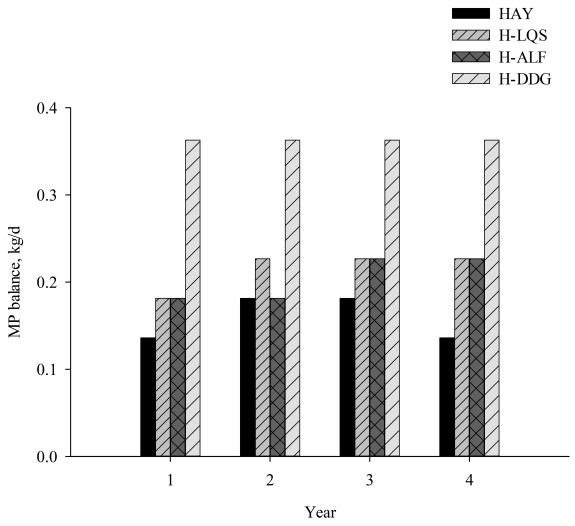


FIGURE 3
Predicted MP balance (dietary MP supply – MP requirements, kg/d) in cattle grazing grass hay (HAY) or liquid-treated grass hay (H-LQS), grass hay plus alfalfa hay (H-ALF), or grass hay plus corn DDGS (H-DDG).

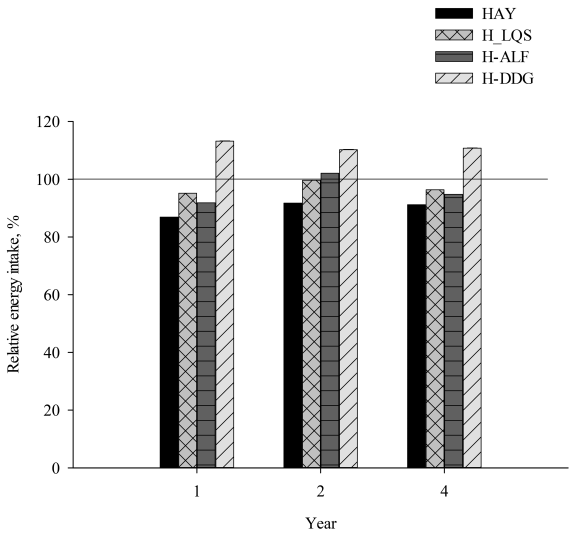


FIGURE 5
Relative energy intake (%) in cattle grazing grass hay (HAY) or liquid-treated grass hay (H-LQS), grass hay plus alfalfa hay (H-ALF), or grass hay plus corn DDGS (H-DDG). Horizontal line represents year 3 liquid supplement energy intake (baseline).

exceeded requirements of dry cows. Based on the 2016 NASEM model, feeding 1.8 kg corn DDGS/head/day to cows bale grazing grass hay supplied energy in excess of cow requirements at all environmental temperatures encountered in this study (Figure 4). Compared to year 3 liquid supplementation as a baseline, supplementation with DDGS supplied energy that exceeded cow energy requirements even in years with severe environmental temperatures (Figure 5). As a supplement, corn DDGS compares favorably with other supplements such as soybean meal and canola meal since corn DDGS is a good source of protein, fat, phosphorus, and readily digestible fiber (Klopfenstein et al., 2008). The low starch content of corn DDGS makes corn DDGS a suitable as supplement for grazing cattle (Klopfenstein et al., 2008). Benefits of supplementing cattle on pasture with corn DDGS have been reported in other studies (Wilson et al., 2015; Lardner et al., 2018; Smith et al., 2020). Among the supplements evaluated, corn DDGS is the only supplement that supplied adequate energy for pregnant beef cows that were bale grazing in all environmental conditions encountered in this study.

Animal response to supplementation was influenced by treatment as well as environmental conditions in different years. This response was clearly demonstrated by changes in ADG due to supplementation strategy and environmental temperatures over the four-year period. Environmental conditions can have a significant effect on variation in supplement intake by grazing beef cattle consuming a protein supplement in winter (Wyffels et al., 2020). Supplementation strategies successfully improved animal gains in more favorable environmental conditions such as those in year 2. In more inclement weather conditions, corn DDGS supplementation was more effective in maintaining or improving animal gains.

Supplementation costs ranged from $1.33 to $1.90/head/day for different strategies (Table 5). Predictably, bale grazing grass hay alone resulted in the lowest system costs. Minimizing use of

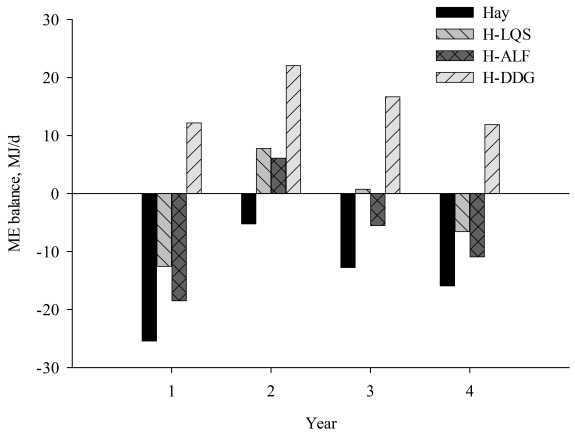


FIGURE 4
Predicted ME balance (dietary ME supply – ME requirements, MJ/d) in cattle grazing grass hay (HAY) or liquid-treated grass hay (H-LQS), grass hay plus alfalfa hay (H-ALF), or grass hay plus corn DDGS (H-DDG).

TABLE 5 Cost comparison following bale grazing of grass hay, or grass hay supplemented with a liquid, alfalfa hay, or corn DDGS.

	Cost ($/head/day)			
	HAY[1]	H-LQS	H-ALF	H-DDG
Grass hay	1.29	1.29	0.97	1.29
Supplement	–	0.26	0.57	0.51
Labor[2]	0.04	0.04	0.04	0.10
Total cost	1.33	1.59	1.58	1.90

[1]HAY, grass hay; H-LQS, liquid-treated grass hay; H-ALF, grass hay plus alfalfa hay; H-DDG, grass hay plus corn DDGS.
[2]Crossfencing and supplement delivery.

purchased hay, transportation costs, and grazing hay bales in the field from which the hay was baled would keep costs of bale grazing low. Liquid supplementation increased grazing costs by $0.26/head/day over grass hay due to the cost of the liquid supplement. Supplementation with alfalfa hay increased costs by $0.25/head/day over grass hay (Table 5). The highest cost ($1.90/head/day), occurred when corn DDGS was offered as a supplement mainly due to the cost of corn DDGS as well as labor required for twice-weekly visits to deliver corn DDGS to cattle on pasture. Limiting delivery frequency to one visit per week would reduce the cost of corn DDGS supplementation.

5 Conclusion

Alfalfa hay or molasses-based liquids may be utilized as supplements during mild winters but severely cold winters require higher energy supplements such as corn DDGS. Despite the relatively higher cost, supplementation with corn DDGS may be required in situations such as severe winters where cattle require extra energy to maintain or improve performance. Supplement selection should consider supplement effectiveness to meet animal nutrient requirements particularly in adverse winter conditions such as those encountered in the US Northern Great Plains.

Data availability statement

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

Ethics statement

The animal study was approved by North Dakota State Animal Care &Use Committee (IACUC). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MU: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. KS: Conceptualization, Funding acquisition, Investigation, Writing – review & editing, Writing – original draft. JB: Data curation, Investigation, Methodology, Writing – review & editing, Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by North Dakota State University, Central Grasslands Research Extension Center Hatch Project (ND06152).

Acknowledgments

We thank the late Rodney Schmidt, the late Dwight Schmidt, as well as Stephanie Becker, Scott Alm, Tim Long, Cody Wieland, Thomas Mittleider, and Rick Bohn for technical assistance. Special thanks to QLF personnel, Tom Lere and Curt Lahr, for technical support and to QLF for partial support through a reduced (research) price for Range-40. We also thank Janet Patton for reviewing this manuscript.

Conflict of interest

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

Publisher's note

References

Abney, C. S., and Galyean, M. L. (2006). Seasonal and environmental effects on energy requirements and performance of high-producing cattle. *Mid-South Ruminant Nutr. Conf.*, 43–50. Available online at: https://www.txanc.org/Proceedings/2006/Abney-manuscript-final.pdf.

Alemu, A. W., Doce, R. R., Dick, A. C., Basarab, J. A., Kröbel, R., Haugen-Kozyra, K., et al. (2016). Effect of winter feeding systems on farm greenhouse gas emissions. *Agric. Syst.* 148, 28–37. doi: 10.1016/j.agsy.2016.06.008

Asem-Hiablie, S., Rotz, C. A., Stout, R., and Stackhouse-Lawson, K. (2016). Management characteristics of beef cattle production in the Northern Plains and Midwest regions of the United States. *Prof. Anim. Sci.* 32, 736–749. Available at: https://www.appliedanimalscience.org/action/showPdf?pii=S1080-7446%2816%2930082-1.

Association of Official Analytical Chemists (AOAC) (2005). *Official methods of analysis* (Arlington, VA: AOAC).

Bernier, J. N., Undi, M., Ominski, K. H., Donohoe, G., Tenuta, M., Flaten, D., et al. (2014). Nitrogen and phosphorus utilization and excretion by beef cows fed a low-quality forage diet supplemented with dried distillers' grains with solubles under thermal neutral and prolonged cold conditions. *Anim. Feed Sci. Technol.* 193, 9–20. doi: 10.1016/j.anifeedsci.2014.03.010

Cappellozza, B. I., Bohnert, D. W., Schauer, C. S., Falck, S. J., Vanzant, E. S., Harmon, D. L., et al. (2013). Daily and alternate day supplementation of urea or soybean meal to ruminants consuming low-quality cool-season forage: II. Effects on ruminal fermentation. *Livest. Sci.* 155, 214–222. doi: 10.1016/j.livsci.2013.05.002

DelCurto, T., Cochran, R. C., Nagaraja, T. G., Corah, L. R., Beharka, A. A., and Vanzant, E. S. (1990). Comparison of soybean meal/sorghum grain, alfalfa hay and dehydrated alfalfa pellets as supplemental protein sources for beef cattle consuming dormant tallgrass-prairie forage. *J. Anim. Sci.* 68, 2901–2915. doi: 10.2527/1990.6892901x

DelCurto, T., Hess, B. W., Huston, J. E., and Olson, K. C. (2000). Optimum supplementation strategies for beef cattle consuming low-quality roughages in the western United States. *J. Anim. Sci.* 77, 1–16. doi: 10.2527/jas2000.77E-Suppl1v

Fox, D. G., Sniffen, C. J., and O'Connor, J. D. (1988). Adjusting nutrient requirements of beef cattle for animal and environmental variations. *J. Anim. Sci.* 66, 1475–1495. doi: 10.2527/jas1988.6661475x

Horney, M. R., DelCurto, T., Stamm, M. M., Bailey, R. K., and Brandyberry, S. D. (1996). Early-vegetative tall fescue hay versus alfalfa hay as a supplement for cattle consuming low-quality roughages. *J. Anim. Sci.* 74, 1959–1966. doi: 10.2527/1996.7481959x

Jaderborg, J. P., Bird, S. L., Crawford, G. I., Walker, R. S., and DiCostanzo, A. (2021). Influence of hay feeding method, supplement moisture, or access time on intake and waste by beef cows. *Transl. Anim. Sci.* 5, 1–11. doi: 10.1093/tas/txab069

Jungnitsch, P., Schoenau, J. J., Lardner, H. A., and Jefferson, P. G. (2011). Winter feeding beef cattle on the western Canadian prairies: impacts on soil nitrogen and phosphorous cycling and forage growth. *Agric. Ecosyst. Environ.* 141, 143–152. doi: 10.1016/j.agee.2011.02.024

Kelln, B. M., Lardner, H. A., McKinnon, J. J., Campbell, J. R., Larson, K., and Damiran, D. (2011). Effect of winter-feeding system on beef cow performance, reproductive efficiency, and system cost. *Prof. Anim. Sci.* 27, 410–421. doi: 10.15232/S1080-7446(15)30513-1

Klopfenstein, T. J., Ericson, G. E., and Bremer, V. R. (2008). Board invited review: Use of distillers by-products in the beef cattle feeding industry. *J. Anim. Sci.* 86, 1223–1231. doi: 10.2527/jas.2007-0550

Lardner, H. B., Larson, K., Christensen, D. A., McKinnon, J. J., Añez-Osuna, F., Damiran, D., et al. (2018). Performance of stocker cattle grazing bales in winter and supplemented with wheat-based dried distillers grains plus solubles or barley grain in western Canada. *Prof. Anim. Sci.* 34, 460–468. doi: 10.15232/pas.2018-01723

Loy, T. W., MacDonald, J. C., Klopfenstein, T. J., and Erickson, G. E. (2007). Effect of distillers grains or corn supplementation frequency on forage intake and digestibility. *J. Anim. Sci.* 85, 2625–2630. doi: 10.2527/jas.2005-750

Manoukian, M., DelCurto, T., Kluth, J., Carlisle, T., Davis, N., Nack, M., et al. (2021). Impacts of rumen degradable or undegradable protein supplementation with or without salt on nutrient digestion, and VFA concentrations. *Animals.* 11, 3011. doi: 10.3390/ani11113011

McGeough, E. J., Cattani, D. J., Koscielny, Z., Hewitt, B., and Ominski, K. H. (2018). Annual and perennial forages for fall/winter grazing in western Canada. *Can. J. Plant Sci.* 98247–254. doi: 10.1139/cjps-2017-0228

NASEM (2016). *Nutrient requirement of beef cattle. 8th rev. ed* (Washington, DC: Natl. Acad. Press). doi: 10.17226/19014

North Dakota Agricultural Weather Network (2021). Available online at: https://ndawn.ndsu.nodak.edu/ (Accessed June 22, 2022).

Rasby, R. J., Stalker, A., and Funston, R. N. (2014).Body condition scoring beef cows: A tool for managing the nutrition program for beef herds. Available online at: https://extensionpublications.unl.edu/assets/pdf/ec281.pdf (Accessed February 17, 2019).

SAS Institute Inc (2008). *SAS user's guide. 4th ed. Version 9.1* (Gary, NC: SAS Institute, Inc.).

Schauer, C. S., Bohnert, D. W., Ganskopp, D. C., Richards, C. J., and Falck, S. J. (2005). Influence of protein supplementation frequency on cows consuming low-quality forage: performance, grazing behavior, and variation in supplement intake. *J. Anim. Sci.* 83, 1715–1725. doi: 10.2527/2005.8371715x

Smith, W. B., Banta, J. P., Forster, J. L., Redmon, L. A., MaChado, T. J., Tedeschi, L. O., et al. (2020). Evaluation of growth performance and carcass characteristics of beef stocker cattle grazing Tifton 85 Bermudagrass supplemented with dried distiller's grains with solubles then finished in the feedlot. *App. Anim. Sci.* 36, 308–319. doi: 10.15232/aas.2019-01907

U.S. Department of Agriculture, Natural Resources Conservation Service (NRCS) (2021). Soil web survey. 2021. Available online at: https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm (Accessed October 20, 2021).

Vanzant, E. S., and Cochran, R. (1994). Performance and forage utilization by beef cattle receiving increasing amounts of alfalfa hay as a supplement to low-quality, tallgrass-prairie forage. *J. Anim. Sci.* 72, 1059–1067. doi: 10.2527/1994.7241059x

Villalobos, G., Adams, D. C., Klopfenstein, T. J., Nichols, J. T., and Lamb, J. B. (1997). Grass hay as a supplement for grazing cattle: I. Animal performance. *J. Range Manage.* 50, 351–356. doi: 10.2307/4003298

Walker, R. S., LaMay, D., Davis, J. R., and Bandyk, C. (2013). Method of feeding a liquid protein supplement with low- to medium-quality hay affects hay waste and cow performance. *Prof. Anim. Sci.* 29, 552–558. doi: 10.15232/S1080-7446(15)30277-1

Warner, J. M., Schneider, C. J., Erickson, G. E., Klopfenstein, T. J., and Rasby, R. J. (2015). Applying corn condensed distillers solubles to hay windrows before baling: effects on bale temperature, nutrient composition, and growing steer-calf performance. *Prof. Anim. Sc.* 31, 207–215. doi: 10.15232/pas.2014-01354

Weder, C. E., DelCurto, T., Svejcar, T., Jaeger, J. R., and Bailey, R. K. (1999). Influence of supplemental alfalfa quality on the intake, use, and subsequent performance of beef cattle consuming low-quality roughages. *J. Anim. Sci.* 77, 1266–1276. doi: 10.2527/1999.7751266x

Wickersham, T. A., Titgemeyer, E. C., Cochran, R. C., Wickersham, E. E., and Moore, E. S. (2008). Effect of frequency and amount of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. *J. Anim. Sci.* 86, 3089–3099. doi: 10.2527/jas.2007-0326

Wilson, T. B., Schroeder, A. R., Ireland, F. A., Faulkner, D. B., and Shike, D. W. (2015). Effects of late gestation distillers' grains supplementation on fall-calving beef cow performance and steer calf growth and carcass characteristics. *J. Anim. Sci.* 93, 4843–4851. doi: 10.2527/jas.2015-9228

Wyffels, A., Dafoe, J. M., Parsons, C. T., Boss, D. L., DelCurto, T., and Bowman, J. P. G. (2020). The influence of age and environmental conditions on supplement intake by beef cattle winter grazing northern mixed-grass rangelands. *J. Anim. Sci.* 98, 1–8. doi: 10.1093/jas/skaa217

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 03 January 2025
DOI 10.3389/fanim.2024.1469850

OPEN ACCESS

EDITED BY
David L. Harmon,
University of Kentucky, United States

REVIEWED BY
Assar Ali Shah,
Jiangsu University, China
Ravikanth Reddy Poonooru,
University of Missouri, United States

*CORRESPONDENCE
Wenchun Qian
cbb2068003@sina.com

†These authors have contributed equally to this work

RECEIVED 24 July 2024
ACCEPTED 18 November 2024
PUBLISHED 03 January 2025

CITATION
Guo L, Shi X, Cao F, Hu S and Qian W (2025) Effects of dietary addition of mulberry leaf powder on blood metabolites and fecal microbiota composition in Hu sheep. *Front. Anim. Sci.* 5:1469850. doi: 10.3389/fanim.2024.1469850

Effects of dietary addition of mulberry leaf powder on blood metabolites and fecal microbiota composition in Hu sheep

Liangyong Guo[1†], Xingyun Shi[1†], Fang Cao[2], Shilei Hu[3] and Wenchun Qian[1*]

[1]Huzhou Key Laboratory of Innovation and Application of Agricultural Germplasm Resources, Huzhou Academy of Agricultural Sciences, Huzhou, China, [2]College of Life Science, Huzhou Teachers College, Huzhou, China, [3]Central Plains Environmental Protection Co. Ltd, Treatment Plant Dispatch Center, Zhengzhou, China

In livestock production, ruminant feed resources are often scarce, and numerous challenges arise during production, such as immune disorders and oxidative stress. Mulberry leaves are rich in various nutrients and exhibit significant antioxidant and immune-regulating properties. Therefore, they can be used as an unconventional feed resource in livestock production. This study investigated the effects of mulberry leaves (ML) as a feed supplement on the blood biochemical parameters and hindgut microbial structure of Hu sheep. Sixteen Hu sheep were randomly divided into two groups and fed either 0 or 60 g/d of ML. Compared to the control group, sheep fed ML showed a significant increase in AKP (P = 0.027) and GPT (P = 0.002) levels in the blood, while TP (P = 0.001) levels decreased significantly. Additionally, there was an increasing trend in GSH-Px (P = 0.082) and CAT (P = 0.058) levels. After the addition of ML, the abundance of Campylobacterota, *Campylobacter*, and *Mailhella* in the hindgut significantly increased (P < 0.05), while the abundance of *Alloprevotella*, *Roseburia*, and *Prevotellaceae UCG-003* significantly decreased (P < 0.05). Therefore, ML can serve as a natural feed supplement to regulate the immune status of animals, thereby promoting the healthy production of ruminants.

KEYWORDS

Hu sheep, mulberry leaf powder, blood metabolism, fecal microbiota, healthy

1 Introduction

Mulberry leaves are rich in protein, fiber, minerals, and other nutrients, making them an excellent source of feed with high biological yield, comprehensive nutritional value, good palatability, and high digestibility. They can serve as a new type of protein feed resource to address feed shortages (Geng et al., 2024). Mulberry leaves grow in regions with diverse climatic conditions worldwide, ranging from temperate to tropical areas. The annual yield

of mulberry leaves can reach 25 to 30 tons per hectare, with a protein content of 18-25% (on a dry matter basis) (Guha et al., 2010; Iqbal et al., 2012; Wang et al., 2022). Moreover, the digestibility of protein in mulberry leaves for animals can reach 75-85% (on a dry matter basis) (Chan et al., 2016; Chen et al., 2018). Analysis reveals that mulberry leaves possess a relatively high crude fat content and low crude fiber content, positioning them as a valuable source of high-quality protein for livestock feed. Additionally, it is also rich in protein, containing 218.6 g/kg (Trabi et al., 2017). They are rich not only in amino acid content but also in diversity. Some essential amino acids, which may be lacking in other feeds, are present in sufficient quantities in mulberry leaves, meeting the amino acid requirements of livestock and poultry and helping to balance amino acid ratios (Leterme et al., 2005). Mulberry leaves are also high in minerals, with 100 g containing 2.699% calcium and 3.101% potassium (Batiha et al., 2023). The vitamin content in mulberry leaves is also abundant, especially in B-complex and C vitamins, which are beneficial for improving the immune systems of animals (Chen et al., 2021). Additionally, mulberry leaves contain various physiologically active substances such as polysaccharides, flavonoids, steroids, volatile oils, and alkaloids, which play important physiological roles (Liu et al., 2001). Multiple studies have demonstrated that incorporating mulberry leaves as a feed ingredient can significantly improve the health status, productivity, meat quality, and flavor of ruminant animals. Supplementing 600 g/d of mulberry leaf feed in a rice straw-based diet can significantly increase the dry matter intake of beef cattle (Tan et al., 2012). Moreover, adding 2 g of air-dried mulberry leaves per head in the daily ration does not adversely affect the feed intake of sheep (Chen et al., 2015). According to Liu et al. (2001), supplementing ammonia-treated rice straw with varying levels of mulberry leaf powder as a replacement for rapeseed meal can enhance feed intake in lambs and improve their growth performance. These findings suggest that mulberry leaf products can be added as a feed ingredient in the diets of ruminant animals.

In recent years, the use of unconventional plant feed ingredients has become increasingly popular, with beneficial plant-based feed ingredients being widely adopted in animal husbandry, especially in intensive farming systems (Hashem et al., 2020; Zhao et al., 2022a). Reports indicate that supplementing animal diets with plant feed can improve animal productivity and immune response while reducing oxidative stress and inflammation (Zhao et al., 2023a). These substances act on the gut microbiota by neutralizing free radicals and preventing their interaction with cellular DNA, while also enhancing nutrient digestion and absorption, thereby exerting immunomodulatory effects (Yu et al., 2023a). Therefore, adding feed ingredients containing natural plant nutrients such as polyphenols to animal diets is considered an optimal alternative for improving animal productivity (Yu et al., 2023b; Zhao et al., 2023b). In organic farms, a diverse grassland with high plant diversity ensures adequate intake of polyphenolic compounds. Achieving such results in intensive farming requires supplementing feed with polyphenol-rich food additives (Huang et al., 2018). This strategy has been supported by research conducted on some farms where the addition of natural plant extract antioxidants to animal feed has improved the quality of animal products (Ji et al., 2018). Additionally, these natural plant extracts play an important role in the hindgut of animals. Therefore, a comprehensive exploration of the effects of natural plant components on the blood and hindgut health of ruminant animals in intensive farming is a focal point of the modern livestock industry. Considering these factors, the aim of this study is to evaluate the effects of supplementing fattening Hu sheep with mulberry leaf powder and retrograde diets on blood parameters and hindgut microbiota.

2 Materials and methods

2.1 Source of dried tea residue

Mulberry leaves purchased from Huzhou, China, were tested for their nutritional levels, yielding the following results: moisture 11.46%, crude protein 19.30%, crude fat 8.25%, crude ash 7.46%, neutral detergent fiber 34.20%, acid detergent fiber 16.38%, calcium 1.54%, phosphorus 0.10%.

2.2 Animals and treatments

This study selected 16 male Hu sheep with a weight of 28.96 ± 1.04 kg at three months of age. These 16 sheep were randomly divided into a control group and a treatment group, with 8 sheep in each group. The control group was fed a basal diet (CON, n=8), while the treatment group was fed with 60 g of mulberry leaf powder (ML, n=8) daily. The dosage of mulberry leaf powder was determined based on the results of an unpublished *in vitro* experiment. The basal diet chosen for the experiment (Table 1) was a complete mixed ration, with a concentrate-to-roughage ratio of 7:3, meeting the requirements of Chinese sheep feeding standards

TABLE 1 Composition and nutrient levels of the basal diet (dry matter basis %).

Ingredients		Nutrient levels[2]	
Corn/%	20.40	ME/(MJ/kg)	10.14
Wheat bran/%	3.56	CP/%	14.09
Soybean meal/%	12.80	EE/%	5.06
Corn silage/%	10.00	NDF/%	50.30
Peanut straw/%	50.00	ADF/%	22.4
NaCl/%	0.28	Ca/%	1.09
$NaCO_3$/%	0.64	P/%	0.43
$CaCO_3$/%	0.24		
$CaHCO_3$/%	0.08		
Premix[1]/%	2.00		

ME, metabolic energy; CP, crude protein; EE, ether extract, NDF, neutral detergent fiber; ADF, acid detergent fiber.
[1]The premix provided the following per kilogram of the diet: Cu 16.0 mg, Fe 35.0 m, Mn 30.0 mg, Zn 80.0 mg, I 0.5 mg, Se 0.10 mg, Co 0.03 mg, VA 14400 IU, VD 4 400 IU, VE 30 mg.
[2]The metabolic energy was calculated and the rest was measured.

(NY/T816-2004). The Hu sheep in the experiment were individually housed in pens and fed the basal diet twice daily (8:00 am and 5:00 pm). They had ad libitum access to feed and water throughout the experiment, which lasted for 56 days, including a 14-day adaptation period and a formal experimental period of 42 days. All experimental techniques followed the rules established by the Experimental Animal Management Committee of the Zhejiang Academy of Agricultural Sciences.

2.3 Sample collection

2.3.1 Blood sample collection

On the morning of the 56th day, 2 hours after feeding, blood samples were collected from the jugular vein of the Hu sheep using non-anticoagulant vacuum tubes. The samples were then centrifuged at 3,000 × g for 15 minutes at 4°C to collect serum. Commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and a microplate reader (Multiskan FC; Thermo Fisher Scientific, Waltham, MA, USA) were used to analyze the concentrations of catalase (CAT), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), total antioxidant capacity (T-AOC), free fatty acids (FFA), albumin (ALB), total protein (TP), low-density lipoprotein (LDL), high-density lipoprotein (HDL), alkaline phosphatase (AKP), acid phosphatase (ACP), glutamate pyruvate transaminase (GPT), triglycerides (TG), total cholesterol (TCH), glutamate oxaloacetate transaminase (GOT), and lactate dehydrogenase (LD). All ELISA data were recorded using a microplate reader (Multiskan FC; Thermo Fisher Scientific, Waltham, MA, USA) and analyzed according to the instructions provided by the supplier using commercial ELISA assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.3.2 Fecal sample collection, 16S rRNA amplicon sequencing and analysis

On the morning of the 56th day, fecal samples were collected 2 hours after feeding and stored in liquid nitrogen until analysis. Genomic DNA was extracted from the collected fecal microbiota using the E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, USA). The quantity and quality of the extracted DNA were evaluated using a ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA), and validation was performed by running samples on a 1% agarose gel. Sequencing of the 16S rRNA gene was conducted using the Illumina MiSeq platform. For bacteria, the V3-V4 region of the 16S rRNA gene was amplified using primers 338F (5'-barcode-ACTCCTRCGGGAGGCAGCAG-3') and 806R (5'-GGACTACCVGGGTATCTAAT-3'). PCR amplification was performed in a 25 μL reaction system, including 2 μL of DNA template, 12.5 μL of 2× Taq PCR MasterMix, 2.5 μL of each primer, and ddH2O to adjust the final volume. PCR products were evaluated by 2% agarose gel electrophoresis and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Amplified products were then mixed at equimolar ratios to generate the amplicon library. Paired-end sequencing (2×300 bp) was performed on the Illumina MiSeq sequencing system. Paired-end reads were merged using FLASH (version 1.2.11). Sequence reads were processed and analyzed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline software (version 1.9.1). Operational Taxonomic Units (OTUs) were assigned to reads based on 97% similarity using average-linkage clustering. OTU raw read counts were normalized against the total number of quality-filtered reads to calculate relative abundances. Taxonomic assignments were performed for each OTU using the RDP classifier (version 2.13) against the SILVA 16S rRNA database (version 138). Alpha diversity indices, including ACE, Chao1, Shannon, and Simpson, were calculated using Mothur (version 1.30.2). Beta diversity analysis was conducted using PCoA based on weighted and unweighted UniFrac distances in QIIME. ANOSIM statistical tests were performed to identify the statistical significance of between-group beta diversity.

2.4 Statistical analyses

The differences in blood biochemical parameters and microbial relative abundances observed in the experiment were analyzed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). After conducting the Shapiro-Wilk test for normality, a two-tailed t-test was employed for analysis. A significance level of $P < 0.05$ was considered to indicate a significant difference, $0.10 < P \leq 0.05$ indicated a trend, and $P \geq 0.10$ indicated no significant difference.

3 Results

3.1 Serum index

From Table 2, it can be observed that the addition of ML to the diet of Hu sheep does not significantly affect the concentrations of MDA, T-AOC, and T-SOD in the blood ($P > 0.05$). However, there is a trend of increased concentrations of GSH-Px ($P = 0.082$) and CAT ($P = 0.058$). This indicates that the addition of ML to the diet has the potential to enhance the antioxidant capacity of Hu sheep. From Table 3, it can be seen that the concentration of TP in the blood significantly decreases after adding ML to the diet ($P = 0.001$), while the concentrations of AKP ($P = 0.027$) and GPT ($P = 0.002$)

TABLE 2 Effects of ML on the antioxidant capacity of blood in Hu sheep.

Item	Treatments		SEM	*P*-value
	CON	ML		
GSH-Px (nmol/min/mL)	223.01	243.70	5.961	0.082
MDA (nmol/mL)	3.23	3.35	0.080	0.483
T-AOC (μmol/mL)	0.06	0.07	0.004	0.738
T-SOD (U/mL)	6.02	5.47	0.331	0.426
CAT (μmol/mL)	1.73	1.92	0.052	0.058

The control group was fed a basal diet (CON, n=8), while the treatment group was fed with 60 g of mulberry leaf powder (ML, n=8) daily. CAT, catalase; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; T-AOC, total antioxidant capacity.

TABLE 3 Effect of ML on hematological biochemical indices in Hu sheep.

Item	Treatments		SEM	*P*-value
	CON	ML		
ALB (g/L)	0.17	0.18	0.013	0.619
TP (mg/mL)	3.92	3.69	0.040	0.001
LD (nmol/min/mL)	28.70	29.96	0.883	0.495
ACP (μmol/min/mL)	0.02	0.01	0.003	0.277
AKP (μmol/min/mL)	0.12	0.17	0.011	0.027
HDL-C (mmol/L)	1.34	1.035	0.505	0.770
LDL-C (mmol/L)	1.00	0.99	0.017	0.938
FFA (μmol/L)	138.50	144.81	4.417	0.495
GPT (nmol/min/mL)	126.77	127.41	0.116	0.002
GOT (nmol/min/mL))	29.65	25.38	1.294	0.100
TG (mmol/L)	0.19	0.21	0.018	0.619
TCH (mmol/L)	1.26	1.20	0.084	0.728

The control group was fed a basal diet (CON, n=8), while the treatment group was fed with 60 g of mulberry leaf powder (ML, n=8) daily. FFA, free fatty acids; ALB, albumin; TP, total protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AKP, alkaline phosphatase; ACP, acid phosphatase; GPT, glutamate pyruvate transaminase; TG, triglycerides; TCH, total cholesterol; GOT, glutamate oxaloacetate transaminase; and LD, lactate dehydrogenase.

significantly increase. However, there are no significant differences in the concentrations of ALB, LD, ACP, HDL-C, LDL-C, FFA, GOT, TG, and TCH ($P > 0.05$).

3.2 Structural changes in the gut microbiota

In Figure 1, the microbial composition of the CON and ML groups is presented. As seen in Figure 1A, the dilution curves of the sequencing samples reach a plateau, indicating that the sequencing depth meets the requirements. Figure 1B shows the PCA scores of the two groups, indicating that there is no significant separation between them. Further analysis of the microbial composition at the phylum level reveals that both the CON and ML groups have similar compositions, primarily composed of Firmicutes, Bacteroidota, Spirochaetota, Proteobacteria, Fibrobacterota, Verrucomicrobiota, Desulfobacterota, Cyanobacteria, Actinobacteriota, and Campylobacterota (Figure 1C). At the genus level, the microbial composition of both groups is also consistent, comprising *Rikenellaceae RC9 gut group*, *UCG-005*, *Bacteroides*, *Treponema*, *Alistipes*, *Christensenellaceae R-7 group*, *Monoglobus*, *Lachnospiraceae NK4A136 group*, *Succinivibrio*, and *Prevotellaceae UCG-003* (Figure 1D). Additionally, according to Table 4, there are no significant differences in the α-diversity of the microbiota ($P > 0.05$). This suggests that the addition of ML to the diet does not have a negative impact on the composition of hindgut microbiota.

We compared the specific microbial abundances and found that after adding ML to the diet, the abundance of Campylobacterota at the phylum level significantly increased ($P < 0.05$). At the genus level, the abundances of *Campylobacter* and *Mailhella* significantly increased ($P < 0.05$), while the abundances of *Alloprevotella*, *Roseburia*, and *Prevotellaceae UCG-003* significantly decreased ($P < 0.05$). There was a decreasing trend in the abundances of *Rikenellaceae RC9 gut group* ($P = 0.067$) and *UCG-002* ($P = 0.081$, Figure 2).

Figure 3 displays the core composition of gut microbiota in the CON and ML groups. In the CON group, the significant core microbiota include *Ruminococcaceae* uncultured, *Hydrogenoanaerobacterium*, *Succinivibrio*, *Acetitomaculum*, *Clostridia* norank, *UCG-009*, *NK4A214* group, and *Alloprevotella*. In the ML group, the significant core microbiota consist of *Lachnoclostridium*, *Rickettsiales* norank, *UCG-005*, *Marinbryantia*, *Oscillibacter*, *Ruminococcaceae* norank, *Papillibacter*, *Ruminiclostridium*, *Gastranaerophilales*, *Quinella*, and *Ruminococcus*.

3.3 Correlation between gut microbiota and blood indices

We utilized Spearman correlation analysis to examine the correlation between trending microbiota and all detected blood indices (Figure 4). We found a significant negative correlation ($P < 0.05$, $R < -0.5$) between *Prevotellaceae UCG-003* and ABL, TG, LD, and FFA. *Mailhella* exhibited a significant positive correlation ($P < 0.05$, $R > 0.5$) with LD, FFA, and ACP. *UCG-002* showed a significant positive correlation ($P < 0.05$, $R > 0.5$) with MDA and ACP, and a significant negative correlation ($P < 0.05$, $R < -0.5$) with T-AOC. ACP exhibited a significant positive correlation ($P < 0.05$, $R > 0.5$) with *Campylobacter* and a significant negative correlation ($P < 0.05$, $R < -0.5$) with *Alloprevotella*. *Rikenellaceae RC9 gut group* showed a significant positive correlation ($P < 0.05$, $R > 0.5$) with ALB.

4 Discussion

Adding food, industrial by-products, or unconventional feed products to animal diets is becoming an important approach in the development of animal husbandry today. For example, adding apple pomace (Maslovarić et al., 2017), citrus peel (Zhao et al., 2022b), and other vegetable and fruit waste to feed has become increasingly common (Sahoo et al., 2021). Previous studies have demonstrated that mulberry leaves are rich in various bioactive compounds, including flavonoids and phenolic acids, which contribute to their efficacy as a high-quality immune modulator (Cui X. et al., 2023). These bioactive components, such as flavonoids, can effectively improve the health status of the host through pathways like the PI3K-Akt signaling pathway and the AGE-RAGE pathway (Lv et al., 2022). Additionally, many studies have reported that mulberry leaves also have a certain growth-promoting effect, which will be an important focus of our future research (Ding et al., 2021; Mengistu et al., 2020).

In the animal biological system, the redox system plays a crucial role in the host's immune and health status. GSH-Px, MDA, T-AOC, T-SOD, and CAT are the main indicators used to evaluate the antioxidant capacity of animals. Among these, GSH-Px reduces

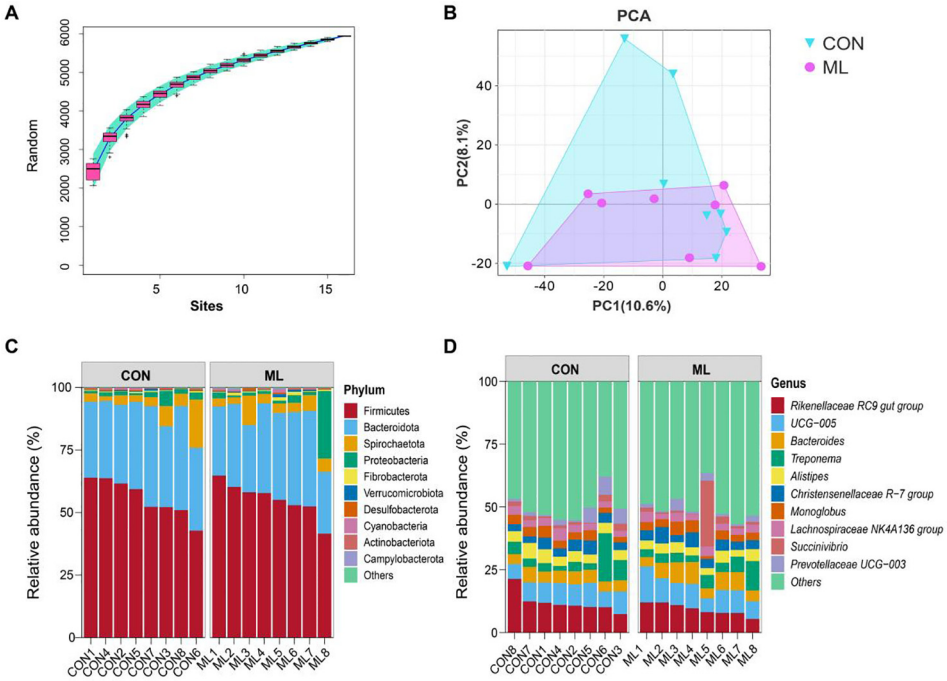


FIGURE 1
Overview of the structure and composition of gut microbiota. **(A)** Dilution curves of all samples. **(B)** PCA score plot based on unweighted analysis. **(C)** Composition of bacteria at the phylum level across all samples. **(D)** Composition of bacteria at the genus level across all samples. The control group was fed a basal diet (CON, n=8), while the treatment group was fed with 60 g of mulberry leaf powder (ML, n=8) daily.

toxic hydrogen peroxide and organic peroxides to relatively harmless water and corresponding alcohols, playing a role in clearing peroxides within cells (Czyżowska et al., 2023; Sharma et al., 2021). CAT, on the other hand, is another antioxidant enzyme responsible for breaking down hydrogen peroxide (a type of oxygen free radical) into water and oxygen, thus protecting cells from oxidative stress damage, especially in tissues with active aerobic metabolism such as the liver and lungs (Kang et al., 2013). In this study, there was an increasing trend in GSH-Px and CAT after adding mulberry leaves to the diet, indicating that mulberry leaves have a positive effect on improving the immune status of sheep. This finding is consistent with previous research results (Jin et al., 2022; Li et al., 2022; Shi et al., 2022). This effect may be attributed to the various bioactive components abundant in mulberry leaves, similar to the significant antioxidant capacity reported previously for polysaccharides and flavonoids in mulberry leaves (Kim et al., 2020; Liao et al., 2017). Our current research does not offer a comprehensive analysis of antioxidant indicators. While some indicators show an improving trend, this does not fully substantiate the antioxidant effects of ML. It merely suggests that ML does not negatively impact the antioxidant system of Hu sheep. The specific effects will be explored in greater detail in future studies.

TABLE 4 Effects of ML on the α diversity of fecal bacteria in Hu sheep.

Item	Treatments		SEM	*P*-value
	CON	ML		
Observed_species	2418.38	2460.75	56.638	0.722
Chao1	2897.90	2941.67	61.076	0.733
ACE	2948.43	3009.75	59.821	0.625
Shannon	6.41	6.38	0.087	0.904
Simpson	0.99	0.99	0.002	0.547

The control group was fed a basal diet (CON, n=8), while the treatment group was fed with 60 g of mulberry leaf powder (ML, n=8) daily.

During the growth and development of animals, the concentrations of AKP and GPT reflect the growth and remodeling of bone and muscle tissues (Eriksson et al., 2016; Sobhani et al., 2021; Wang et al., 2017). In bone tissue, AKP is primarily secreted by osteoblasts and plays a critical role in promoting the deposition of calcium and phosphate, thereby facilitating bone mineralization and formation. AKP catalyzes the hydrolysis of phosphate esters, converting organic phosphate esters into inorganic phosphate and alcohols or phenols, a process essential for bone mineralization (Ansari et al., 2022). GPT catalyzes the transamination reaction between glutamate and pyruvate, converting glutamate to α-ketoglutarate and pyruvate to alanine. This process is essential for amino acid metabolism and nitrogen transport (Wei et al., 2022). In this study, the addition of mulberry leaves to the animal diet resulted in increased concentrations of AKP and GPT, suggesting that mulberry leaves may influence certain blood hormones to regulate the host's development. However, the specific mechanisms underlying this effect warrant further investigation. The concentration of TP also reflects the metabolism within the animal's body. A slight decrease in TP levels may indicate metabolic adjustments within the animal's

10.3389/fanim.2024.1469850

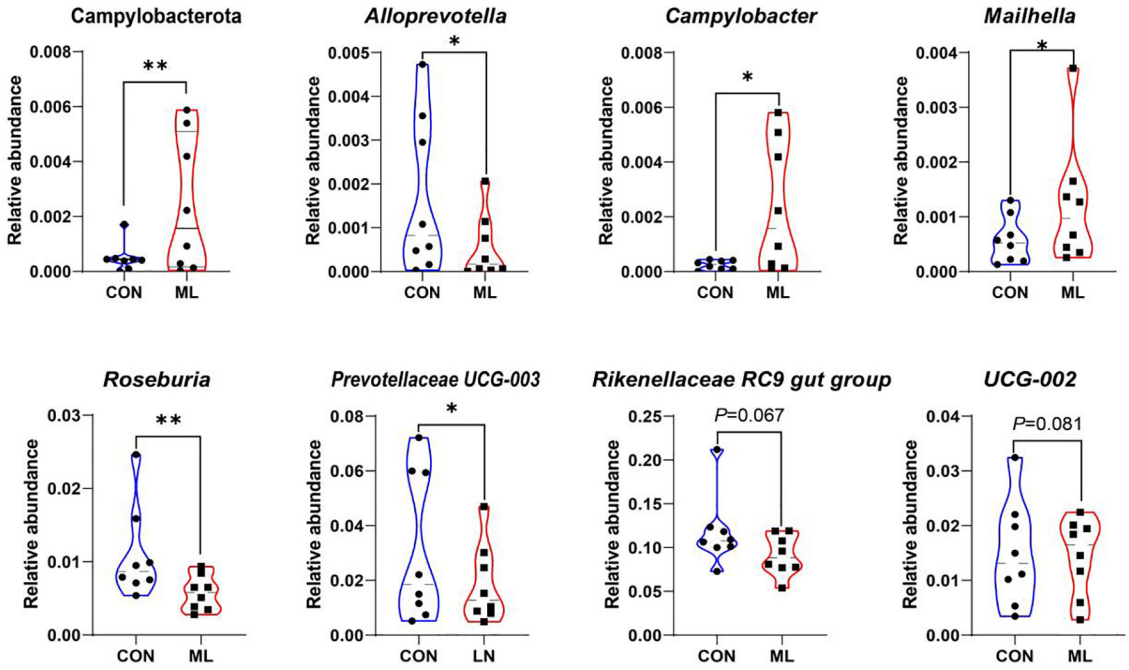


FIGURE 2
Comparative analysis of microbial relative abundance. * denotes $P < 0.05$, ** denotes $P < 0.01$. Data were presented as means ± SEM (n=8 per group). The control group was fed a basal diet (CON, n=8), while the treatment group was fed with 60 g of mulberry leaf powder (ML, n=8) daily.

body (Mapfumo and Muchenje, 2015; Rogaly et al., 1982). In this study, the observed decrease in TP levels may be associated with the liver and kidney functions of Hu sheep. However, it remains unclear whether ML have a beneficial or detrimental effect on TP regulation. Some studies suggest that a moderate reduction in TP may be linked to immune system modulation, potentially lowering hyperactive immune responses and reducing inflammation-related damage (Azzini et al., 2022; Fuellen et al., 2023). Although there are indirect reports indicating that supplementing Hu sheep diets with ML may enhance protein utilization efficiency in intensive farming, the specific mechanisms underlying this effect remain unexplored and require further investigation (Ariyarathne et al.,

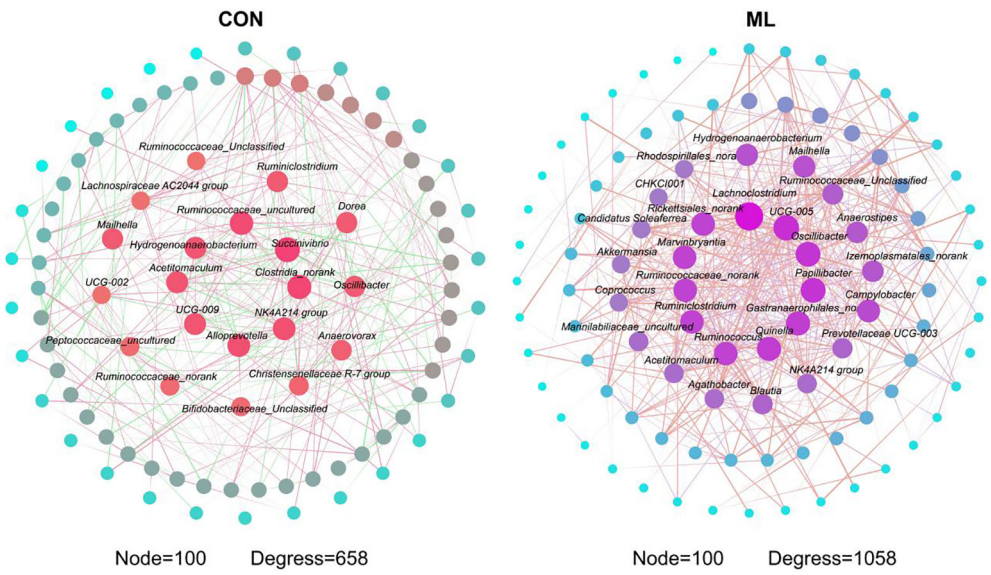


FIGURE 3
Overview of the composition of core microbiota network interactions. Data were presented as means ± SEM (n=8 per group). The control group was fed a basal diet (CON, n=8), while the treatment group was fed with 60 g of mulberry leaf powder (ML, n=8) daily.

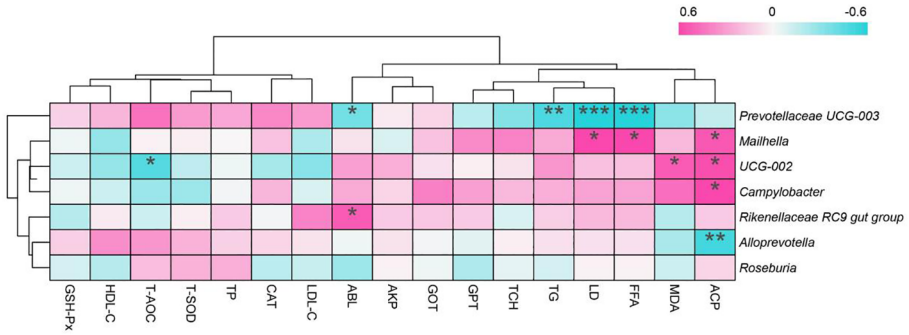


FIGURE 4
Correlation analysis between blood indices and microbiota showing trends of change. * denotes $P < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.001$. The control group was fed a basal diet (CON, n=8), while the treatment group was fed with 60 g of mulberry leaf powder (ML, n=8) daily. CAT, catalase; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; T-AOC, total antioxidant capacity. FFA, free fatty acids; ALB, albumin; TP, total protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AKP, alkaline phosphatase; ACP, acid phosphatase; GPT, glutamate pyruvate transaminase; TG, triglycerides; TCH, total cholesterol; GOT, glutamate oxaloacetate transaminase, and LD, lactate dehydrogenase.

2021; Laroche et al., 2022). This suggests that the addition of mulberry leaves to the diet has the potential to influence the host's physiological processes. However, further investigation is needed to determine whether all these effects are indeed beneficial (Cui W. et al., 2023).

In this study, we focused on the influence of mulberry leaves on the composition of the intestinal microbiota. At the phylum level, there was a significant increase in the abundance of Campylobacterota, which may be due to the relative decrease in the abundance of other phyla. At the genus level, *Campylobacterin* the intestine may play a role in food degradation and digestion, potentially secreting enzymes that break down complex food components and facilitate nutrient absorption in the host (Burnham and Hendrixson, 2018). However, most studies identify *Campylobacter* as a harmful pathogen responsible for animal diseases. The observed increase in abundance in this study might be linked to the host's nutrient metabolism. *Campylobacter* possesses unique metabolic pathways, primarily utilizing amino acids and organic acids as carbon and energy sources, instead of relying on carbohydrate fermentation. This adaptation enables their survival in low-oxygen environments, such as the gut and foodborne microbial niches (Stahl et al., 2012). Moreover, there are few reports regarding *Mailhella*, but it may contribute to maintaining the diversity and stability of the intestinal microbiota, interacting with other microbial communities to collectively maintain normal intestinal function. The significant increase in the abundance of *Campylobacterota*, *Campylobacter*, and *Mailhella* in this study may be related to changes in certain indicators in the blood, as demonstrated in the correlation analysis. Meanwhile, the observed decrease in the abundance of *Alloprevotella*, *Roseburia*, and *Prevotellaceae UCG-003* may be attributed to changes in the microbial network. Additionally, their changes are strongly correlated with immune metabolic capacity (Shen et al., 2022; Ye et al., 2022; Yu et al, 2023c). Finally, we observed a significant increase in the complexity of the microbial network in the hindgut following the addition of mulberry leaves to the diet. More complex microbial networks are known to possess greater resistance to adverse external factors (Yu et al., 2023b; Yu et al., 2024), further supporting the notion that mulberry leaves can modulate intestinal microbiota structure and enhance host health.

5 Conclusions

This study offers new insights into the use of ML in ruminant production. The inclusion of ML can elevate the levels of AKP and GPT in the blood, and showing potential to enhance the host's antioxidant capacity and improve the hindgut microbiota structure of Hu sheep. These findings are significant for the development of alternative feed resources. ML is a promising feed ingredient that benefits both animal health and environmental sustainability. Further investigation into ML's impact on rumen fermentation, metabolism, and microbial communities in ruminants will support its wider adoption in ruminant production.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI BioProject, accession PRJNA1191173.

Ethics statement

The animal study was approved by Animal Care and Use Committee at Zhejiang Academy of Agricultural Sciences. The study was conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

LG: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. XS: Software, Supervision, Validation, Visualization, Writing – original draft. FC: Data curation, Methodology, Project administration, Resources, Software, Writing – original draft. SH: Investigation, Software, Supervision, Validation, Visualization, Writing – original draft. WQ: Conceptualization, Formal analysis, Funding acquisition, Investigation, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the China Agriculture Research System of MOF and MARA (CARS-18-SYZ05) and Zhejiang Province Industrial Technology Team Project (2022CSSFD08).

Conflict of interest

Author SH was employed by the company Central Plains Environmental Protection Co. Ltd.

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

Publisher's note

References

Ansari, S., Ito, K., and Hofmann, S. (2022). Alkaline phosphatase activity of serum affects osteogenic differentiation cultures. *ACS Omega* 7, 12724–12733. doi: 10.1021/acsomega.1c07225

Ariyarathne, H., Correa-Luna, M., Blair, H. T., Garrick, D. J., and Lopez-Villalobos, N. (2021). Identification of genomic regions associated with concentrations of milk fat, protein, urea and efficiency of crude protein utilization in grazing dairy cows. *Genes* 12, 456. doi: 10.3390/genes12030456

Azzini, E., Peluso, I., Intorre, F., Barnaba, L., Venneria, E., Foddai, M. S., et al. (2022). Total and plant protein consumption: the role of inflammation and risk of non-communicable disease. *Int. J. Mol. Sci.* 23, 8008. doi: 10.3390/ijms23148008

Batiha, G. E., Al-Snafi, A. E., Thuwaini, M. M., Teibo, J. O., Shaheen, H. M., Akomolafe, A. P., et al. (2023). Morus alba: a comprehensive phytochemical and pharmacological review. *Naunyn. Schmiedebergs. Arch. Pharmacol.* 396, 1399–1413. doi: 10.1007/s00210-023-02434-4

Burnham, P. M., and Hendrixson, D. R. (2018). Campylobacter jejuni: collective components promoting a successful enteric lifestyle. *Nat. Rev. Microbiol.* 16, 551–565. doi: 10.1038/s41579-018-0037-9

Chan, E. W., Lye, P., and Wong, S. (2016). Phytochemistry, pharmacology, and clinical trials of morus alba. *Chin. J. Nat. Med.* 14, 17–30. doi: 10.3724/SP.J.1009.2016.00017

Chen, C., Mohamad Razali, U. H., Saikim, F. H., Mahyudin, A., and Mohd Noor, N. Q. I. (2021). Morus alba l. Plant: bioactive compounds and potential as a functional food ingredient. *Foods* 10, 689. doi: 10.3390/foods10030689

Chen, D., Chen, X., Tu, Y., Wang, B., Lou, C., Ma, T., et al. (2015). Effects of mulberry leaf flavonoid and resveratrol on methane emission and nutrient digestion in sheep. *Anim. Nutr.* 1, 362–367. doi: 10.1016/j.aninu.2015.12.008

Chen, X., Zhang, T., Wang, X., Hamann, M. T., Kang, J., Yu, D., et al. (2018). A chemical investigation of the leaves of morus alba l. *Molecules* 23, 1018. doi: 10.3390/molecules23051018

Cui, W., Luo, K., Xiao, Q., Sun, Z., Wang, Y., Cui, C., et al. (2023). Effect of mulberry leaf or mulberry leaf extract on glycemic traits: a systematic review and meta-analysis. *Food Funct.* 14, 1277–1289. doi: 10.1039/d2fo02645g

Cui, X., Yang, Y., Zhang, M., Liu, S., Wang, H., Jiao, F., et al. (2023). Transcriptomics and metabolomics analysis reveal the anti-oxidation and immune boosting effects of mulberry leaves in growing mutton sheep. *Front. Immunol.* 13. doi: 10.3389/fimmu.2022.1088850

Czyżowska, A., Brown, J., Xu, H., Sataranatarajan, K., Kinter, M., Tyrell, V. J., et al. (2023). Elevated phospholipid hydroperoxide glutathione peroxidase (gpx4) expression modulates oxylipin formation and inhibits age-related skeletal muscle atrophy and weakness. *Redox Biol.* 64, 102761. doi: 10.1016/j.redox.2023.102761

Ding, Y., Jiang, X., Yao, X., Zhang, H., Song, Z., He, X., et al. (2021). Effects of feeding fermented mulberry leaf powder on growth performance, slaughter performance, and meat quality in chicken broilers. *Animals* 11, 3294. doi: 10.3390/ani11113294

Eriksson, M., Strandberg, G., Lipcsey, M., and Larsson, A. (2016). Evaluation of intraosseous sampling for measurements of alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, creatinine kinase, gamma-glutamyl transferase and lactate dehydrogenase. *Scand. J. Clin. Lab. Invest.* 76, 597–600. doi: 10.1080/00365513.2016.1230774

Fuellen, G., Walter, U., Henze, L., Bohmert, J., Palmer, D., Lee, S., et al. (2023). Protein biomarkers in blood reflect the interrelationships between stroke outcome, inflammation, coagulation, adhesion, senescence and cancer. *Cell. Mol. Neurobiol.* 43, 1413–1424. doi: 10.1007/s10571-022-01260-1

Geng, B., Gao, J., Cheng, H., Guo, G., and Wang, Z. (2024). Effects of dietary mulberry leaves on growth, production performance, gut microbiota, and immunological parameters in poultry and livestock: a systematic review and meta-analysis. *Anim. Biosci.* 37, 1065–1076. doi: 10.5713/ab.23.0449

Guha, A., Sengupta, D., and Reddy, A. R. (2010). Physiological optimality, allocation trade-offs and antioxidant protection linked to better leaf yield performance in drought exposed mulberry. *J. Sci. Food. Agric.* 90, 2649–2659. doi: 10.1002/jsfa.4135

Hashem, N. M., Gonzalez-Bulnes, A., and Simal-Gandara, J. (2020). Polyphenols in farm animals: source of reproductive gain or waste? *Antioxidants* 9, 1023. doi: 10.3390/antiox9101023

Huang, Q., Liu, X., Zhao, G., Hu, T., and Wang, Y. (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Anim. Nutr.* 4, 137–150. doi: 10.1016/j.aninu.2017.09.004

Iqbal, S., Younas, U., Sirajuddin,, Chan, K. W., Sarfraz, R. A., and Uddin, M. K. (2012). Proximate composition and antioxidant potential of leaves from three varieties of mulberry (morus sp.): A comparative study. *Int. J. Mol. Sci.* 13, 6651–6664. doi: 10.3390/ijms13066651

Ji, Z. K., Zhao, Y., Yu, S. S., and Zhao, H. (2018). The application progress of 3d printing technology in ophthalmology. *Chung-Hua Yen K'o Tsa Chih.* 54, 72–76. doi: 10.3760/cma.j.issn.0412-4081.2018.01.014

Jin, Y., Tu, J., Han, X., Zhuo, J., Liu, G., Han, Y., et al. (2022). Characteristics of mulberry leaf powder enriched with gamma-aminobutyric acid and its antioxidant capacity as a potential functional food ingredient. *Front. Nutr.* 9. doi: 10.3389/fnut.2022.900718

Kang, M. Y., Kim, H., Piao, C., Lee, K. H., Hyun, J. W., Chang, I., et al. (2013). The critical role of catalase in prooxidant and antioxidant function of p53. *Cell Death Differ.* 20, 117–129. doi: 10.1038/cdd.2012.102

Kim, M., Nam, D., Ju, W., Choe, J., and Choi, A. (2020). Response surface methodology for optimization of process parameters and antioxidant properties of mulberry (morus alba l.) Leaves by extrusion. *Molecules* 25, 5231. doi: 10.3390/molecules25225231

Laroche, J. P., Gervais, R., Lapierre, H., Ouellet, D. R., Tremblay, G. F., Halde, C., et al. (2022). Milk production and efficiency of utilization of nitrogen, metabolizable protein, and amino acids are affected by protein and energy supplies in dairy cows fed alfalfa-based diets. *J. Dairy Sci.* 105, 329–346. doi: 10.3168/jds.2021-20923

Leterme, P., Londo O, A. M., Estrada, F., Souffrant, W. B., and Buldgen, A. (2005). Chemical composition, nutritive value and voluntary intake of tropical tree foliage and cocoyam in pigs. *J. Sci. Food Agric.* 85, 1725–1732. doi: 10.1002/jsfa.2177

Li, R., Zhu, Q., Wang, X., and Wang, H. (2022). Mulberry leaf polyphenols alleviated high-fat diet-induced obesity in mice. *Front. Nutr.* 9. doi: 10.3389/fnut.2022.979058

Liao, B., Zhu, D., Thakur, K., Li, L., Zhang, J., and Wei, Z. (2017). Thermal and antioxidant properties of polysaccharides sequentially extracted from mulberry leaves (morus alba l.). *Mol. (Basel Switzerland)* 22, 2271. doi: 10.3390/molecules22122271

Liu, J. X., Yao, J., Yan, B., Yu, J. Q., and Shi, Z. Q. (2001). Effects of mulberry leaves to replace rapeseed meal on performance of sheep feeding on ammoniated rice straw diet. *Small Ruminant Res.* 39, 131–136. doi: 10.1016/S0921-4488(00)00180-2

Lv, Q., Lin, J., Wu, X., Pu, H., Guan, Y., Xiao, P., et al. (2022). Novel active compounds and the anti-diabetic mechanism of mulberry leaves. *Front. Pharmacol.* 13. doi: 10.3389/fphar.2022.986931

Mapfumo, L., and Muchenje, V. (2015). Comparative changes in monthly blood urea nitrogen, total protein concentrations, and body condition scores of nguni cows and heifers raised on sweetveld. *S. Afr. J. Anim. Sci.* 45, 96–103. doi: 10.4314/SAJAS.V45I1.12

Maslovarić, M. D., Vukmirović, Đ., Pezo, L., Čolović, R., Jovanović, R., Spasevski, N., et al. (2017). Influence of apple pomace inclusion on the process of animal feed pelleting. *Food Additives Contaminants: Part A.* 34, 1353–1363. doi: 10.1080/19440049.2017.1303851

Mengistu, G., Assefa, G., and Tilahun, S. (2020). Noug seed (guizotia abyssinica) cake substituted with dried mulberry (morus indica) andvernonia amygdalina mixed leaves' meal on growth performances of bonga sheep at teppi, Ethiopia. *J. Nutr. Metab.* 2020, 1–10. doi: 10.1155/2020/9308761

Rogaly, E., Clague, M. B., Carmichael, M. J., Wright, P. D., and Johnston, I. D. A. (1982). Comparison of body protein metabolism during total parenteral nutrition using glucose or glucose and fat as the energy source. *Clin. Nutr.* 1, 81–90. doi: 10.1016/0261-5614(82)90008-5

Sahoo, A., Sarkar, S., Lal, B., Kumawat, P., Sharma, S., and De, K. (2021). Utilization of fruit and vegetable waste as an alternative feed resource for sustainable and eco-friendly sheep farming. *Waste Manage.* 128, 232–242. doi: 10.1016/j.wasman.2021.04.050

Sharma, G., Shin, E., Sharma, N., Nah, S., Mai, H. N., Nguyen, B. T., et al. (2021). Glutathione peroxidase-1 and neuromodulation: novel potentials of an old enzyme. *Food. Chem. Toxicol.* 148, 111945. doi: 10.1016/j.fct.2020.111945

Shen, Z., Luo, W., Tan, B., Nie, K., Deng, M., Wu, S., et al. (2022). Roseburia intestinalis stimulates tlr5-dependent intestinal immunity against crohn's disease. *Ebiomedicine* 85, 104285. doi: 10.1016/j.ebiom.2022.104285

Shi, Y., Zhong, L., Fan, Y., Zhang, J., Zhong, H., Liu, X., et al. (2022). The protective effect of mulberry leaf flavonoids on high-carbohydrate-induced liver oxidative stress, inflammatory response and intestinal microbiota disturbance in monopterus albus. *Antioxidants* 11, 976. doi: 10.3390/antiox11050976

Sobhani, S., Aryan, R., AkbariRad, M., Ebrahimi, M. E., Alinezhad-Namaghi, M., Sobhani, S. R., et al. (2021). The association between anthropometry indices and serum concentrations of gamma-glutamyl transferase, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. *BioMed. Res. Int.* 2021, 2365399. doi: 10.1155/2021/2365399

Stahl, M., Butcher, J., and Stintzi, A. (2012). Nutrient acquisition and metabolism by campylobacter jejuni. *Front. Cell. Infect. Microbiol.* 2. doi: 10.3389/fcimb.2012.00005

Tan, N. D., Wanapat, M., Uriyapongson, S., Cherdthong, A., and Pilajun, R. (2012). Enhancing mulberry leaf meal with urea by pelleting to improve rumen fermentation in cattle. *Asian Australas. J. Anim. Sci.* 25, 452–461. doi: 10.5713/ajas.2011.11270

Trabi, E. B., Yuan, X., Li, J., Dong, Z., Shah, A. A., and Shao, T. (2017). Effect of glucose and lactic acid bacteria on the fermentation quality, chemical compositions effect of glucose and lactic acid bacteria on the fermentation quality, chemical compositions and in *vitro* digestibility of mulberry (morus alba) leaf silage. *Pak. J. Zool.* 49, 2271–2277. doi: 10.17582/journal.pjz/2017.49.6.2271.2277

Wang, F., Wang, X., Wang, K., Han, L., Wang, J., and Xue, C. (2017). Phosphorylated peptides from antarctic krill (euphausia superba) improve fracture healing in mice with ovariectomy induced osteoporosis. *J. Food Biochem.* 41, e12408. doi: 10.1111/jfbc.12408

Wang, L., Gao, H., Sun, C., and Huang, L. (2022). Protective application of morus and its extracts in animal production. *Animals* 12, 3541. doi: 10.3390/ani12243541

Wei, P., Bott, A. J., Cluntun, A. A., Morgan, J. T., Cunningham, C. N., Schell, J. C., et al. (2022). Mitochondrial pyruvate supports lymphoma proliferation by fueling a glutamate pyruvate transaminase 2-dependent glutaminolysis pathway. *Sci. Adv.* 8, eabq117. doi: 10.1126/sciadv.abq0117

Ye, M., Hou, M., Peng, Q., Jia, S., Peng, B., Yin, F., et al. (2022). The microbiota and cytokines correlation between the jejunum and colon in altay sheep. *Animals* 12, 1564. doi: 10.3390/ani12121564

Yu, S., Li, L., Zhao, H., Liu, M., Jiang, L., and Zhao, Y. (2023a). Citrus flavonoid extracts alter the profiling of rumen antibiotic resistance genes and virulence factors of dairy cows. *Front. Microbiol.* 14. doi: 10.3389/fmicb.2023.1201262

Yu, S., Li, L., Zhao, H., Tu, Y., Liu, M., Jiang, L., et al. (2023b). Characterization of the dynamic changes of ruminal microbiota colonizing citrus pomace waste during rumen incubation for volatile fatty acid production. *Microbiol. Spectr.* 11, e351722. doi: 10.1128/spectrum.03517-22

Yu, S., Li, L., Zhao, H., Zhang, S., Tu, Y., Liu, M., et al. (2023c). Dietary citrus flavonoid extract improves lactational performance through modulating rumen microbiome and metabolites in dairy cows. *Food Funct.* 14, 94–111. doi: 10.1039/d2fo02751h

Yu, S., Zhao, Y., Li, L., Zhao, H., Liu, M., and Jiang, L. (2024). Flavonoids from citrus peel display potential synergistic effects on inhibiting rumen methanogenesis and ammoniagenesis: a microbiome perspective. *Environ. Sci. pollut. Res.* 31, 21208–21223. doi: 10.1007/s11356-024-32509-5

Zhao, Y., Yu, S., Li, L., Zhao, H., Li, Y., Jiang, L., et al. (2023a). Feeding citrus flavonoid extracts decreases bacterial endotoxin and systemic inflammation and improves immunometabolic status by modulating hindgut microbiome and metabolome in lactating dairy cows. *Anim. Nutr.* 13, 386–400. doi: 10.1016/j.aninu.2023.03.007

Zhao, Y., Yu, S., Zhang, S., Li, Y., Tu, Y., Liu, M., et al. (2022a). Microbiome-metabolomics insights into the milk of lactating dairy cows to reveal the health-promoting effects of dietary citrus peel extracts on the mammary metabolism. *Foods* 11, 4119. doi: 10.3390/foods11244119

Zhao, Y., Yu, S., Zhao, H., Li, L., Li, Y., Liu, M., et al. (2023b). Integrated multi-omics analysis reveals the positive leverage of citrus flavonoids on hindgut microbiota and host homeostasis by modulating sphingolipid metabolism in mid-lactation dairy cows consuming a high-starch diet. *Microbiome* 11, 236. doi: 10.1186/s40168-023-01661-4

Zhao, Y., Yu, S., Zhao, H., Li, L., Li, Y., Tu, Y., et al. (2022b). Lipidomic profiling using gc and lc-ms/ms revealed the improved milk quality and lipid composition in dairy cows supplemented with citrus peel extract. *Food Res. Int.* 161, 111767. doi: 10.1016/j.foodres.2022.111767

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 06 February 2025
DOI 10.3389/fanim.2024.1458762

OPEN ACCESS

EDITED BY
Anusorn Cherdthong,
Khon Kaen University, Thailand

REVIEWED BY
Chompunut Lumsangkul,
National Chung Hsing University, Taiwan
Jessada Rattanawut,
Prince of Songkla University, Thailand

*CORRESPONDENCE
Ning Liu
ningliu68@163.com

RECEIVED 03 July 2024
ACCEPTED 26 December 2024
PUBLISHED 06 February 2025

CITATION
Li Y, Liu D, Li W, Zhao L, Cao P, Ma Y, Ding K, Zhang C and Liu N (2025) Optimizing *Flammulina velutipes* residue use: impact on health metrics and performance in three-yellow chickens. *Front. Anim. Sci.* 5:1458762. doi: 10.3389/fanim.2024.1458762

Optimizing *Flammulina velutipes* residue use: impact on health metrics and performance in three-yellow chickens

Yuanxiao Li[1,2], Dan Liu[1], Wang Li[1,2], Longmei Zhao[1], Pinghua Cao[1], Yanbo Ma[1,2], Ke Ding[1], Cai Zhang[1,2] and Ning Liu[1]*

[1]College of Animal Science, Henan University of Science and Technology, Luoyang, China, [2]International Joint Laboratory of Animal Welfare and Healthy Breeding in Henan Province, Luoyang, China

Introduction: This study investigated the effects of *Flammulina velutipes* residue (FVR) on performance, antioxidant function, immunity, and intestinal flora of broilers.

Methods: A total of 192 one-day-old three-yellow chickens were divided into four groups of 48 chickens per group, 6 replicates per group and 8 chickens per replicate. The control group (CON) was fed a basal diet, while the remaining three groups were supplemented with FVR in the basal diet, adding 2%, 4% and 6% of the basal diet, respectively. The experiment lasted for 48 days. Blood samples were collected from the jugular vein on days 28 and 48 to determine serum biochemical indices. Caecum contents were collected on day 48 to assess flora diversity.

Results and discussion: No significant differences were observed in dry matter intake (DMI), average daily gain (ADG), or feed conversion ratio (FCR) between the 2% and 4% group and the CON. However, the 6% FRV group showed significantly reduced DMI and FCR. The FVR groups exhibited significantly increased levels of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPC-PX) and total antioxidant capacity (T-AOC), along with significantly decreased malondialdehyde (MDA) content. Additionally, serum interleukin-1 (IL-1) levels decreased, while immunoglobulin G (IgG), immunoglobulin A (IgA) and interleukin 10 (IL-10) levels significantly increased in the FVR groups. The caecal flora diversity test revealed that FVR altered the flora structure, with increased proportions of *Bacteroides*, *Ruminococcus* and *Faecalibacterium* in the 6% FVR groups. In conclusion, FVR can significantly enhance the antioxidant capacity and immunity of broilers and enrich the structure of intestinal flora. The impact on growth performance is limited and dosage-dependent. Further research is needed to optimize its use in poultry diets.

KEYWORDS

***Flammulina velutipes* residue, performance, antioxidant, immunity, cecal flora**

1 Introduction

Poultry feed, predominantly consisting of corn, soybean meal, and other grains, serves as the foundation of poultry farming and constitutes approximately one-third of the global food resources (Silver et al., 2021). By 2025, the consumption of animal-based food is projected to increase by 50% (McIntyre et al., 2009). Over the past few decades, the global population has grown significantly, reaching 7.43 billion in 2015, with food demand doubling and surpassing the Earth's regenerative capacity (Staniškis, 2012; Michiel et al., 2021). Furthermore, the Food and Agriculture Organization estimates a 70% increase in global food demand by 2050 (Bruinsma, 2003). Given the continuous global population growth and climate change-induced food shortages, the conflict between feed resource supply and demand is intensifying, posing a significant threat to the sustainable development of poultry farming (Mottet and Tempio, 2017).

China, home to 20% of the world's population but only 6% of its land area (Ziegler, 2006; Zhang, 2011), faces significant challenges. The rapid development of its poultry industry has dramatically increased food demand, particularly for grain, which has doubled, posing a substantial impact on the global food market and potentially leading to a food security crisis (Rosegrant et al., 2001; Trostle, 2008; Cao and Li, 2013; Chen and Nie, 2016).

As a producer of edible fungi, China boasts a diverse range of these resources (Falandysz, 2013; Yadav and Samadder, 2018), with an annual output of nearly 2 million tons that will continue to increase (Aida et al., 2009; Amin et al., 2014). Approximately 5 kg of residue is produced per kilogram of edible fungi (Lin et al., 2014; Zisopoulos et al., 2016). These residues are rich in proteins, vitamins, and trace elements (Medina et al., 2009; Zhu et al., 2012), cellulose and lignin, promoting food digestion (Fazaeli et al., 2014) and offering potential health benefits (Manila et al., 2000). As a foodborne mushroom, the *Flammulina mushroom* is deeply loved by Asian people because of its unique fragrance (Leifa et al., 2001; Ko et al., 2007; Jing et al., 2014). In addition, it contains various immune-active substances such as fungal ribosome inactivation protein (RIP) and fungal immunomodulatory protein (FIP), which deserve more attention. RIP from *Flammulina velutifolia*, with a molecular weight of 30 kDa, effectively inhibits tumor cells (Ng, 2006). FVPB2, a new polysaccharide from *Flammulina vellum*, enhances the immune response in mice, enabling B cells to induce high immunoglobulin (Ig) M and IgG levels (Wang et al., 2018). Polysaccharides extracted from *Flammulina velutipes* residue (FVR) also demonstrate strong antioxidant activity (Lin et al., 2016). Despite these benefits, edible mushroom residues are often discarded (Chiu et al., 1998; Williams et al., 2001).

Development and utilization of FVR and its rational use in broiler production will be conducive to increasing the diversity of feed, reducing the pressure of "human and animal competition for food", and support the sustainable development of animal husbandry. The three-yellow chicken, a renowned breed in China for its meat and nutritional qualities, has been under-researched regarding the effects of FVR. The three-yellow chicken is typically raised for approximately 60–70 days to reach a market weight of 1.5–2.0 kg. This study examines the impact of varying FVR concentrations on the antioxidant activity, immunity, and intestinal flora of three-yellow chickens, evaluating the feasibility of incorporating FVR into their basal diet.

2 Materials and methods

2.1 Treatments, experimental diet and management

One-day old three-yellow chickens were selected as the experimental subjects. A total of 192 healthy three-yellow chickens (96 males and 96 females) with similar body weight were purchased and randomly divided into four treatment groups, each comprising 48 chickens. Each treatment group comprised six replicates, with eight chickens per replicate. The control (CON) group was fed a basal diet, while the other three groups received FVR supplementation at levels of 2%, 4%, and 6% relative to the basal diet, respectively. The FVR used in this study was provided by the Shanghai Academy of Agricultural Sciences. Before feeding, the residue was dried and crushed for inclusion in the diet. The entire experiment lasted 48 d.

FVR was derived from the residual material after the harvest. This material was utilized after being dried at 80°C. The nutritional composition was 90.40% dry matter, 9.63% crude protein, 0.20% crude fat, 17.20% crude fiber, 13.50% crude ash, 3.20% calcium, 0.38% total phosphorus, and 6.3% total amino acids.

During the experiment, the chickens were raised in two-layer cages with eight chickens in each cage, and specially assigned individuals were responsible for feeding the chickens and maintaining natural ventilation in the cages. The dietary formulations and nutritional levels of the basic feed are presented in Table 1. The nutritional levels were formulated in accordance with standard poultry nutrition guidelines and were further refined based on data specific to local poultry breeds, including the three-yellow chicken. The experimental feed, consisting of a powdered mixture, was prepared on-site using a dedicated mixer and ingredients procured specifically for the study. Experimental animals were provided with ad libitum access to both feed and water throughout the trial period.

All animals experiment were conducted in accordance with the Guidelines for the Care and Use of Experimental Animals of the Chinese Academy of Agricultural Sciences and approved by the Review of Experimental Animal Welfare and Ethics of the Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences (SV-20230526- Y01).

2.2 Sample collection and analysis

Feed intake and weight were systematically recorded throughout the study periods. Feed intake was calculated the difference between the amount of feed provided and the amount of feed refused at each replicate. ADG was determined by starting weight and ending weight every periods. FCR was then calculated

10.3389/fanim.2024.1458762

TABLE 1 Composition and nutrient levels of basal diets (dry matter basis).

Items	0–4 week	5–7 week
Ingredient (%)		
Corn	58.76	62.47
Wheat middling	4.00	5.00
Soyabean meal	27.08	21.56
Corn gluten meal	3.00	
Rapeseed meal		3.00
Fish meal	2.00	1.00
Calcium hydrogen phosphate	1.47	1.34
Limestone	1.17	1.35
NaCl	0.31	0.31
Plant oils	0.90	2.69
premixes	1.00	1.00
Lys	0.18	0.19
DL-Met	0.13	0.09
Total	100	100
Nutrient level (%)		
ME (MJ/kg)	12.13	12.35
CP	20.06	17.10
Ca	0.84	0.90
Available P	0.45	0.40
NaCl	0.38	0.37
Lysine	1.09	0.94
Methionine	0.48	0.38
Threonine	0.81	0.69
Tryptophan	0.26	0.22

CON, broilers fed with a basic diet; 2% FVR, broilers fed with a basic diet supplemented with 20 g/kg *Flammulina velutipes* residue; 4% FVR, broilers fed with a basic diet supplemented with 40 g/kg *Flammulina velutipes* residue; 6% FVR, broilers fed with a basic diet supplemented with 60 g/kg *Flammulina velutipes* residue. ME, metabolizable energy; CP, crude protein; Ca, calcium; Available P, Available phosphorus.

The premix provides following for 1kg of feed: VA,10000 IU; VD_3, 2400 IU; VE, 10 IU; VK, 1.6 mg; VB_1,1.0 mg; VB_2, 6.8 mg; VB_6,1.0 mg; VB_{12},0.01 mg; Biotin,0.08mg; Folic acid, 1.02 mg; Nicotinic acid,28.0mg; Choline chloride,500mg; Cu,16mg; Fe,90mg; Zn,45mg; Mn, 70 mg; I,0.3 mg; Se, 0.15 mg.

The nutritional levels of the diet were calculated based on the nutrient content of the ingredients and the nutrient content of the ingredients determined through laboratory analysis.

by dividing the total feed intake by the corresponding weight gain achieved during each specific period average daily.

One chicken was randomly selected from each replicate on 28 and 48 days. Blood was collected from the jugular vein, stored at 4°C overnight, and centrifuged at 8000g for 30 min. The upper serum was absorbed and stored at −80°C. Cecal contents of 2–3 g was collected aseptically on 48 day and frozen at −80 °C for microbial flora diversity detection.

According to the kit instructions (provided by Shanghai Yinuopai Biotechnology Co., Ltd.), the following serum antioxidant indices were determined: catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPC-PX), total antioxidant capacity (T-AOC), and malondialdehyde (MDA); The serum immune indices were immunoglobulin A(IgA), Immunoglobulin G(IgG). The Serum inflammatory factors were interleukin-1 (IL-1), interleukin 10 (IL-10), and tumor necrosis factor alpha (TNF-α).

After first completing the genomic DNA extraction, the extracted genomic DNA was detected using 1% agarose gel electrophoresis. For formal experiments, three replicates of each sample were mixed with the polymerase chain reaction (PCR) products from the same sample and detected using 2% agarose gel electrophoresis. The PCR products were recovered by cutting glue using an E.Z.N.A. ® Gel Extraction Kit (Omega Bio-Tek, Guangzhou Feiyang Biological Engineering Co., LTD) and eluted with Tris HCl and 2% agarose gel electrophoresis. The abovementioned PCR sequencing region was 338F_806R, 338F (5′-ACTCCTACGGGAGGCAGCAG-3′), and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). According to the preliminary quantitative results of electrophoresis, the PCR products were determined using QuantiFluor™ -ST blue fluorescence quantification system (Promega). Sequencing was performed using an Illumina's MiSeq PE300 platform. The Flash (version 1.2.11) software was used for pair-end double-ended sequence splicing, and sequence quality was controlled and filtered simultaneously. Operational taxonomic unit (OTU) cluster and taxonomic analyses of species were performed after distinguishing the samples. Based on OTU cluster analysis results, α and β diversities were calculated, and the groups were compared. Differences in species at different levels in each group were analyzed using the Kruskal–Wallis H test.

2.3 Statistical analysis

All data were preliminarily sorted using EXCEL 2010, and SPSS 26.0. was used to test the significance between different groups using one-way analysis of variance and Duncan's multiple comparison test. The results were expressed as means ± SD. Statistical significance was set at $P < 0.05$. The GraphPad Prism 9.5 software was used for mapping.

3 Results and discussion

3.1 Growth performance

The results of performance are shown in Table 2. The results indicated that the low-dose addition of FVR (2%) tended to increase dry matter intake compared to the control group, although this difference was not statistically significant. There was no significant difference in average daily weight gain between the control and low-

10.3389/fanim.2024.1458762

TABLE 2 Effects of *Flammulina velutipes* residue on growth performance of three-yellow chicken.

Items	CON	2% FVR	4% FVR	6% FVR
BW(g)				
D1	28.27 ± 0.89	29.20 ± 1.08	28.71 ± 1.31	28.19 ± 1.12
D28	265.94 ± 10.01	276.25 ± 11.09	262.50 ± 15.55	260.50 ± 4.08
D48	755.92 ± 53.50^{a}	696.67 ± 50.33^{a}	682.08 ± 55.83^{a}	601.79 ± 38.95^{b}
D1–28				
ADG (g)	8.80 ± 0.35	9.15 ± 0.44	8.66 ± 0.59	8.59 ± 0.14
ADFI(g)	14.94 ± 0.66^{ab}	15.06 ± 0.24^{ab}	15.37 ± 0.28^{a}	14.48 ± 0.77^{b}
FCR	1.70 ± 0.08	1.65 ± 0.08	1.78 ± 0.14	1.69 ± 0.10
D29–48				
ADG(g)	25.79 ± 2.98^{a}	22.13 ± 3.07^{ab}	22.08 ± 2.33^{ab}	17.99 ± 2.23^{b}
ADFI(g)	48.29 ± 0.50^{a}	48.36 ± 0.36^{a}	47.76 ± 0.58^{a}	46.30 ± 0.61^{b}
FCR	1.89 ± 0.24^{b}	2.22 ± 0.29^{ab}	2.18 ± 0.20^{b}	2.60 ± 0.30^{a}
D1–48				
ADG(g)	15.48 ± 1.15^{a}	14.20 ± 1.06^{a}	13.09 ± 1.21^{a}	12.20 ± 0.85^{b}
ADFI(g)	27.62 ± 0.37^{ab}	28.07 ± 0.35^{a}	27.03 ± 0.60^{b}	25.97 ± 0.28^{c}
FCR	1.79 ± 0.13^{b}	1.98 ± 0.12^{ab}	1.95 ± 0.16^{ab}	2.14 ± 0.15^{a}

CON, broilers fed with a basic diet; 2%FVR, broilers fed with a basic diet supplemented with 20 g/kg *Flammulina velutipes* residue; 4%FVR, broilers fed with a basic diet supplemented with 40 g/kg *Flammulina velutipes* residue; 6%FVR, broilers fed with a basic diet supplemented with 60 g/kg *Flammulina velutipes* residue. BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.
Superscript letters within each row show significant differences of mean values at $P < 0.05$.

dose groups. The feed conversion ratio (FCR) was higher in the low-dose group than in the control group, but not significant, indicating a potential decrease in feed conversion efficiency. Conversely, the high-dose addition of FVR (6%) significantly reduced body weight at 48 days of age (P<0.05). Additionally, dry matter intake was significantly lower in the high-dose group during both the 29 to 48 days period and the entire 1 to 48 days period (P<0.05). The high-dose group also exhibited a significantly increased FCR during both the 29 to 48 days and 1 to 48 days periods, indicating reduced feed conversion efficiency (P<0.05).

3.2 Blood serum antioxidant capacity

As shown in Table 3, the serum antioxidant content of broilers added with FVR was significantly changed compared with that in the CON group. On D28, serum CAT levels in the 4% and 6% FVR groups were significantly higher than those in the control group (P<0.05), whereas the 2% FVR group showed no significant difference. By D48, all experimental groups demonstrated significantly elevated serum CAT levels compared to the control group (P<0.05). Throughout the experimental period, SOD levels in the experimental groups consistently remained significantly higher than those in the control group (P<0.05). On D28, serum GSH-PX levels in the 6% FVR group were significantly higher than those in the control group (P<0.05). By D48, GSH-PX levels in all experimental groups exceeded those in the control group (P<0.05). During the trial period, T-AOC in the 2% and 4% FVR groups was significantly higher than that in the control group (P<0.05), while the 6% FVR group exhibited an extremely significant increase (P<0.01). On D28, serum MDA levels in the 4% and 6% FVR groups were significantly lower than those in the control group (P<0.05). By D48, MDA levels in the 2% and 4% FVR groups showed a significant reduction (P<0.05), while the 6% FVR group exhibited an extremely significant decrease compared to the control group (P<0.01).

3.3 Blood serum immunoglobulin and inflammatory factors

As illustrated in Figures 1A–D, serum immunoglobulin levels indicated no significant change in IgA in the FVR groups compared with that in the CON group at 28 days, and IgA levels in the 6% FVR groups were significantly higher than those in the CON group at 48 days and increased in the 2% and 4% FVR groups (P<0.05). At 28 d and 48 d, the IgG levels in the 6% FVR groups were significantly higher than those in the CON group (P<0.05). As presented in Figures 1E–J, the levels of inflammatory factors changed. The FVR groups demonstrated lower serum IL-1 contents than the CON group at 48 days. The content of IL-10 in the 4% FVR and 6% FVR groups significantly increased compared to that in the CON group (P<0.05). For TNF-α activity, no significant change was observed between groups.

3.4 Cecal microbiota of broilers

As presented in Figure 2A, the Venn plot demonstrates 1249 OTUs in the four groups, with 84, 49, 110 and 66 OTUs unique to the CON, 2% FVR and 4% FVR, and 6% FVR, respectively. Based on α diversity, Ace, Chao, Shannon, and Simson indices changed in Figures 2B–E, although the difference was not significant (P>0.05). Assessing β-diversity as in Figures 2F, G, the microbial composition was slightly altered at the genus and phylum level (P>0.05). The dominant flora of each group was further analyzed at the phylum and genus level. At the phylum level, Firmicutes and Bacteroidetes were the dominant bacteria. As in Figures 2H, I, Firmicutes were upregulated in 2% and 4% FVR groups, and the proportion of Bacteroidetes was significantly upregulated in 6% FVR groups (P<0.05). At the genus level, *Bacteroides* and *Lactobacillus* accounted for a high proportion (Figures 2H, I). The total proportion of *Bacteroides* and *Lactobacillus* in the FVR groups decreased, whereas those of other bacteria were upregulated, including *Ruminococcus* and *Faecalibacterium*, which were slightly upregulated. However, *Bacteroides* was significantly upregulated in the 6% FVR groups (P<0.05).

TABLE 3 Effects of *Flammulina velutipes* residue on antioxidant functions of three-yellow chicken.

AGE	Items	CON	2% FVR	4% FVR	6% FVR
D28	CAT (U/mL)	50.91 ± 2.56^{a}	55.84 ± 2.49^{ab}	57.42 ± 2.48^{b}	58.38 ± 1.61^{b}
	SOD (U/ml)	322.15 ± 2.26^{a}	334.28 ± 3.80^{b}	344.40 ± 1.97^{b}	385.01 ± 5.70^{b}
	GSH-PX (nmol/min/mL)	793.19 ± 1.87^{a}	790.87 ± 6.44^{a}	794.29 ± 9.98^{a}	912.01 ± 4.37^{b}
	T-AOC (U/Trolox/mL)	0.18 ± 0.04^{a}	0.22 ± 0.01^{b}	0.21 ± 0.01^{b}	0.25 ± 0.02^{c}
	MDA (nmol/mL)	12.10 ± 1.68^{b}	12.06 ± 2.49^{b}	9.10 ± 1.10^{a}	8.37 ± 2.79^{a}
D48	CAT (U/mL)	49.30 ± 4.70^{a}	62.03 ± 6.66^{b}	60.24 ± 3.30^{b}	59.02 ± 6.04^{b}
	SOD (U/ml)	323.54 ± 2.25^{a}	345.81 ± 2.25^{b}	365.61 ± 0.72^{b}	502.13 ± 0.12^{c}
	GSH-PX (nmol/min/mL)	644.59 ± 9.77^{a}	781.06 ± 1.93^{b}	737.28 ± 1.67^{c}	953.29 ± 4.87^{d}
	T-AOC (U/Trolox/mL)	0.19 ± 0.01^{a}	0.21 ± 0.09^{b}	0.21 ± 0.01^{b}	0.24 ± 0.03^{c}
	MDA (nmol/mL)	11.57 ± 1.01^{b}	12.21 ± 2.14^{b}	11.53 ± 1.51^{b}	8.64 ± 1.73^{a}

Superscript letters (a–c) within each row show significant differences of mean values at $P < 0.05$.
CON, broilers fed with a basic diet; 2% FVR, broilers fed with a basic diet supplemented with 20 g/kg *Flammulina velutipes* residue; 4% FVR, broilers fed with a basic diet supplemented with 40 g/kg *Flammulina velutipes* residue; 6% FVR, broilers fed with a basic diet supplemented with 60 g/kg *Flammulina velutipes* residue.CAT, catalase; SOD, superoxide dismutase; GPC-PX, glutathione peroxidase; T-AOC, total antioxidant capacity; MDA, malondialdehyde.

4 Discussion

In animal production, growth performance is a critical determinant of economic benefits. Key indicators such as ADG and FCR are often researched to optimize feeding strategies. Studies have shown that dietary supplementation with certain mushrooms can improve these performance metrics. For instance, higher body weight gain and lower FCR were observed in broilers fed a 2% level of *Agaricus bisporus* mushroom (Giannenas et al., 2010). In our research, the addition of FVR at low doses (2%, 4%) did not significantly affect the growth performance of broiler. These findings align with previous research by Hassan et al. (2020) and Mahfuz et al. (2020), who observed similar results. The limited impact of FVR at low doses could be attributed to the nutrient composition and bioactive substances in the residue. *Flammulina velutipes* mycelium produces cellulolytic enzymes during growth, which break down fibrous structures, releasing nutrients that aid in digestion and absorption. The presence of phenolic compounds and glucans in mushrooms enhances antioxidant capacity in animals. However, the active substances in fungal residue are limited, which might explain the negligible effect on broiler chickens' production performance when FVR is used in small quantities. The experiment revealed that a high dose of FVR (6%) significantly reduced body weight, dry matter intake, and feed conversion efficiency. These adverse effects are likely due to the lower protein content and higher levels of indigestible fiber and crude ash in the fungal residue compared to the basal diet. The direct addition of high doses of FVR diluted the overall nutrient content, inhibiting dry matter intake and thus negatively impacting growth performance. These findings suggest that while FVR has potential as a feed ingredient, careful consideration of its dosage and nutrient content is essential to avoid negative impacts on broiler performance.

Edible fungi contain numerous active substances (Meng et al., 2018) with antioxidative properties and tumor-inhibiting abilities (Muszyń et al., 2018). Polysaccharides, the most abundant macromolecules present in edible fungi, significantly scavenge hydroxyl and superoxide anion free radicals (Lin et al., 2014). By influencing the Keap1-Nrf2/ARE signaling pathway, these polysaccharides regulate antioxidant enzyme expression and enhance the body antioxidant capacity (Lin et al., 2016; Wu et al., 2020). Li et al. (2018) reported that polysaccharides can increase the expression of Nrf2 and ARE in cyclophosphamide-treated mouse testicular tissue, reduce Keap1 expression, and promote antioxidant enzyme production. These polysaccharides also regulate apoptosis pathway genes expression and promote antioxidant production, thereby mitigating oxidative damage. Additionally, polysaccharides from sea buckthorn increase SOD activity in mice by inhibiting BAX expression (Chen et al., 2020). Polyphenols in edible fungi act as natural antioxidants, clearing free radicals and quenching reactive oxygen species. Wang et al. (2012) found that sesquiterpenoids from *Flammulinella magnolia* prevent lipid peroxidation by chelating OH^{-} with Fe^{2+} or Cu^{2+} in the polyphenol ring, thus reducing oxidative stress. Feeding FVR to broilers significantly increased antioxidant enzyme activity in the serum and decreased harmful products, highlighting the role of bioactive substances in FVR. However, the

specific antioxidant effects of *edible mushroom* residues require further elucidation.

Additionally, the immune capacities of livestock and poultry should not be ignored, and the concentration of immune factors is an important index for measuring poultry immunity (Criste et al., 2020). FVR supplementation significantly increased IgG and IgA contents in serum of 48-day-old broilers, decreased IL-1 levels, and increased IL-10 levels, which had positive effects on the immunity of broilers. Active substances of edible fungi are known to enhance macrophage phagocytosis and stimulate the release of cytokines such as NO, TNF-α, and IL-6 (Guo et al., 2023). Among them, edible polysaccharide regulates the secretion and expression of cytokines and other factors through nuclear factor-κB and mitogen-activated protein kinases signaling pathways, thereby exerting its immunomodulatory activity (Wu et al., 2019). In addition, edible fungi contain various active proteins, the most common of which are lectins (12–190 kDa) and FIP (12.7 kDa), which are involved in various physiological functions such as anticancer, antiviral, antibacterial, and immune regulation (Ng and Wang, 2004). Polypeptides from mycelium of *Pleurotus eryngii* have been demonstrated to inhibit the proliferation of cancer cells (cervical, breast, and stomach cancer cells) while promoting the proliferation of macrophages (Ana-1 cells), TNF-α, and IL-6 and expression of TLR2 and TLR4 (Sun et al., 2017).

The gut microbiota plays an important role in nutrient digestion, metabolism, maintenance of intestinal barrier function, and immune

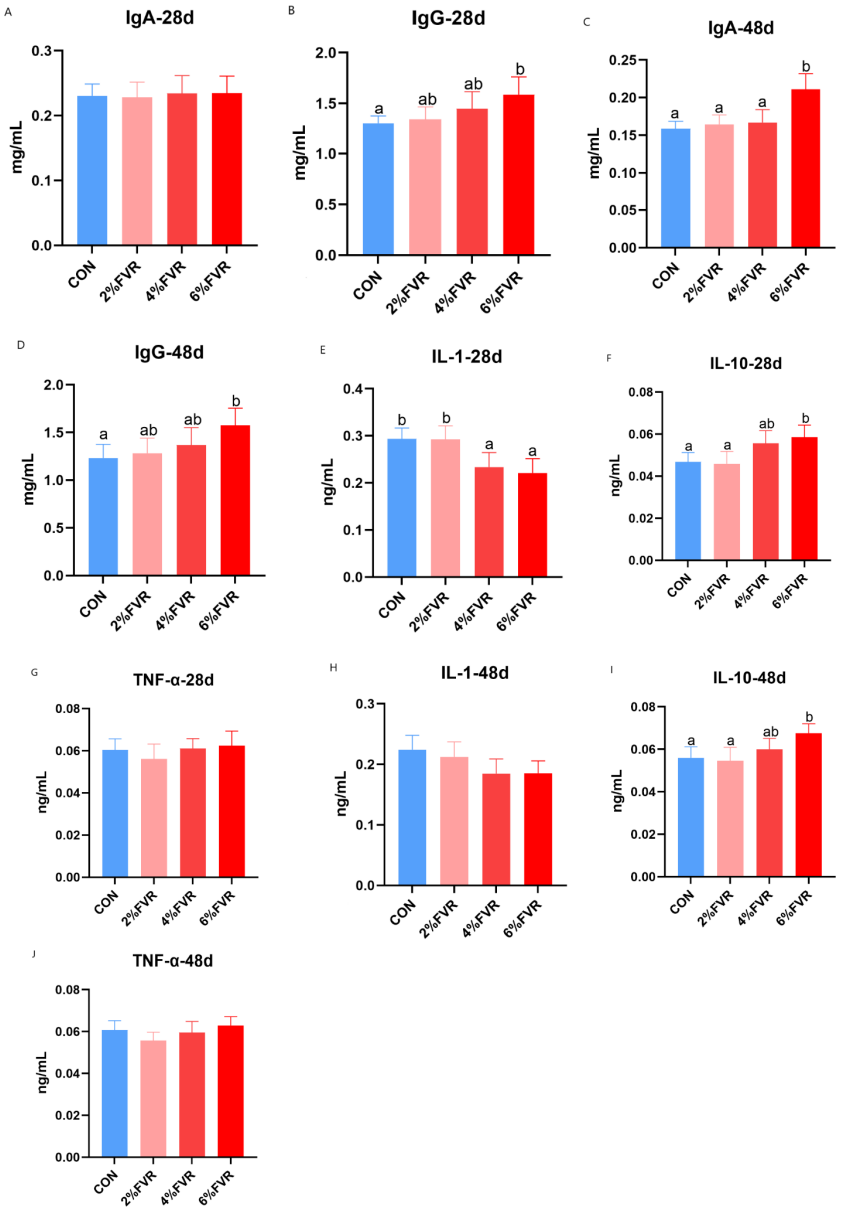


FIGURE 1
Effects of *Flammulla velutipes* residue on serum immunoglobulin and inflammatory factors of broilers. **(A, B)** IgA and IgG content in d28; **(C, D)** IgA and IgG content in d48; **(E–G)** IL-1, IL-10 and INF-α content in d28; **(H–J)** IL-1, IL-10 and INF-α content in d48. Lower-case letters show significant differences of mean values at $P < 0.05$.

development (Fu et al., 2021). Active substances of edible fungi, which can regulate the structure and diversity of the intestinal microbial community and affect the production of short-chain fatty acids in the intestine and intestinal barrier to exert benefits (Zhao et al., 2018). According to the results of the fecal microflora diversity analysis, FVR affected the regulation of intestinal microflora and its number. *Firmicutes* and *Bacteroides* constitute was the main of microbial communities at the phylum level (Ahir et al., 2010), and *Firmicutes* and *Bacteroidetes* were the dominant bacteria in this study, consistent with the results of previous studies (Choi et al., 2014). However, in contrast to that in the CON group, the abundance of *Bacteroidetes* significantly increased at the phylum and genus levels in the 6% FVR groups. However, *Bacteroidetes* promote the production of short-chain fatty acids during biological metabolism, maintain intestinal environmental stability, and effectively prevent inflammation (Zhang et al., 2019). *Bacteroidetes* also respond significantly to changes in the intestinal environment (Flint and Duncan, 2014). This indicates that the intestinal environment is changed by the addition of FVR, which makes the above-mentioned immunity improvement in broilers feasible. Other studies have demonstrated that Bacteroides can increase the relative abundance of plant polysaccharides through degradation them (Tamura et al., 2017). In addition, at the genus level, while the total proportion of Bacteroides and Lactobacillus decreased in the FVR groups, the proportion of other beneficial bacterial genera, such as *Ruminococcus* and *Faecalibacterium*, increased. *Ruminococcus* is known to be one of the most efficient bacterial genera for decomposing carbohydrates, and can degrade and ferment cellulose and hemicellulose. Fibers can further increase the viscosity of the digestive fluid and thickness of the mucous layer, reduce the number of mucus-degrading bacteria, and increase the number of cellulose-degrading bacteria, which are considered a line of defense against pathogens in the intestinal tract (La Reau and Suen, 2018).

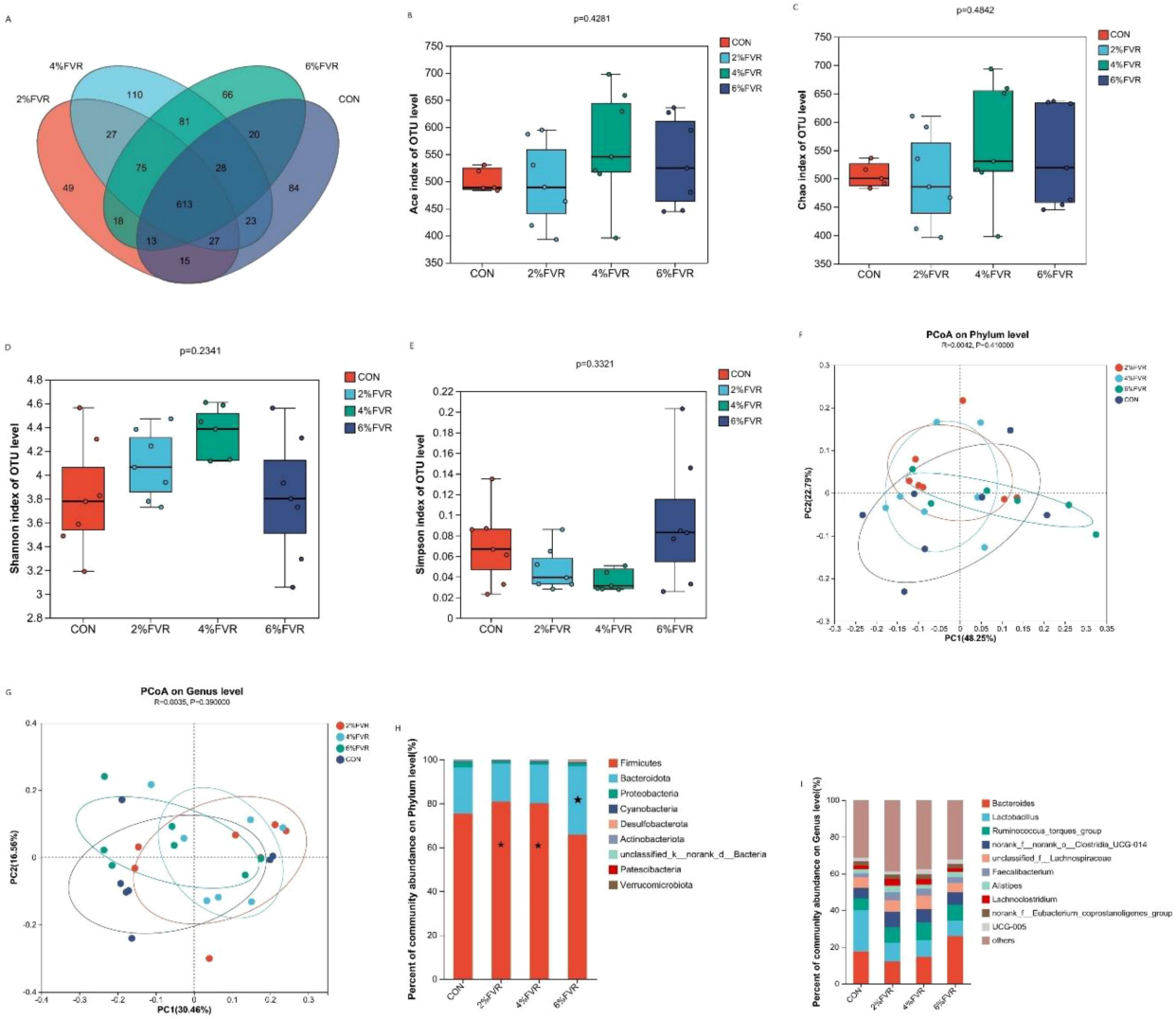


FIGURE 2
Effect of *Flammulla velutipes* residue on cecal microbiota of broilers. **(A)** Venn diagram of broilers cecal microbiota in different treatments: **(B)** Ace index; **(C)** Chao index; **(D)** Shannon index; **(E)** Simpson index of OUT level of broilers cecal microbiota. **(F, G)** β-diversity (PCoA) on Phylum level and Genus level. **(H, I)** relative abundance of bacteria at the phylum and genus levels. * indicates significant differences ($P<0.05$).

Ruminococcus is a probiotic that can enhance the overall immunity and growth indices of cultured fish (Gayed et al., 2021). As a member of *Firmicutes*, *Faecalibacterium* plays an important role in promoting the production of butyrate in the intestine, anti-inflammation, maintenance of bacterial enzyme activity, and protection of the digestive system from intestinal pathogens. Farhadfar et al. (2021) have reported that *Faecalibacterium* is actively involved in the metabolism of the host and regulates the intestinal immune system, oxidative stress, and colon cell metabolism through butyrate production. The addition of FVR has been demonstrated to increase the diversity of cecal flora and increase the number of beneficial bacteria.

5 Conclusion

Our results demonstrate that supplement 2% or 4% FVR to three-yellow chickens has no significant effect on performance, but supplement 6% FVR can reduce performance of broiler. But supplement FVR can improve antioxidant capacity and immunity, change intestinal probiotic flora to a certain extent, and enrich the diversity of intestinal flora.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The animal study was approved by Review of Experimental Animal Welfare and Ethics of the Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences (SV-20230526-Y01). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

YL: Investigation, Writing – original draft, Writing – review & editing. DL: Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. WL: Data curation, Writing – review & editing. LZ: Resources, Writing – review & editing. PC: Data curation, Resources, Software, Writing – review & editing. YM: Conceptualization, Funding acquisition, Methodology, Writing – review & editing. KD: Resources, Writing – review & editing. CZ: Conceptualization, Methodology, Resources, Writing – review & editing. NL: Funding acquisition, Project administration, Resources, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was supported the Program for International S&T Cooperation Projects of Henan (232102521012), the Science Foundation for Expat Scientist Studio for Animal Stress and Health Breeding of Henan Province (GZS2021006), and the Key Scientific Research Foundation of the Higher Education Institutions of Henan Province (22A230001).

Acknowledgments

We gratefully acknowledge the help from The Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, offers laboratory animal sites and services.

Conflict of interest

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

Publisher's note

References

Ahir, V. B., Koringa, P. G., Bhatt, V. D., Ramani, U. V., Tripathi, A. K., Singh, K. M., et al. (2010). Metagenomic analysis of poultry gut microbes. *Indian J. Poult. Sci.* 45, 111–114.

Aida, F., Shuhaimi, M., Yazid, M., and Maaruf, A. G. (2009). Mushroom as a potential source of prebiotics: a review. *Trends Food Sci. Technol.* 20, 567–575. doi: 10.1016/j.tifs.2009.07.007

Amin, M. Z., Harun, A., and Wahab, M. A. (2014). Status and potential of mushroom industry in Malaysia. *Econ Technol. Manag. Rev.* 9b, 103–111. Available at: http://etmr.mardi.gov.my/Content/ETMR%20Vol.9b%20(2014)/Vol9b_2_.pdf.

Bruinsma, J. (2003). *World agriculture: towards 2015/2030-An FAO perspective* (London: FAO/Earthscan), 432.

Cao, Y., and Li, D. (2013). Impact of increased demand for animal protein products in Asian countries: Implications on global food security. *Anim. Front.* 3, 48–55. doi: 10.2527/af.2013-0024

Chen, T., Song, T., and Sun, X. (2020). Regulatory effect of angelica sinensis polysaccharide on bcl-2/bax/caspase-3 signal pathway in spleen of rats with radiation injury. *J. Biobased Mater Bioenergy* 14, 579–583. doi: 10.1166/jbmb.2020.1994

Chen, Y., and Nie, F. (2016). "Analysis of China's Food Supply and Demand Balance and Food Security," in *Food Security and Industrial Clustering in Northeast Asia. New Frontiers in Regional Science: Asian Perspectives*. Eds. L. Kiminami and T. Nakamura (Springer, Tokyo), 47–59. doi: 10.1007/978-4-431-55282-6_4

Chiu, S. W., Ching, M. L., Fong, K. L., and Moore, D. (1998). Spent oyster mushroom substrate performs better than many mushroom mycelia in removing the biocide pentachlorophenol. *Mycol. Res.* 102, 1553–1562. doi: 10.1017/S0953756298007588

Choi, J. H., Kim, G. B., and Cha, C. J. (2014). Spatial heterogeneity and stability of bacterial community in the gastrointestinal tracts of broiler chickens. *Poult. Sci.* 93, 1942–1950. doi: 10.3382/ps.2014-03974

Criste, A., Urcan, A. C., and Corcionivoschi, N. (2020). Avian IgY antibodies, ancestors of mammalian antibodies–production and application. *Rom. Biotechnol. Lett.* 25, 1311–1319. doi: 10.25083/rbl/25.2/1311.1319

Falandysz, J. (2013). On published data and methods for selenium in mushrooms. *Food Chem.* 138, 242–250. doi: 10.1016/j.foodchem.2012.10.046

Farhadfar, N., Gharaibeh, R. Z., Lyon, D., Whitlock, J. A., Murthy, H. S., Weaver, M. T., et al. (2021). Microbiota phylogenic analysis revealed decreased abundance of Faecalibacterium prausnitzii, an anti-inflammatory commensal bacterium, in patients with chronic graft-versus-host disease. *Hematology/oncol. Stem Cell Ther.* 14, 263–265. doi: 10.1016/j.hemonc.2021.03.004

Fazaeli, H., Shafyee-Varzeneh, H., Farahpoor, A., and Moayyer, A. (2014). Recycling of mushroom compost wheat straw in the diet of feedlot calves with two physical forms. *Int. J. Recycl. Org. Waste Agric.* 3, 65. doi: 10.1007/s40093-014-0065-z

Flint, H. J., and Duncan, S. H. (2014). "Bacteroides, Prevotella," in *Encyclopedia of Food Microbiology*, *2nd ed*. Eds. C. A. Batt and M. L. Tortorello (Academic Press, Elsevier).

Fu, J., Wang, T., Cheng, Y., Cheng, Y., Wang, F., Jin, M., et al. (2021). Clostridium butyricum ZJU-F1 benefits the intestinal barrier function and immune response associated with its modulation of gut microbiota in weaned piglets. *Cells* 10, 527. doi: 10.3390/cells10030527

Gayed, M. A., Elabd, H., Tageldin, M., and Abbass, A. (2021). Probiotic Zado® (Ruminococcus Flavefaciens) boosts hematology, immune, serum proteins, and growth profiles in Nile tilapia (Oreochromis niloticus). *Fish Shellfish Immunol. Rep.* 2, 100021. doi: 10.1016/j.fsirep.2021.100021

Giannenas, I., Pappas, I. S., Mavridis, S., Kontopidis, G., Skoufos, J., Kyriazakis, I., et al. (2010). Performance and antioxidant status of broiler chickens supplemented with dried mushrooms (Agaricus bisporus) in their diet. *Poult. Sci.* 89, 303–311. doi: 10.3382/ps.2009-00207

Guo, Q., Liang, S. G., Xiao, Z. C., and Ge, C. R. (2023). Research progress on extraction technology and biological activity of polysaccharides from edible fungi: A review. *Food Rev. Int.* 39, 4909–4940. doi: 10.1080/87559129.2022.2039182

Hassan, R. A., Shafi, M. E., Attia, K. M., and Assar, M. H.. (2020). Influence of oyster mushroom waste on growth performance, immunity and intestinal morphology compared with antibiotics in broiler chickens. *Front. Vet. Sci.* 7. doi: 10.3389/fvets.2020.00333

Jing, P., Zhao, S. J., Lu, M. M., Cai, Z., Pang, J., and Song, L. H. (2014). Multiple-fingerprint analysis for investigating quality control of Flammulina velutipes fruiting body polysaccharides. *J. Agric. Food Chem.* 62, 12128–12133. doi: 10.1021/jf504349r

Ko, W. C., Liu, W. C., Tsang, Y. T., and Hsieh, C. W.. (2007). Kinetics of winter mushrooms (Flammulina velutipes) microstructure and quality changes during thermal processing. *J. Food Eng.* 81, 587–598. doi: 10.1016/j.jfoodeng.2006.12.009

La Reau, A. J., and Suen, G. (2018). The Ruminococci: key symbionts of the gut ecosystem. *J. Microbiol.* 56, 199–208. doi: 10.1007/s12275-018-8024-4

Leifa, F., Pandey, A., and Soccol, C. R. (2001). Production of Flammulina velutipes on coffee husk and coffee spent-ground. *Braz. Arch. Biol. Technol.* 44, 205–212. doi: 10.1590/S1516-89132001000200015

Li, S., Song, Z., and Liu, T. (2018). Polysaccharide from Ostrea rivularis attenuates reproductive oxidative stress damage via activating Keap1-Nrf2/ARE pathway. *Carbohydr. Polym.* 186, 321–331. doi: 10.1016/j.carbpol.2018.01.075

Lin, L., Cui, F. Y., Zhang, J. J., and Gao, X. (2016). Antioxidative and renoprotective effects of residue polysaccharides from Flammulina velutipes. *Carbohydr. Polym.* 146, 388–395. doi: 10.1016/j.carbpol.2016.03.071

Lin, Y., Ge, X., and Li, Y. (2014). Solid-state anaerobic co-digestion of spent mushroom substrate with yard trimmings and wheat straw for biogas production. *Biores. Technol.* 169, 468–474. doi: 10.1016/j.biortech.2014.07.020

Mahfuz, S., He, T., Ma, J., Liu, H., Long, Q., Shang, Q., et al (2020). Mushroom (Flammulina velutipes) stem residue on growth performance, meat quality, antioxidant status and lipid metabolism of broilers. *Ital. J. Anim. Sci.* 19, 803–812. doi: 10.1080/1828051X.2020.1797545

Manila, P., Suonpaa, K., and Piironen, V. (2000). Functional properties of edible mushrooms. *Nutrition* 16, 694–696. doi: 10.1016/S0899-9007(00)00341-5

McIntyre, B. D., Herren, H. R., Wakhungu, J., and Watson, R. (2009). *IAASTD-International Assessment of Agricultural Knowledge, Science and Technology for Development. Global Report* (Washington, DC: Island Press).

Medina, E., Paredes, C., Pérez-Murcia, M. D., Bustamante, M. A., and Moral, R. (2009). Spent mushroom substrates as component of growing media for germination and growth of horticultural plants. *Biores. Technol.* 100, 4227–4232. doi: 10.1016/j.biortech.2009.03.055

Meng, Y., Yan, J. M., Yang, G., Han, Z., Tai, G. H., Cheng, H. R., et al. (2018). Structural characterization and macrophage activation of a hetero-galactan isolated from Flammulina velutipes. *Carbohydr. Polym.* 183, 207–218. doi: 10.1016/j.carbpol.2017.12.017

Michiel, D., Morley, T., Rau, M. L., and Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nat. Food* 2, 494–501. doi: 10.1038/s43016-021-00322-9

Mottet, A., and Tempio, G. (2017). Global poultry production: current state and future outlook and challenges. *Worlds Poult. Sci. J.* 73, 245–256. doi: 10.1017/S0043933917000071

Muszyń, B., Agata, G. K., Katarzyna, K., and Gdula-qrgasinska, J. (2018). Anti-inflammatory properties of edible mushrooms: A review. *Food Chem.* 243, 373–381. doi: 10.1016/j.foodchem.2017.09.149

Ng, T. B. (2006). "Fungal Ribosome Inactivating Proteins," in *Handbook of Biologically Active Peptides*. Ed. A. J. Kastin (Academic Press), 145–149. doi: 10.1016/B978-0-12-369442-3.X5001-6

Ng, T. B., and Wang, H. X. (2004). Flammin and velin: new ribosome inactivating polypeptides from the mushroom Flammulina velutipes. *Peptides* 25, 929–933. doi: 10.1016/j.peptides.2004.03.007

Rosegrant, M. W., Paisner, M. S., Meijer, S., and Witcover, J. (2001). *Global Food Projections to 2020: Emerging Trends and Alternative Futures* (Washington, DC: International Food Policy Research Institute).

Silver, W. L., Perez, T., Mayer, A., and Jones, A. R. (2021). The role of soil in the contribution of food and feed. *Philos. Trans. R. Soc. B* 376, 20200181. doi: 10.1098/rstb.2020.0181

Staniškis, J. K. (2012). Sustainable consumption and production: how to make it possible. *Clean Techn Environ. Policy* 14, 1015–1022. doi: 10.1007/s10098-012-0535-9

Sun, Y. N., Hu, X. L., and Li, W. X. (2017). Antioxidant, antitumor and immunostimulatory activities of the polypeptide from Pleurotus eryngii mycelium. *Int. J. Biol. Macromol.* 97, 323–330. doi: 10.1016/j.ijbiomac.2017.01.043

Tamura, K., Hemsworth, G. R., and Déjean, G. (2017). Molecular mechanism by which prominent human gut Bacteroidetes utilize mixed-linkage beta-glucans, major health-promoting cereal polysaccharides. *Cell Rep.* 21, 417–430. doi: 10.1016/j.celrep.2017.09.049

Trostle, R. (2008). *Global Agricultural Supply and Demand: Factors Contributing to the Recent Increase in Food Commodity Prices* (United States Department of Agriculture), 30. International Agriculture and Trade Outlook. https://www.govinfo.gov/content/pkg/GOVPUB-A93-PURL-gpo10109/pdf/GOVPUB-A93-PURL-gpo10109.pdf.

Wang, S. J., Li, Y. X., Bao, L., Han, J. J., Yang, X. L., Li, H. R., et al. (2012). Eryngiolide A, a cytotoxic macrocyclic diterpenoid with an unusual cyclododecane core skeleton produced by the edible mushroom Pleurotus eryngii. *Org. Lett.* 14, 3672–3675. doi: 10.1021/ol301519m

Wang, W. H., Zhang, J. S., and Feng, T. (2018). Structural elucidation of a polysaccharide from Flammulina velutipes and its immunomodulation activities on mouse B lymphocytes. *Sci. Rep.* 8, 3120. doi: 10.1038/s41598-018-21375-0

Williams, B. C., McMullan, J. T., and McCahey, S. (2001). An initial assessment of spent mushroom compost as a potential energy feedstock. *Biores. Technol.* 79, 227–230. doi: 10.1016/S0960-8524(01)00073-6

Wu, Q., Liu, L. T., and Wang, X. Y. (2020). Lycium barbarum polysaccharides attenuate kidney injury in septic rats by regulating Keap1-Nrf2/ARE pathway. *Life Sci.* 242, 117240. doi: 10.1016/j.lfs.2019.117240

Wu, Y. J., Wei, Z. X., Zhang, F. M., Linhardt, R. J., Sun, P. L., and Zhang, A. Q. (2019). Structure, bioactivities and applications of the polysaccharides from Tremella fuciformis mushroom: A review. *Int. J. Biol. Macromol.* 121, 1005–1010. doi: 10.1016/j.ijbiomac.2018.10.117

Yadav, P., and Samadder, S. R. (2018). A critical review of the life cycle assessment studies on solid waste management in Asian countries. *J. Clean Prod.* 185, 492–515. doi: 10.1016/j.jclepro.2018.02.298

Zhang, J. H. (2011). China's success in increasing per capita food production. *J. Exp. Bot.* 62, 3707–3711. doi: 10.1093/jxb/err132

Zhang, H., Wang, Q., Liu, S., Huo, D., Zhao, J., Zhang, L., et al. (2019). Genomic and metagenomic insights into the microbial community in the regenerating intestine of the sea cucumber Apostichopus japonicus. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.01165

Zhao, R. Q., Yang, W. J., Pei, F., Zhao, L., and Hu, Q. (2018). *In vitro* fermentation of six kinds of edible mushrooms and its effects on fecal microbiota composition. *LWT* 96, 627–635. doi: 10.1016/j.lwt.2018.06.012

Zhu, H., Sheng, K., Yan, E., Qiao, J., and Lv, F. (2012). Extraction, purification and antibacterial activities of a polysaccharide from spent mushroom substrate. *Int. J. Biol. Macromol.* 50, 840–843. doi: 10.1016/j.ijbiomac.2011.11.016

Ziegler, E. H. (2006). China's cities, globalization, and sustainable development: comparative thoughts on urban planning, energy, and environmental policy. *Wash U Glob Stud. Law Rev.* 5, 295–322.

Zisopoulos, F. K., Ramírez, H. A., and Boom, R. M. (2016). A resource efficiency assessment of the industrial mushroom production chain: the influence of data variability. *J. Clean Prod.* 126, 394–408. doi: 10.1016/j.jclepro.2016.03.066

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 15 April 2025
DOI 10.3389/fanim.2025.1430709

OPEN ACCESS

EDITED BY
Marcos Inacio Marcondes,
William H. Miner Agricultural Research Institute, United States

REVIEWED BY
Gemma A Miller,
Scotland's Rural College, United Kingdom
Andrea Vitali,
University of Tuscia, Italy

*CORRESPONDENCE
Vibeke Lind
vibeke.lind@nibio.no

RECEIVED 10 May 2024
ACCEPTED 27 March 2025
PUBLISHED 15 April 2025

CITATION
Lind V, Hayes M, Waters SM, Kirwan SF and Aasen IM (2025) Methane emissions and growth rate of lambs fed *Laminaria hyperborea* supplemented diet. *Front. Anim. Sci.* 6:1430709. doi: 10.3389/fanim.2025.1430709

Methane emissions and growth rate of lambs fed *Laminaria hyperborea* supplemented diet

Vibeke Lind[1]*, Maria Hayes[2], Sinéad M. Waters[3,4], Stuart F. Kirwan[3] and Inga Marie Aasen[5]

[1]Department of Grassland and Livestock, Norwegian Institute of Bioeconomy Research (NIBIO), Aas, Norway, [2]Teagasc Food Research Centre, Ashtown, Dublin, Ireland, [3]Animal and Bioscience Research Department, Teagasc Grange, Meath, Ireland, [4]School of Biological and Chemical Sciences, Ryan Institute, University of Galway, Galway, Ireland, [5]Department of Biotechnology and Nanomedicine, SINTEF Industry, Trondheim, Norway

Introduction: The discovery of the methane-mitigating effect of the red seaweed *Asparagopsis taxiformis* has triggered a search for other seaweed species with similar effects. Brown seaweeds constitute the largest production volume of seaweeds in Europe. Some brown algae are known to inhibit methanogens and could potentially reduce enteric methane emissions. Use of by-products generated from industrial processing of plants are typically inedible for human consumption but well known as ruminant feeds. As fractions from *Laminaria hyperborea* showed significant reductions in methane emissions *in vitro*, a *L. hyperborea* by-product was chosen for an *in vivo* trial with sheep. The aim was to investigate the effect of *L. hyperborea* by-product inclusion in the diet of growing lambs on dry matter intake, methane emissions, growth rate and nitrogen digestibility.

Methods: Twenty-four Norwegian White Sheep lambs (12 ewe and 12 male lambs, 4 months; 36.8 kg live weight) were fed a Control diet (grass silage and control concentrate) or an Algae diet (grass silage and algae concentrate 2% inclusion rate). Lambs were fed a basic diet (grass silage and neutral concentrate) and, in staggered order, introduced to their respective diets for five weeks before entering one of six open circuit respiration chambers. Methane production was measured for three consecutive days. All lambs entered the chambers three time (Periods 1, 2 and 3). Feed intake was measured four consecutive days a week, and live weight (kg) was measured every two weeks. Twelve male lambs were used to investigate *in vivo* nitrogen digestibility using metabolism crates.

Results: The inclusion rate of *L. hyperborea* by-product was above the target and ended at 2.5% of DM. There was an increase in feed intake and live weight over the experimental period, consistent with the growth of the lambs. Methane production, yield, or intensity was not affected by diet, overall, but the Algae diet reduced methane in Period 1. Male lambs produced more methane than female lambs. Algae inclusion affected live weight negatively.

Discusssion: It is concluded that use of *L. hyperborea* by-products as a feed additive to sheep needs further investigation due to inconclusive results in the present study.

KEYWORDS

methane abatement, sheep, seaweed, feed intake, ruminants

1 Introduction

Livestock supply chains account for approximately 14.5% of global anthropogenic greenhouse gas (GHG) emissions (FAO, 2023), of which Approximately two-thirds results from rumen fermentation during digestion of feed by ruminants. As a result, strategies to reduce enteric methane (CH_4) emissions from ruminants is of high priority. Participants in the Global Methane Pledge have commitments to take voluntary actions to reduce global CH_4 emissions by 30% from 2020 levels by 2030. The discovery of the methane-mitigating effect of the red seaweed *Asparagopsis taxiformis* (9 to 98% reduction; Wasson et al., 2022) has initiated a search for other seaweed species with similar effects. The CH_4 reducing compound in this alga is bromoform, which inhibits methanogens without affecting other bacteria. However, bromoform is classified by the U.S Environmental Protection Agency (EPA) as a probable human carcinogen and is reported to be transferred to milk when fed to dairy cows (Muizelaar et al., 2021). Brown seaweeds, known as the *Phaeophyceae*, constitute the largest production volume of seaweeds in Europe. These species do not contain significant levels of bromoform, but other bioactive components including carbohydrates, lipids, peptides, iodine, and polyphenols known as phlorotannins. Some brown algae are known to inhibit methanogens (Archaea) and therefore could potentially reduce enteric CH_4 emissions (Min et al., 2021). Exploration and characterization of any mitigating effects of these species are therefore of high interest.

To date, different species have been screened *in vitro*, using rumen fluid from sheep or cattle, as a cost-efficient and ethical first step to select a seaweed species for use in animal trials. Abbot et al. (2020) identified 21 seaweeds as candidates capable of mitigating CH_4 *in vitro*, while other species had no mitigating effect. Wang et al. (2008) observed a 15% reduction in CH_4 when *Ascophyllum nodosum* was included in an *in vitro* diet at 11%, but Roskam et al. (2022) observed no reduction in CH_4 emission adding 10g/kg DM. Including 2% of daily DM intake of *A. nodosum* extract in diet to sheep, Roskam et al. (2024) found inconsistent results on CH_4 emissions which was suggested to be due to differences in the content of phlorotannins in the diets.

Use of by-products generated from industrial processing of plants are well known as ruminant feeds, such as the protein-rich residues after oil extraction from oil seeds, grain residues from breweries and distilleries, and byproducts from sugar production (Salami et al., 2019). Typically, these by-products are not fit for human consumptions but used as feed by-products they can increase sustainable production of meat and milk. Some by-products contain bioactive compounds of interest for livestock. The seaweed industry extract hydrocolloids such as alginate, as well as high-value products for use as techno-functional food, pharmaceutical and cosmetic ingredients. Following hydrocolloid extraction process, a biomass residue or by-product remains, which usually has no economic value. Seaweed by-products have only, to a limited degree, been explored as feed additives in ruminant diets.

The by-product resulting from alginate extraction from *Laminaria hyperborea* contains residual alginate, cellulose, insoluble protein, and membrane lipids. Most water-soluble compounds, such as the carbohydrates used for storage of energy, are removed during the extensive washing steps, thus the composition of the by-product is less influenced by seasonal variations than the unprocessed biomass. However, minerals and other low-molecular weight compounds that are bound to, or embedded in, the insoluble parts of the seaweed will remain, including some polyphenols. The concentrations of iodine present in seaweed by-product resulting from alginate extractions were found to be in the same range and even higher, than concentration found in fresh biomass (Lind et al., 2023). However, Lind et al. (2023) investigated the iodine intake and excretion from sheep supplemented with the *L. hyperborea* by-product and found that most of the iodine was excreted in feces without compromising animal health.

In vitro analyses oxf CH_4 and total gas production of various brown algae, including the commercial species *A. nodosum*, *L. hyperborea*, *Saccharina latissima*, and *Alaria esculenta*, performed at the Swedish Agricultural University, Umeå (data not published) showed significant reductions in CH_4 for *A. nodosum* and one *L. hyperborea* stipe sample, however, with a tendency towards lower emissions for all *L. hyperborea* samples. Since the *L. hyperborea* byproduct used in this study is generated mainly from the stipes and has no current application or value, demonstration of a CH_4 mitigating effect of this would be of scientific interest. The aim of this study was to investigate the effect of *L. hyperborea* by-product inclusion in the diet of growing lambs on dry matter intake (DMI), CH_4 emissions, growth rate, and nitrogen digestibility. The hypotheses were 1) *L. hyperborea* inclusion in the diet of sheep reduces CH_4 emissions; 2) *L. hyperborea* inclusion does not affect DMI or growth rate.

2 Material and methods

2.1 Concentrate feed

Laminaria hyperborea by-product from industrial alginate production were provided by IFF N&H Norway AS (Haugesund, Norway) as described in Lind et al. (2023). Briefly, the by-products were pressed and then dried from 3% solid dry weight to 93% dry weight. The dried biomass was ground to 2 mm and incorporated into a concentrate feed manufactured by Felleskjøpet Agri (Rindsem Mølle, Verdal, Norway). Two types of concentrate were produced, a control (Control) and an experimental (Algae). The concentrate composition was designed for this study and was based on commonly used organic concentrate for sheep available for Norwegian farmers. Dried *L. hyperborea* by-product was included in the concentrate at 6% (w/w) inclusion rate. The ingredient composition of the two concentrates and the chemical composition of the *L. hyperborea* by-product are presented in Lind et al. (2023) and in the Supplementary Material.

2.2 Diet composition

Lambs were offered first cut grass silage composed of *Bromus inermis*, *Poa pratensis*, *Festuca pratensis* and *Trifolium repens*. The grass was wilted for 24 hours and subsequently baled in large bales (700 kg raw weight) without the use of an additive. During the experiment all animals received a fixed amount of concentrate with grass silage offered ad libitum. From week 1-2 the animals received 300g of concentrate, week 3-9 they received 400g and from week 10-17 they received 600g.

The inclusion rate of *L. hyperborea* by-product was limited by the iodine content of the by-product (Lind et al., 2023) and aimed to be 2% of the total diet (fresh matter). The inclusion was thus adjusted by increasing the concentrate level in parallel with the increased level of grass silage.

2.3 Animal trial

2.3.1 Animals, diets, and methane emissions

An *in vivo* sheep trial was initiated using 24 Norwegian White Sheep lambs (4 months of age). The trial was carried out in accordance with the Norwegian Ministry of Agriculture and Food regulation for use of animals in experiments and approved by the Ethics Commission on Animal Use application number FOTS ID 19314. It complies with the EU Directive 2010/63/EU on the use of experimental animals.

The animals consisted of 12 male lambs (37.8 kg live weight (LW) +/- SD 1.2 kg) and 12 female lambs (35.8 kg LW +/- SD 0.6 kg). The lambs were born in April 2021, milk fed for two months, then weaned and let out on a pasture where they additionally were fed a commercial concentrate. In the middle of August, 4 months of age, the lambs were collected and individually housed in stalls in an uninsulated barn at NIBIO Tjøtta in Nordland County, Norway (65° 49'22 N 12° 25'37 E). They were adapted to the indoor conditions fed grass silage *ad libitum*, 300 g commercial concentrate and given free access to water. The lambs were weighed and within sex, randomly allocated to either a Control diet (grass silage and Control concentrate, 36.4 kg LW +/- 1 kg) or an Algae diet (grass silage and Algae concentrate, 37.2 kg LW +/- 1 kg). This resulted in six male and six female lambs receiving each of the diets. Animals were fed their daily allowances in two equal portions at 08:30 and 14:30 h. Concentrate was offered separately from the grass silage. Grass silage was weighed for four consecutive days each week (Monday through to Thursday) and refused grass silage was measured before morning feeding (Tuesday through to Friday) to calculate daily feed intake. Samples of grass silage were taken weekly and dried for 48 h at 60°C for dry matter (DM) analysis. Fresh grass silage and concentrate samples were collected weekly and stored at -18 °C for further analysis.

Six open circuit respiration chambers at NIBIO Tjøtta were used during the experiment. Each chamber had a 25 x 25 mm frame of coated steel covered with a steel mesh that protected the exterior polycarbonate sheeting (Hart et al., 2014). The chambers measured 1.8 m wide, 1.8 m deep and 1.5 m high, comprising a total volume of approximately 5 m^3. Enteric CH_4 was sampled into a seven port Servopro Multiexact 4100 Analyzer (Servomex Group Inc., Woburn, MA, USA) as described in Lind et al. (2020). Methane concentrations in ambient air and in exhaust gas leaving each chamber (in total seven ports) were measured on a rotational basis, taking one gas sample reading after 2.5 min per port, measuring ambient air and each chamber every 17.5 min. The gas Servomex analyzer was calibrated before the animals entered the chambers using a zero gas (pure nitrogen) and a span gas (50 ppm CH_4). The average CH_4 recovery rate for the chambers was determined before and after the experiment and was 89%.

Animals were kept on the basic diet until five weeks prior to their entry to one of the respiration chambers. In a staggered order over four weeks, lambs (six each week) were introduced and adapted to their diets with three lambs receiving the Control and three lambs receiving the Algae diet each week. The lambs were kept in the chambers for 72 h, during which they were fed and managed in a similar way as in the barn. Grass silage intake was measured daily. Concentrate was offered separately from the grass silage and increased from 300 g/animal per day to 400 g/animal per day during Period 1 (see below) and further increased to 600 g/animal per day during Period 2 and 3. The increase in concentrate was following the nutritional needs for growing lambs and regulated according to grass silage intake to reach the target of 2% inclusion rate of *L. hyperborea* in the total diet. Water was available in buckets. Chambers were cleaned daily during morning feeding.

All the animals entered the respiration chambers in the same order, three times (Period 1, 2 and 3) to record enteric methane emissions. The total experimental period was 17 weeks including the adaptation period of five weeks and three periods of four weeks. Lambs were weighted every two weeks during the experimental period in an electronic scale (BioControl, WSS3000, Rakkestad, Norway).

2.3.2 Nitrogen digestibility

In vivo nitrogen digestibility was determined using the 12 male lambs assigned to the study as previously described by Lind et al. (2023). Briefly, lambs were placed individually in metabolism crates for three consecutive days during week 5 and week 10 of the present experiment and fed as described above. Samples of grass silage and refusals were collected daily, dried at 48 °C for 48 h and ground to pass 1-mm screen using a Tecator Cyclotec Sample Mill (Foss Analytical Co., Ldt., Suzhou, China). Samples were stored in plastic zipped bags at -18 °C until further analysis.

Buckets were placed under each of the metabolism crates to collect facilitate the separate collection of feces and urine One hundred milliliters (ml) of 10% sulfuric acid were added to each urine bucket to prevent loss of ammonia (Özkan-Gülzari et al., 2019). Acidity of urine was measured using a pH indicator strips non-bleeding pH 0 – 6.0 (Teststrips, pH, MColorpHast™, product number: 1.09531.0001, VWR International, Merck KGaA, Darmstadt, Germany). Feces and urine were collected, and total amount measured from each animal in the morning before feeding.

Samples of feces (g) and urine (l), 10% of total volume was collected and stored at −18°C for later analysis. Frozen fecal samples were course ground and freeze-dried for 48 h, using a Labconco FreeZone 4.5 Plus® freeze-drier (Kansas City, Missouri, USA). The samples were then ground to pass through a 1-mm screen using a Tecator Cyclotec Sample Mill® (Foss Analytical Co., Ltd., Suzhou, China) and stored in plastic zipped bags at -18°C until further analyses.

2.4 Analysis

2.4.1 Chemical composition grass silage, refusals, feces and urine

Samples of grass silage were analyzed using NIR by Eurofins Norway performed at Eurofins Agro Testing Wageningen (Binnenhaven 5, NL-6709 PD, Wageningen ISO/IEC 17025:2005 RvA L122) for DM, ash, crude protein (CP) and neutral detergent fiber (NDF) content. Nitrogen in feed, refusals, feces, and thawed urine was measured as Kjeldahl N (AOAC, 2002; method 2001.11, KjeltecTM 8400; Foss Electric, Hillerød, Denmark) by the laboratory at NMBU, Ås, Norway.

2.4.2 Polyphenol content of *L. hyperborea* by-product

The total polyphenol content (TPC) of the algal concentrate and control (without algae) was determined as described previously by Kupina et al. (2018) and is based on the AOAC recognized method of Singleton and Rossi (1965). Test extracts with algae and without (control) were prepared as follows: 100 mg of the test extract was transferred into a 100 mL volumetric flask. To this, 75 mL of water was added. The solution was sonicated for 10 min until all solids were dissolved. 5.0 mL of this solution was added to a 100 mL volumetric flask and diluted to volume with water. The weight of the test material was adjusted to achieve an absorbance within the calibration curve range. Phenolic content was determined by measuring the absorbance at 765 nm of the test extract in solution and comparing it with a calibration curve using Gallic acid (GA) as a standard. The concentration of Gallic acid for stock solutions was calculated using the equation:

$$\text{Stock standard solution (mg/L)} = M \times P \times (1 - W) \times 1000$$

where; M = mass of Gallic acid (g); P = purity of Gallic acid (%); and W = water content of Gallic acid. The concentration of Gallic acid of the stock standard solutions was then used to calculate the actual concentration of each calibration standard solution as follows: Calibration standard solution (mg/L) = s v mL × 25 where S = the concentration of the stock standard solution (mg/L) and V = the volume of stock standard solution (mL). Absorbance at 765 nm vs concentration of the calibration standards was used to determine the mg/L GAE for test samples. Results were determined as statistically different using the two-tailed P value test where the value was less than 0.0001 (P<0.0001) calculated using GraphPad Prism and are reported as mg GAE/100 mg of extract (the amount of extract used in the study).

2.5 Statistical analysis

To determine daily dry matter intake, and enteric methane emissions between diets and periods, data were analyzed using the ANOVA repeated measures mixed model in Minitab for Windows version 19.2. Diet, period, and sex were considered fixed effects and animal a random effect. Individual animal were nested under the respective diets.

Animal live weight and average daily weight gain were analyzed using the ANOVA general linear model in Minitab. Diet and sex were considered fixed and individual animal random effects. Initial start weight was used as covariate when calculating average daily weight gain.

Nitrogen digestibility was analyzed using the ANOVA general linear model in Minitab. Diet was considered fixed and individual animal random effects.

When a significant effect of diet, period or sex was found, *post hoc* comparison of means was made using the Tukey test. Differences were considered significant at $P < 0.05$.

3 Results

3.1 Feed composition

The chemical composition (g/kg DM) of grass silage and concentrate offered within each of the three periods and overall trial, during the 17 weeks is presented in Table 1. The two concentrates were formulated to be isonitrogenous and isocaloric.

3.1.1 Total polyphenols

As shown in Figure 1, the content of polyphenols in the algae fed diet (84.7 mg GAE/100 mg extract) was greater than the control (51.3 mg GAE/100 mg extract; $P < 0.0001$).

3.2 Feed intake

The inclusion of *L. hyperborea* in the Algae diet on fresh matter, including concentrate and grass silage, was on average 2.5% per animal per day during the 17 weeks of experiment (Table 2). The inclusion was significantly higher in Period 3 compared to Period 1 and Period 2 ($P < 0.001$) and higher for female lambs (maximum intake was 3.9%) compared to male lambs (maximum intake was 3.3%; $P < 0.01$), due to increased intake of concentrate relatively to the silage. No refusals of concentrate were observed throughout the experiment from any lambs. The proportion of concentrate consumed relative to grass silage was higher in female lambs ($P < 0.001$) compared to male lambs (43% versus 35% DM).

Dry matter intake (DMI) of grass silage and total DMI (TDMI) over the three periods, within period and sex is presented in Table 3. The differences in total DMI (TDMI) between diets (total and within Periods) are related to differences in DMI from grass silage, as the DMI from concentrate was the same for all animals within

TABLE 1 Average chemical composition (g/kg DM) of grass silage in Period 1, 2 and 3 and average over the three periods, Control and Algae concentrates.

	Grass silage				Concentrate	
	Period 1	Period 2	Period 3	Average periods	Control	Algae
DM, g/kg	414	446	355	405	881	881
Ash	55.2	57.6	56.2	56.3	97.6	95.3
OMD	69.7	67.7	70.7	69.4	11.6	11.7
Crude Protein	106.6	118.6	114.8	113.3	174.8	178.2
NDF	495.6	507.8	507.8	503.7	212.3	210.0
Sugar	100.0	105.0	75.2	93.4	55.6	52.2
Fat	24.4	24.0	26.0	24.8	45.4	44.3
pH	4.38	4.68	4.40	4.49	–	–
NH_3-N	88.8	93.4	110.4	97.5	–	–

Period. The DMI from concentrate increased from Period 1 (0.44 kg DM/lamb per day) to Period 2 and 3 (0.56 g DM/lamb per day). The proportion of concentrate (DM basis) in the diet was 38.0% in the Control diet and 39.8% in the Algae diet ($P = 0.153$). the proportion of concentrate in Period 1, 2, and 3 was 38.9%, 36.3% and 41.6% respectively ($P < 0.003$). Dry matter intake was similar between diets ($P = 0.16$). However, there were differences in DMI between diets within Periods ($P < 0.001$) with animals having the highest intake in Period 2, followed by Period 3 and Period 1. The inclusion of concentrate was highest in Period 3 (41% DM), followed by Period 2 (36% DM) and Period 1 (33% DM).

Total dry matter intake of grass silage and concentrate by male and female lambs for Period 1, 2, 3, respectively, are shown in Figure 2. The highest intake was by female lambs during Period 2 (1.02 kg DMI/lamb per day) and the lowest by female lambs during Period 1 (0.56 g DMI/lamb per day).

3.3 Methane emission

Table 3 shows the CH_4 production (g CH_4/animal per day), CH_4 yield (g CH_4/kg DMI) and intensity (g CH_4/kg LW) as an

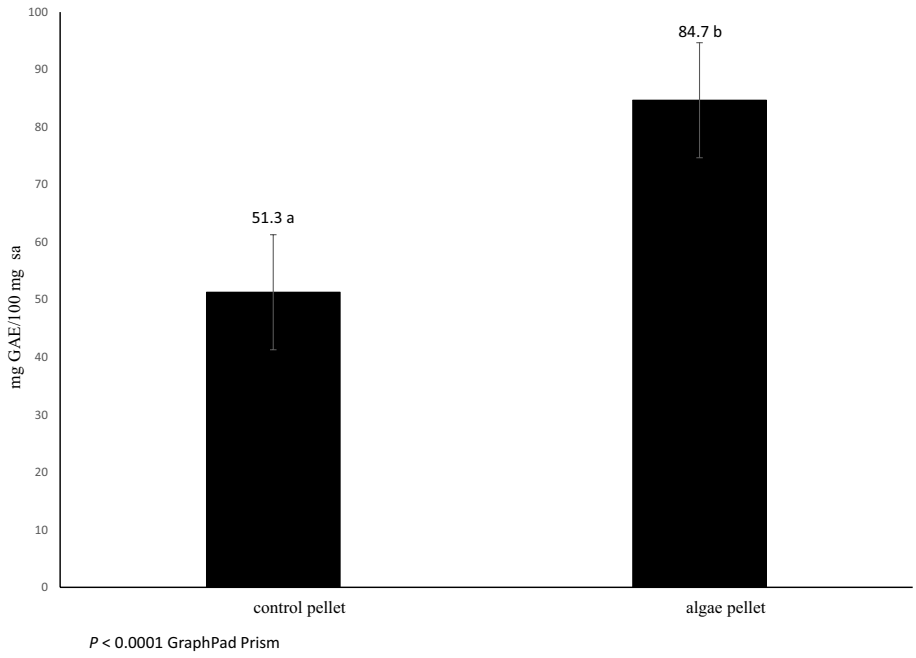


FIGURE 1
Total phenolic content (mg GAE/100 mg extract) in Control and Algae concentrates (n=3).

TABLE 2 Average percentage (%) *L. hyperborea* by-product in the Algae diet consumed by male (M) and female (F) lambs, and all lambs for Period 1, 2 and 3.

	Period 1		Period 2		Period 3		SEM	P-value		
	M	F	M	F	M	F		Period	Sex	PxS
% *L. hyperborea*	2.0^{d}	3.0^{b}	2.4^{bc}	2.3^{cd}	2.5^{bc}	2.9^{ab}	0.138	<0.001	0.01	<0.001
	2.5 ^{b}		2.4^{b}		2.7^{a}			<0.001		

Standard Error of the Means and P-values.
SEM, Standard error of the means; ^{a,b} superscript indicate significant difference between treatments.

average over the 3 periods and within each period, split on diets and on male and female lambs. There were no differences in CH_4 production, yield and intensity overall between diets. However, between periods, there were significant differences, where lambs emitted more CH_4 in Period 1 compared to Period 3 ($P < 0.001$). Methane production was highest for lambs during Period 2 ($P < 0.001$). Male lambs had greater CH_4 production (g CH_4/animal; $P = 0.006$), yield (g CH_4/kg DMI; $P = 0.001$) and intensity (g CH_4/kg LW; $P = 0.009$) compared to female lambs.

3.4 Live weight

Initial and final LW (kg) and average daily growth rate (g/d) for lambs in the Control and Algae treatments and for male and female lambs, are shown in Table 3. There were no differences in start weight between diets ($P = 0.41$) or sex ($P = 0.20$) (Table 3, Figure 3). Lambs fed the Control diet had a higher daily growth rate ($P = 0.034$) and final LW ($P = 0.026$). Male lambs were heavier ($P = 0.001$) at the end of the experiment and had higher growth rate ($P = 0.002$) than female lambs.

3.5 Nitrogen digestibility

Total N intake (g/day) was similar between diets ($P = 0.388$) and no differences in N output (feces or urine) were observed (Table 4).

4 Discussion

Seaweed is considered a natural product and as such can be referred to as a supplement rather than a feed additive. Inclusion of seaweed, or natural by-products from seaweed, in a diet to livestock,

TABLE 3 Average daily dry matter intake (DMI, kg) of grass silage, total dry matter intake (kg), average CH_4 production (g/d), CH_4 yield (g/kg DMI) and CH_4 intensity (g/kg live weight) for lambs fed Control and Algae diet in Period 1, 2 and 3, and male and female lambs.

												P-value			
	Control				Algae				Male	Female	SEM	Period	Diet	Sex	PxS
	P1	P2	P3	Average	P1	P2	P3	Average							
DMI grass silage	0.78^{bc}	1.01^{a}	0.86^{bc}	0.88	0.73^{c}	0.94^{ab}	0.79^{c}	0.82	0.95^{a}	0.74^{b}	0.017	0.000	0.158	0.000	0.000
Total DMI	1.21^{de}	1.56^{a}	1.42^{bc}	1.40	1.17^{e}	1.49^{ab}	1.36^{cd}	1.34	1.46^{a}	1.23^{b}	0.018	0.000	0.224	0.001	0.000
g methane/ day	33.3^{a}	29.3^{bc}	29.3^{bc}	30.4	24.4^{c}	33.4^{a}	26.5^{c}	28.1	32.1^{a}	26.4^{b}	0.551	0.001	0.214	0.006	0.7
g methane/ kg DMI	29.0^{a}	21.0^{bc}	19.4^{bc}	23.1	21.4^{b}	23.3^{b}	17.4^{c}	20.7	24.5^{a}	19.3^{b}	0.486	0.000	0.089	0.001	0.9
g methane/ kg live weight	0.67^{a}	0.53^{b}	0.48^{b}	0.57	0.52^{b}	0.65^{a}	0.47^{b}	0.54	0.60^{a}	0.51^{b}	0.011	0.000	0.619	0.009	0.9
Start weight	41.4				40.2				41.8	39.8	0.76	–	0.41	0.20	0.94
Final weight	58.5^{a}				55.2^{b}				59.4^{a}	54.3$^{b0.88}$	0.82	–	0.034	0.001	0.68
Growth rate, g/d	195a				177^{b}				200^{a}	165^{b}	6.7	–	0.026	0.002	0.40

Average initial and final live weight (kg), average daily weight gain (g/d) for lambs fed Control and Algae diet and male and female lambs. Standard Error of the Means and P-values.
SEM, Standard error of the means; ^{a,b} superscript indicate significant difference between treatments.

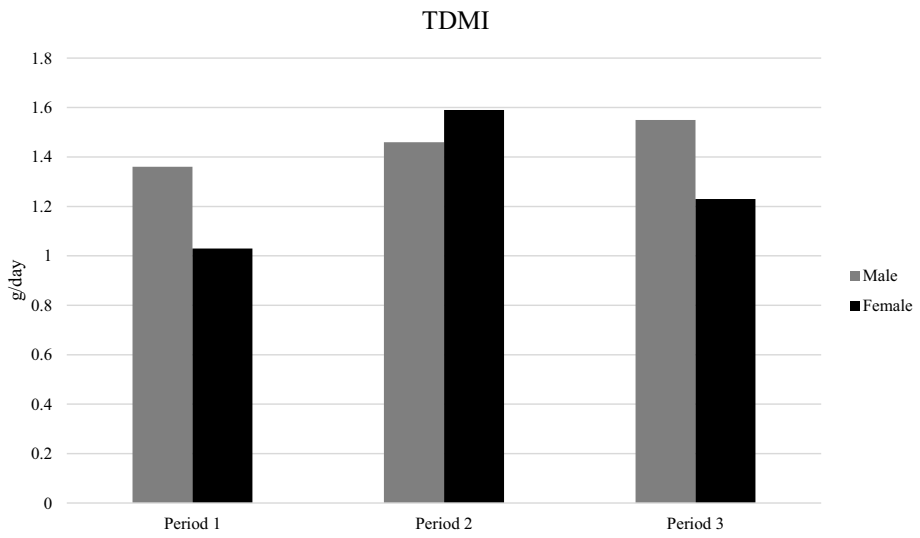


FIGURE 2
Total dry matter intake of grass silage and concentrate by male and female lambs for Period 1, 2, and 3.

thus do not need to go through a time-consuming European Food Safety Authority (EFSA) or FDA regulatory approval processes. Managing seaweed in the diets of livestock, however, needs attention due to toxic levels of heavy metals including arsenic, iodine, or other compounds, such as bromoform, that could affect animal health or if detected as residues in milk and meat products could be unsuitable or dangerous for human consumption. Although there are promising findings describing the use of the red seaweed *Asparagopsis* sp. as a CH_4 mitigation feed supplement in ruminants, there are also report of negative attributes in terms of the bromoform content which is toxic to animal and human health (Glasson et al., 2022). Also, harvested batches of *Asparagopsis* are inconsistent in their mitigating ability, as it ages over time and thus can lose effectiveness and activity in addition to being expensive to purchase (Zhu et al., 2021). Due to these challenges, focus has been changed to brown European coastal species. Several of the brown species have shown contents of polyphenols and other possible bioactive compounds to potentially mitigate enteric CH_4 (Wang et al., 2008), however with inconsistent results (Min et al., 2021; Roskam et al., 2022). Hansen et al. (2003) investigated the primitive sheep breed North Ronaldsay from the Orkney islands in Scotland which survives with seaweeds as their sole feed source. They report the occurrence of *in vitro* gas production by *Laminaria* ssp. which indicate these seaweeds contain phenolic compounds. Furthermore,

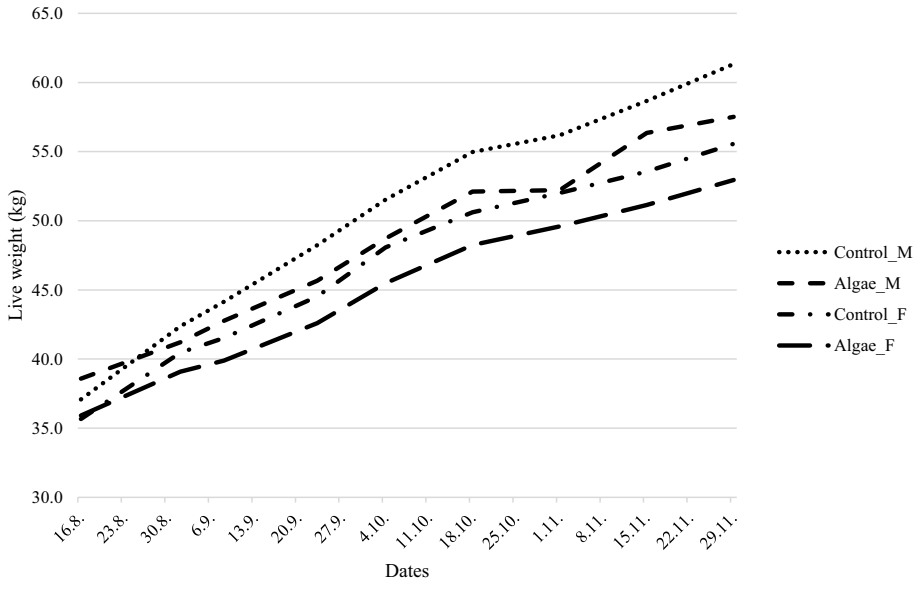


FIGURE 3
Live weight (kg) during the experimental period for male (M) and female (F) lambs fed Control or Algae diet.

TABLE 4 Average intake (g/d) and excretion (g/g intake) of nitrogen in urine and feces of male lambs fed Control and Algae diet.

	Control	Algae	SEM	*P*-value
Total N intake	29.4	28.2	0.687	0.39
N output feces	9.46	9.22	0.254	0.65
N output urine	12.9	12.7	0.491	0.86
N feces/total N intake	0.33	0.33	0.086	0.85
N urine/total N intake	0.45	0.47	0.019	0.74
N excrete/N intake	0.78	0.80	0.025	0.68

SEM, Standard error of the means.

the authors show that *Laminaria* spp. accounted for approximately 90% of the sheep daily feed intake and they therefore suggest that *Laminaria* spp have the potential to be used as an alternative feed source for other sheep breeds. North Ronaldsay sheep have adapted to these extreme conditions, and their metabolism may differ from larger intensive breeds which are bred for composite purposes (meat and wool) and as such are not comparable. Most of the results reported to date on use of brown seaweed species to mitigate enteric CH_4 from ruminants are from *in vitro* studies as few *in vivo* studies have been reported (e.g., Roskam et al., 2024). Due to the inconsistencies associated with dietary supplementation with whole brown seaweeds, there has been a renewed focus on the examination of by-products and bioactives derived from seaweed for their ability to consistently mitigate enteric CH_4 emissions.

4.1 Feed intake

In the current study, a by-product from *L. hyperborea*, was incorporated into a concentrate pellet. The inclusion rate of the by-product in the diets to sheep was 2.5%, but varying between male and female animals, and during the experiment. The increase in feed intake over time is explained by the growth of the lambs even though feed intake was highest in Period 2. The amount of concentrate inclusion in the total diet increased from Period 1 to Period 2 and was unchanged from Period 2 to Period 3. The largest differences are related to intake of grass silage but is not explained by differences in quality of the silages over the three periods (results not shown). As lambs grow, their demand for intake of high-quality feed increases. There were numerical differences in feed intake between animals of the two diets. Roskam et al. (2024) observed no differences in DMI with mature ewes supplemented with the brown seaweed *Ascophyllum nodosum*. Palatability was not an issue in the present experiment as all offered concentrate (which contained the seaweed biomass) was consumed.

4.2 CH_4 emissions

Overall, there were no difference in CH_4 emissions between the diets but differences were observed between experimental periods. The proportion of concentrate consumed was lower in period 1 which would explain the higher CH_4 emissions and yield observed in Period 1.,Similar differences were observed between male and female lambs with male lambs emitting more CH_4 compared to the female lambs. The proportion of concentrate in the diet to females were higher (42% DM) than that of diet to the male lambs (35% DM).

Ascophyllum nodosum is the most tested brown seaweed for nutritional and CH_4 mitigation purposes. The mitigation effect of inclusion as seaweed or as an extract are inconclusive as shown for Roskam et al. (2024) who in one experiment found that inclusion of the seaweed and extract, independently in the diet of sheep, reduced CH_4 emissions while similar results were absent in a repeat experiment. Use of *L. hyperborea* to mitigate CH_4 emissions in ruminants has not been previously reported. Literature referring to this brown seaweed species are focused on their effects in anaerobic digesters on biogas production. Inclusion of *L. hyperborea* by-product in the current study showed inconsistent results. In Period 1, algae fed lambs had a significant reduction in enteric CH_4 emission and yield compared to those fed the control diet. However, in Period 2, this was reversed and for Period 3, no differences were discovered. The rumen microbiome is the main driver for CH_4 production (Tapio et al., 2017) and via the diet it is possible to manipulate their efficiency. The reported effects of polyphenols to mitigate CH_4 thus is assumed to have an effect on the lower emissions and yield for Period 1.

Bioactive substances like polyphenols can modify metabolism in the rumen as identified previously by Vasta et al. (2019). Polyphenols, like phlorotannins, that are known to be exclusively found in brown seaweeds (e.g., *Laminaria hyperborea*), are thought to impact enteric CH_4 emissions through their bioactive antimicrobial or antioxidant actions, which can interfere with the metabolism of microorganisms including methanogens in the rumen. In the present study, the phlorotannin content of the polyphenols in *L. hyperborea* were not quantified specifically. Further characterization work on the algal extracts is therefore required. In An *in vitro* study, Vissers et al. (2017) examined phlorotannin extracts from *Laminaria digitata* at various concentrations and observed reductions in CH_4 production at dosages of 40 g/kg and greater (38-72% reduction). The concentrations of polyphenols reported in this work, are in line with values reported in previous studies focusing on the polyphenol content of *L. hyperborea*. For example, Wekre et al. (2022) found the content of polyphenols in *L. hyperborea* (expressed as mg GAE/g DW) to vary from 5.51 to 8.32 mg GAE/g DW. Concentrations of polyphenols were identified using qNMR in our study while the total polyphenol content (TPC) was determined by Wekre et al. (2019).

Considering it has been reported that the microorganisms adapt to the new environment when the diet is altered (McGovern et al., 2020), one would expect to observe less of a difference in the reduction in CH_4 emissions in the lambs fed the algae diet as the rumen microbes had time to adapt. However, higher CH_4 emissions and yield were recorded for lambs fed the algal supplemented diet

compared to the controls in Period 2, which was unexpected. Based on these results, it seems that the main effect on the CH_4 emissions is related to the level of concentrate in the diets rather than the inclusion of *L. hyperborea*. This is consistent with findings of Olijhoek et al. (2018) who found that increased levels of concentrate relative to forage reduced CH_4 emissions by 14 – 30%.

The largest differences in CH_4 emissions between the diets were found in the first period, and could possibly have been influenced by the development of the rumen and the rumen microbiota. However, in the Norwegian sheep production system, lambs are reared by their dams and follow onto pasture for four to five months before they are slaughtered. The rumen development of lambs occurs within the first two months of life with rumen microbial colonization achieved during the first month, rumen function at 2 months and anatomic development after 2 months (Jiao et al., 2015). Weaning time and access to solid feed have implications on the rumen development (Herath et al., 2021). The pre-experimental management of lambs in the present study included milk and concentrate feeding for two months before the lambs had access to pasture where they additionally were fed concentrate. Therefore, it is possible, but unlikely, that weaning time and pre-feeding influenced our results in the present experiment.

4.3 Weight

The inclusion of *L. hyperborea* in the diet affected lambs' growth rate resulting in algae supplemented lambs being 3 kg lighter following feeding for 4 months. Similarly, Roskam et al. (2024) found a tendency towards the inclusion of seaweed or seaweed extract affecting daily weight gain negatively. It is not ideal to feed growing livestock ingredients or feed additives that affects performance negatively as determined in this study. The maintenance of animal performance is essential for farmers as it directly impacts farm economy which, among other things, is based on the carcass weight. However, the lambs were not slaughtered, so this is an assumption based on previous experience of an observed high correlation between animal live weight and fat free carcass weight (Brooks, 2017; Nicol et al., 1968). In the *in vitro* trial by Wang et al. (2008) ruminal fermentation and protein degradation was reduced with inclusion of phlorotannins. As tannins are considered to reduce ruminal degradation of proteins (Frutos et al., 2004) it could be hypothesized that the phlorotannin content in the algae diet resulted in reduced protein utilization. On the other hand, nitrogen digestibility was not affected by the inclusion of *L. hyperborea* in the diet which leaves more questions to be investigated to find the true mechanisms. We did not expect *L. hyperborea* to be a source of protein as previous analysis of different seaweed species show that brown species in general are low in digestible protein (Tayyab et al., 2016) and their amino acid composition is not beneficial for livestock growth (Gaillard et al., 2018). Red species, on the other hand, may have a more beneficial protein content as we previously determined that lambs fed *Porphyra* sp or soybean meal as the primary protein sources had similar growth rate (Lind et al., 2020).

4.4 By-product as feed ingredient

By-products used in diets to livestock are low-input and sustainable feeds. The costs of by-products are often less than that of whole unprocessed materials (Salami et al., 2019) and the authors summarize the benefits of plant by-products for ruminant meat production. Using the by-products from alginate production requires pressing and drying, as the dry matter is below 5%, in our case was only 3%. Most of the water-soluble compounds of the *L. hyperborea* biomass were removed during the extensive washings during the alginate extraction, resulting in a lower ash content, lower content of toxic heavy metals, and higher protein content than in the native seaweed. However, the content of iodine, bound to insoluble parts, was similar to the original material or even higher in concentrations. Lind et al. (2023) investigated the pathway for iodine in sheep fed *L. hyperborea* by-products and found that most of the iodine was excreted in feces and none of the animals' showed signs of clinical intoxication. Costs and energy for the drying process must be considered when calculating the entire cost and benefits of the feed product. However, in a recent LCA analysis, the *L. hyperborea* residue performed better than other seaweed species and fractions due to the minimal processing requirements and lower allocated burdens (Thomas et al., 2025).

Salami et al. (2019) highlight that the content of bioactive compounds such as unsaturated fatty acids and phytochemicals may decrease GHG emissions while improving meat nutritional composition and shelf-life. However, there is little impact of *A. nodosum* inclusion on meat quality (chemical composition and sensory attributes) which was the only species reviewed when Costa et al. (2021) did their review. Salami et al. (2019) suggest that increased use of plant by-products may replace conventional forages and thus improve the environmental performance of meat production. In general, use of plant by-products can be a low-cost and low-input feed.

This study addresses new knowledge in understanding how brown seaweed species may affect enteric CH_4 emissions in small ruminants. To further understand the potential anti-methanogenic effects of brown seaweed species, additional research on the supplementation of the diets of adult ewes with brown seaweeds and their by-products at different level should be performed. This will address how they respond to the seaweed inclusion. As mentioned, the content of iodine and toxic heavy metals are the most limiting factors in the amount of some brown seaweed species that can be included in the diet of ruminants. Extraction of these compounds from the seaweeds must be achieved before we can recommend higher inclusion rates of the brown species.

5 Conclusion

Discovering new feed ingredients or feed additives with anti-methanogenic properties that are applicable at farm level, are essential to meet our global targets in reducing enteric methane emissions from ruminants. This study included a by-product from the seaweed *Laminaria hyperborea* as a potential to mitigate enteric

methane emissions from sheep. We tested if the inclusion of this by-product would reduce methane but have no negative effect on DMI or growth rate. The results showed that while methane was reduced due to dietary supplementation with the algae diet in the first period of the experiment, the overall production, yield, or intensity of methane was not affected by diet over the entire duration of the study. However, factors other than the algae inclusion may have contributed to the observed effects, and more research, with more comprehensive analyses, is needed to understand the effects on growth and methane emissions.

Data availability statement

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

Ethics statement

The animal studies were approved by Norwegian Ministry of Agriculture and Food Ethics Commission on Animal Use. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

VL: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. MH: Conceptualization, Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing. SW: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. SK: Data curation, Writing – original draft, Writing – review & editing. IA: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The project was funded by the SeaSolutions project, through the ERA-GAS cofound under Horizon 2020 with funding from the Research Council in Norway (308942).

Acknowledgments

The authors want to thank Trond Helgerud and team at IFF N&H Norway AS, who provided the *Laminaria hyperborea* by-product for the experiment and Felleskjøpet Fôrutvikling for producing the concentrates.

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2025.1430709/full#supplementary-material

References

Abbot, D., Aasen, I. M., Beauchemin, K. A., Grøndahl, F., Gruninger, R., Hayes, M., et al. (2020). Seaweed and seaweed bioactives for mitigation of enteric methane: Challenges and opportunities. *Animals* 10, 2432. doi: 10.3390/ani10122432

Brooks, C. (2017). *The relationship between lean meat yield and birth weight in New Zealand Romney sheep* (New Zealand: Dissertation. Dep. Agricultural Sciences, Lincoln university). Available at: https://hdl.handle.net/10182/9031. (Assessed May 03, 2024)

Costa, M., Cardoso, C., Afonso, C., Bandarra, N. M., and Prates, J. A. (2021). Current knowledge and future perspectives of the use of seaweeds for livestock production and meat quality: a systematic review. *J. Anim. Physiol. Anim. Nutr.* 105, 1075–1102. doi: 10.1111/jpn.13509

FAO (2023). "Methane emission in livestock and rice systems," in *Sources, quantification, mitigation and metrics*((FAO: Rome, Italy). doi: 10.4060/cc7607en

Frutos, P., Hervás, G., Giráldez, F. J., and Mantocón, A. R. (2004). Review. Tannins and ruminant nutrition. *Span. J. Agric. Res.* 2, 191–202. doi: 10.5424/sjar/2004022-73

Gaillard, C., Bhatti, H. S., Novoa-Garrido, M., Lind, V., Roleda, M. Y., and Weisbjerg, M. R. (2018). Amino acid profiles of nine seaweed species and their in *situ* degradability in dairy cows. *Anim. Feed Sci. Technol.* 241, 210–222. doi: 10.1016/j.anifeedsci.2018.05.003

Glasson, C. R. K., Kinley, R. D., de Nys, R., King, N., Adams, S. L., Packer, M. A., et al. (2022). Benefits and risks of including the bromoform containing seaweed Asparagopsis in feed for the reduction of methane production from ruminants. *Algal Res.* 64, 102673. doi: 10.1016/j.algal.2022.102673

Hansen, H. R., Hector, B. L., and Feldmann, J. (2003). A qualitative and quantitative evaluation of the seaweed diet of North Ronaldsay sheep. *Anim. Feed Sci. Technol.* 105, 21–28. doi: 10.1016/S0377-8401(03)00053-1

Hart, K. J., Yanez-Ruiz, D. R., Martin-Garcia, A. I., and Newbold, C. J. (2014).Chapter 5: Sheep methane chambers at Aberystwyth University (UK) and CSIC (Spain). In: *Technical manual on respiration chambers designs* (New Zealand:

Ministry of Agriculture and Forestry). Available online at: https://www.globalresearchalliance.org/wp-content/uploads/2012/03/GRA-MAN-Facility-BestPract-2012-ch51.pdf (Accessed 09 April 2024).

Herath, H. M. G. P., Pain, S. J., Kenyon, P. R., Blair, H. T., and Morel, P. C. H. (2021). Rumen Development of artificially-reared lambs exposed to three different rearing regimens. *Animals* 11, 3606. doi: 10.3390/ani11123606

Jiao, J., Li, X., Beauchemin, K. A., Ta, Z., Tang, S., and Zhou, C. (2015). Rumen development process in goats as affected by supplemental feeding v. grazing: age-related anatomic development, functional achievement and microbial colonisation. *Br. J. Nutri.* 113, 888–900. doi: 10.1017/S0007114514004413

Kupina, S., Fields, C., Roman, M. C., and Brunelle, S. (2018). Determination of total phenolic content using the Folin-C Assay: Single-Laboratory validation, First Action 2017.13. *J. AOAC Int.* 101, 1466–1472. doi: 10.5740/jaoacint.18-0031

Lind, V., Opheim, M., Sandvik, J. T., and Aasen, I. M. (2023). Iodine intake and excretion from sheep supplemented with macroalgae (*Laminaria hyperborea*) by-product. *Front. Anim. Sci.* 4. doi: 10.3389/fanim.2023.1213890

Lind, V., Weisbjerg, M. R., Jørgensen, G. M., Fernandez-Yepes, J. E., Arbesú, L., and Molina-Alcaide, E. (2020). Ruminal fermentation, growth rate and methane production in sheep fed diets including white clover, soybean meal or Porphyra sp. *Animals* 10, 79. doi: 10.3390/ani10010079

McGovern, E., McGee, M., Byrne, C. J., Kenny, D. A., Kelly, A. K., and Waters, S. M. (2020). Investigation into the effect of divergent feed efficiency phenotype on the bovine rumen microbiota across diet and breed. *Sci. Rep.* 10, 15317. doi: 10.1038/s41598-020-71458-0

Min, B. R., Parker, D., Brauer, D., Waldrip, H., Lockard, C., Hales, K., et al. (2021). The role of seaweed as a potential dietary supplementation for enteric methane mitigation in ruminants: Challenges and opportunities. *Anim. Nutri.* 7, 1371–1387. doi: 10.1016/j.aninu.2021.10.003

Muizelaar, W., Groot, M., Van Duinkerken, G., Peters, R., and Dijkstra, J. (2021). Safety and transfer study: Transfer of bromoform present in *Asparagopsis taxiformis* to milk and urine of lactating dairy cows. *Foods* 10, 584. doi: 10.3390/foods10030584

Nicol, A. M., Thomson, G. G., and McLean, J. W. (1968). The prediction of carcass composition of lambs from live weight and red cell volume. *New Z. J. Agric. Res.* 11, 330–340. doi: 10.1080/00288233.1968.10431432

Olijhoek, D. W., Løvendahl, P., Lassen, J., Hellwing, A. L. F., Höglund, J. K., Weisbjerg, M. R., et al. (2018). Methane production, rumen fermentation, and diet digestibility of Holstein and Jersey dairy cows being divergent in residual feed intake and fed a 2 forage-to-concentrate ratio. *J. Dairy Sci.* 101, 9936–9940. doi: 10.3168/jds.2017-14278

Özkan-Gülzari, S., Lind, V., Aasen, I. M., and Steinshamn, H. (2019). Effect of supplementing sheep diet with macroalgae species in *in vivo* nutrient digestibility, rumen fermentation and blood amino acid profile. *Animal* 13, 2792–2801. doi: 10.1017/S1751731119001502

Roskam, E., Kirwan, S. F., Kenny, D. A., O'Donnell, C., O'Flaherty, V., Hayes, M., et al. (2022). Effect of brown and green seaweeds on diet digestibility, ruminal fermentation patterns and enteric methane emissions using the rumen simulation technique. *Front. Anim. Sci.* 3. doi: 10.3389/fanim.2022.1021631

Roskam, E., O'Donnell, C., Hayes, M., Kirwan, S. F., Kenny, D. A., O'Flaherty, V., et al. (2024). Enteric methane emission reduction potential of natural feed supplements in ewe diets. *J. Anim. Sci.* 11, skad421. doi: 10.1093/jas/skad421

Salami, S. A., Luciano, G., O'Grady, M. N., Biondi, L., Newbold, C. J., Kerry, J. P., et al. (2019). Sustainability of feeding plan by-products: A review of the implications for ruminant meat production. *Anim. Feed Sci. Technol.* 251, 37–55. doi: 10.1016/j.anifeedsci.2019.02.006

Singleton, V. L., and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144–158. doi: 10.5344/ajev.1965.16.3.144

Tapio, I., Snelling, T. J., Strozzi, F., and Wallace, R. J. (2017). The ruminal microbiome associated with methane emission from ruminant livestock. *J. Anim. Sci. Biotechnol.* 8, 7. doi: 10.1186/s40104-017-0141-0

Tayyab, U., Novoa-Garrido, M., Roleda, M. Y., Lind, V., and Weisbjerg, M. R. (2016). Ruminal and intestinal protein degradability of various seaweed species measured in *situ* in dairy cows. *Anim. Feed Sci. Technol.* 213, 44–54. doi: 10.1016/j.anifeedsci.2016.01.003

Thomas, J. B., Xu, V., Krizsan, S. J., Aasen, I. M., Oliveira, A., Ramos, H., et al. (2025). *Seaweed as a climate fix for meat and dairy production: An LCA perspective* submitted.

Vasta, V., Daghio, M., Cappucci, A., Buccioni, A., Serra, A., Viti, C., et al. (2019). Invited review: Plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: Experimental evidence and methodological approaches. *J. Dairy Sci.* 102, 3781–3804. doi: 10.3168/jds.2018-14985

Vissers, A. M., Caligiani, A., Sforza, S., Vincken, J.-P., and Gruppen, M. (2017). Phlorotannin composition of Laminaria digitata. *Phytochem. Anal.* 28, 487–495. Available at: https://edepot.wur.nl/418098. (Assessed May 03, 2024)

Wang, Y., Xu, Z., Bach, S. J., and McAllister, T. A. (2008). Effects of phlorotannins from *Ascophyllum nodosum* (brown seaweed) on *in vitro* ruminal digestion of mixed forage or barley grain. *Anim. Feed Sci. Technol.* 145, 375–395. doi: 10.1016/j.anifeedsci.2007.03.013

Wasson, D. W., Yarish, C., and Hristov, A. N. (2022). Enteric methane mitigation through Asparagopsis taxiformis supplementation and potential algal alternatives. *Front. Anim. Sci.* 3. doi: 10.3389/fanim.2022.999338

Wekre, M. E., Hellesen Brunvoll, S., and Jordheim, M. (2022). Advancing quantification methods for polyphenols in brown seaweeds-applying a selective qNMR method compared with the TPC assay. *Phytochem. Anal.* 33, 1099–1110. doi: 10.1002/pca.3162

Wekre, M. E., Kåsin, K., Underhaug, J., Holmelid, B., and Jordheim, M. (2019). Quantification of polyphenols in seaweeds: A case study of ulva intestinalis. *Antioxidants* 8 (12), 612. doi: 10.3390/antiox8120612

Zhu, P., Li, D., Yang, Q., Su, P., Wang, H., Heimann, K., et al. (2021). Commercial cultivation, industrial application, and potential halocarbon bio synthesis pathway of Asparagopsis sp. *Algal Res.* 56, 102319. doi: 10.1016/j.algal.2021.102319

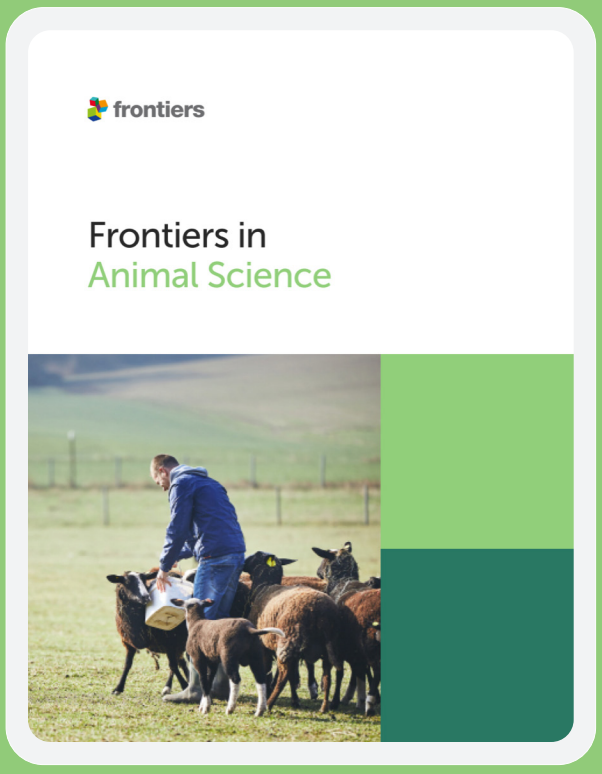
Frontiers in
Animal Science

Understanding the use and management of animals for food production

A multidisciplinary journal that advances our understanding of food and livestock production, while safeguarding animal welfare and environmental sustainability.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact