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Health and welfare problems of farm animals: Prevalence, risk factors, consequences and possible prevention solutions

Edited by
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Health and welfare problems of farm animals: Prevalence, risk factors, consequences and possible prevention solutions

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Table of contents

04 Editorial: Health and welfare problems of farm animals: prevalence, risk factors, consequences and possible prevention solutions
Nikola Čobanović and Luisa Magrin

06 Characteristics of thermal images of the mammary gland and of performance in sows differing in health status and parity
Stephan Rosengart, Bussarakam Chuppava, Lea-Sophie Trost, Hubert Henne, Jens Tetens, Imke Traulsen, Ansgar Deermann, Michael Wendt and Christian Visscher

22 Effect of lameness on feeding behavior of zero grazed Jersey dairy cows
Sandra Gündel, Christian Looft, Leslie Foldager and Peter T. Thomsen

32 Discrepancies between farmers' perceptions and actual animal welfare conditions on commercial pig farms
Eva Nadlučnik, Irena Golinar Oven, Iztok Tomažič, Jan Plut, Alenka Dovč and Marina Štukelj

41 The relationship between common data-based indicators and the welfare of Swiss dairy herds
Barbara Lutz, Sibylle Zwygart, Beat Thomann, Dimitri Stucki and Joan-Bryce Burla

51 Treatment protocols for claw horn lesions and their impact on lameness recovery, pain sensitivity, and lesion severity in moderately lame primiparous dairy cows
Mohammed Babatunde Sadiq, Siti Zubaidah Ramanoon, Wan Mastura Shaik Mossadeq, Rozaihan Mansor and Sharifah Salmah Syed-Hussain

62 Use of qualitative behavioural assessment to investigate affective states of housed dairy cows under different environmental conditions
Alison L. Russell, Laura V. Randall, Jasmeet Kaler, Nikki Eyre and Martin J. Green

79 Stayability in Simmental cattle as affected by muscularity and body condition score between calvings
Giovanni Buonaiuto, Nicolas Lopez-Villalobos, Angela Costa, Giovanni Niero, Lorenzo Degano, Ludovica Maria Eugenia Mammi, Damiano Cavallini, Alberto Palmonari, Andrea Formigoni and Giulio Visentin

91 Welfare implications on management strategies for rearing dairy calves: A systematic review. Part 1–feeding management
Patricia Carulla, Arantxa Villagrá, Fernando Estellés and Isabel Blanco-Penedo

103 Welfare implications on management strategies for rearing dairy calves: A systematic review. Part 2 – Social management
Patricia Carulla, Arantxa Villagrá, Fernando Estellés and Isabel Blanco-Penedo

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Editorial: Health and welfare problems of farm animals: prevalence, risk factors, consequences and possible prevention solutions

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KEYWORDS

Artificial Intelligence, calf welfare, data, farmer perceptions, lameness, muscularity and body condition score, thermography, qualitative behavioral assessment

Editorial on the Research Topic
Health and welfare problems of farm animals: prevalence, risk factors, consequences and possible prevention solutions

According to the forecasts, the global population is expected to grow by two billion by 2050 and, subsequently, the demand for animal products especially those obtained from animal welfare-friendly production systems to satisfy high consumer requirements (1–4). However, the vast literature points out that intensive farming systems aimed at maximizing productivity per animal generate negative impacts on the health and welfare of farm animals, such as increased emotional stress (5, 6), risk of injuries, and physiological and anatomical disorders (*i.e.,* higher prevalence of lameness, etc.) (7), and reduced life expectancy (8, 9). There are many causes often linked to the nutritional and management practices and housing regimes adopted by the farmers (10, 11). Improving the health and welfare of farm animals can enhance their growth rate and reproduction, the quantity and quality of the final marketed product, and, as a consequence, the economic efficiency of the farms (12). Moreover, it may offer significant benefits for human health in the long term, contributing to a reduction in antibiotic use at non-therapeutic levels for growth promotion or disease prevention or in the use of some contaminants (i.e., pesticides) on crops to feed farm animals (13, 14). Despite increasing interest in this research field in the past several decades, the prevalence and consequences of health and welfare problems in intensive farming systems are alarming, and thus, there are still many concerns to be dealt with. Effective preventive and corrective procedures or protocols and new diagnostic methods to be implemented to identify animal welfare risks are crucial in ensuring animal and human health.

This Research Topic consists of a collection of nine studies, two on pigs and seven on dairy cows, that deal with some of the current health challenges for farm animals and with alternative approaches to assessing their welfare. Concerning the existing problems being experienced by farmers, lameness is still one of the most impactful issues regarding animal welfare and economic losses for cattle (15, 16). The potential effects of lameness on animal behavior and a viable treatment protocol for its recovery are some of the topics discussed in two studies of the collection (Gündel et al.; Sadiq et al.). Gündel et al. reported that Jersey cows could behave differently to lameness compared to other breeds and that feeding indicators might not be a useful tool for early detection of lameness. To obtain better recovery rates, treatment protocol consisting of therapeutic trim, hoof block, and pain management, in combination with early detection of cow lameness, was suggested by Sadiq et al.. Among the new alternatives to assess animal welfare, Rosengart et al. reported that thermography, coupled with Artificial Intelligence systems, could be a promising diagnostic tool for detecting diseased sows and piglets at the earliest time. In addition, Lutz et al. explored the accuracy of a quick and cost-effective data-based prediction of dairy cow welfare status. The authors demonstrated that data-based parameters have only potential to provide useful information on specific welfare aspects rather than to provide a comprehensive predictive tool for dairy welfare status at the herd level. In the study reported by Nadlučnik et al., differences between farmers' perceptions and real pig welfare conditions were evaluated. Despite the fact that farmers are aware of animal welfare importance, they follow only minimal statutory requirements, indicating that there is considerable room for improvement, especially regarding biosecurity on pig farms. Another topic covered in this Research Topic concerns the importance of the role of some environmental or resource-related actions as preventive measures to reduce animal stress. Specifically, two detailed systematic reviews reported the best feeding and social management (housing) practices for improving the welfare of pre-weaned calves [Carulla et al. (a); Carulla et al. (b)]. The authors reported that the most important gaps in knowledge regarding dairy calves are the lack of a clear protocol for administering milk replacers to reduce hunger and the best management of weaning to reduce stress, as well as the information regarding optimal time to separate the calf from its mother. One study investigated using the qualitative behavioral assessment whether the provision of different forms of environmental enrichment resources would impact the affective states of housed dairy cows (Russell et al.). The results obtained in this study demonstrated that simple modification to the housed environment, access to a novel object and outdoor space positively influenced the affective lives of commercially housed dairy cows. Lastly, this Research Topic also contains an innovative study, authored by Buonaiuto et al., who provided new predictive indicators (muscularity and body condition score) of the stayability and longevity in a dual-purpose cattle population.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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.

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Characteristics of thermal images of the mammary gland and of performance in sows differing in health status and parity

Stephan Rosengart[1,2], Bussarakam Chuppava[2], Lea-Sophie Trost[3], Hubert Henne[4], Jens Tetens[5], Imke Traulsen[3], Ansgar Deermann[6], Michael Wendt[1] and Christian Visscher[2]*

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Precision livestock farming can combine sensors and complex data to provide a simple score of meaningful productivity, pig welfare, and farm sustainability, which are the main drivers of modern pig production. Examples include using infrared thermography to monitor the temperature of sows to detect the early stages of the disease. To take account of these drivers, we assigned 697 hybrid (BHZP db. Viktoria) sows to four parity groups. In addition, by pooling clinical findings from every sow and their piglets, sows were classified into three groups for the annotation: healthy, clinically suspicious, and diseased. Besides, the udder was thermographed, and performance data were documented. Results showed that the piglets of diseased sows with eighth or higher parity had the lowest daily weight gain [healthy; 192 g ± 31.2, clinically suspicious; 191 g ± 31.3, diseased; 148 g ± 50.3 ($p < 0.05$)] and the highest number of stillborn piglets (healthy; 2.2 ± 2.39, clinically suspicious; 2.0 ± 1.62, diseased; 3.91 ± 4.93). Moreover, all diseased sows showed higher maximal skin temperatures by infrared thermography of the udder ($p < 0.05$). Thus, thermography coupled with Artificial Intelligence (AI) systems can help identify and orient the diagnosis of symptomatic animals to prompt adequate reaction at the earliest time.

KEYWORDS

infrared imaging, postpartum dysgalactia syndrome, precision farming, smart farming, healthcare, welfare monitoring, udder, early disease detection

Introduction

Precision livestock farming (PLF) is key to optimizing farming processes (1, 2). PLF technologies are becoming increasingly important in modern pig production in terms of animal welfare and farm sustainability, with special regard to the survival rate and performance of healthy piglets after birth (1). Recent techniques based on thermal imaging allow estimating the body core temperature by measuring the surface temperature of different body parts without touching the animal (3–5). The temperature is determined indirectly *via* the radiation intensity (5). Previous studies reported using infrared thermography to assess the temperature rise for mastitis diagnosis in dogs, sheep, and cows (6–16) and to detect the disease in pig production (17, 18). In addition, infrared thermography was used in piglets. A correlation was found between the temperature of individual body regions (ear base and back) and rectal temperature (19), and between the temperature of individual body regions (eye, ear base, back, and anus) and the age and growth rate of the piglets (20). To reduce the mortality rate through illness or infection, early disease detection is an important monitor, especially in sows (18, 21). Monitoring of sows' health is the key to preventing and controlling diseases in sows, and it guarantees optimal rearing conditions for piglets (18).

Shortly after farrowing, sows can suffer from postpartum dysgalactia syndrome (PDS), which disturbs the sows' performance and impairs animal welfare (22, 23). The leading symptoms of PDS are a body temperature higher than 39.5°C and reduced feed intake (24). Furthermore, purulent vaginal discharge and inflammation with swelling and reddening of the mammary gland may occur (25). If the udder is painful, the sows rest on it more often without presenting it to the piglets (25–27). Therefore, piglets cannot access teats, and this will reduce their intake of colostrum and milk. Too little colostrum intake negatively impacts growth performance and reduces the survival chances of a suckling piglet until weaning (28). Moreover, milk intake and piglets' daily weight gain until weaning show a highly positive correlation (29). Many healthy piglets with a high daily weight gain can reasonably achieve satisfactory weight at weaning if suckling from sows with high colostrum and milk secretion. Through genetic selection and management improvement in recent years, this is more important than in the past because the number of live-born piglets per sow per year has increased (30, 31). In addition, the increase in litter variation due to large litters is an additional factor that minimizes survival rates of suckling piglets, especially of small suckling piglets (32) and especially in multiparous breed sows. High milk secretion can be expected in healthy sows reared under a favorable farming environment (management and feeding) for optimal expression of genetic potential for neuroendocrine support during gestation and lactation (23, 33–36). During the suckling period, daily weight gain of suckling piglets of about 200 g can then be expected (37). Moreover, when there is a lack of milk, the hungry piglets show restless behavior (33, 38), injuries to the carpal joints or face, and growth retardation (23). In addition, piglets born from PDS-affected sows are more prone to diarrhea, resulting in a higher mortality rate (23, 39). In order to keep sows healthy, it is important to identify and treat sows diagnosed with the disease as soon as possible (40).

As mentioned above, PDS is associated with an increase in core body temperature (35). Mastitis is generally understood to be inflammation of the parenchyma of the udder (26). Inflammation is characterized by redness, swelling, pain, heating, and loss of function (18, 41). In both cases, the increase in temperature is a very frequent symptom. Therefore, the results that using infrared thermography of the mammary gland, in general, can make a helpful contribution to finding diseased sows with poor milk production have been reported in numerous studies (18, 42, 43) because those animals have warmer temperatures in the thermal image of the mammary gland. Whether the age and parity of sows have an influence on the information contained in the thermal image of the mammary gland has not yet been researched. Furthermore, it is known that the age and parity of sows have an influence on the sows' performance (44–46). Therefore, we hypothesized that the temperature in the thermal image of the mammary gland and the information from the thermal images about performance and health status differ between parity groups.

Thus, the aim of this study was to examine whether there are certain parities or parity groups in which infrared thermography of the udder allows differentiation between PDS-affected sows and non-PDS-affected sows and between sows with high and low performance. In addition, whether there are parities or parity groups in which infrared thermography of the udder allows no differentiation regarding this.

Materials and methods

Animals and diets

Data collection and animal housing were carried out on a farm in Lower Saxony, Germany from August 2019 to November 2020 in accordance with German regulations, and the research protocol was approved by the Animal Welfare Officer of the University of Veterinary Medicine Hannover, Hanover, Germany (reference: TVO-2020-V-9). A total of 487 db, Viktoria hybrid sows (BHZP Landrace × BHZP Large White, Bundes Hybrid Zucht Programm (BHZP), Ellringen, Germany) with a parity ranging from 1 to 14 were used in this study. The sows were examined throughout up to three lactations, so a total of 697 sows were examined at birth.

The sows studied were kept in four identical farrowing units, each with 24 ProDromi farrowing pens (about 12 pens

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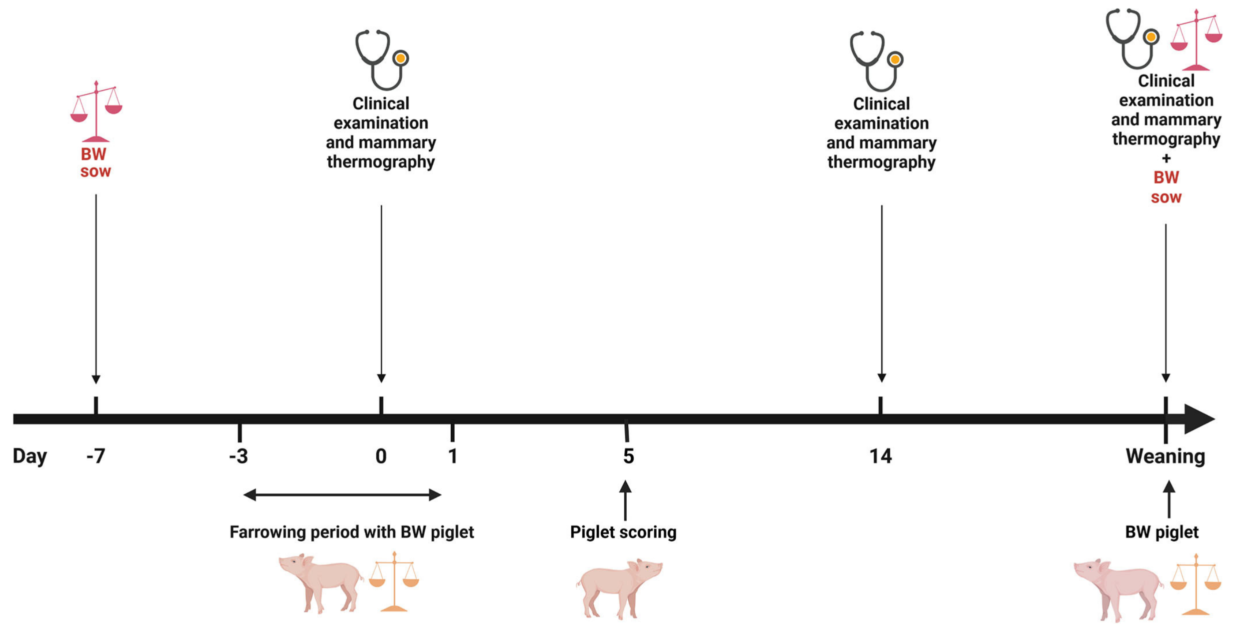

FIGURE 1
Overview of the data collection. BW, bodyweight; (Figure was created with BioRender.com). Adapted from Rosengart et al. (18).

TABLE 1 Nutrient contents in the gestation and lactation diet (pelleted complete feed) in accordance with the analysis (g/kg as fed).

Item	Gestation diet	Lactation diet
Dry matter	887 ± 7.5	889 ± 6.9
Crude protein	142 ± 3.4	163 ± 10.1
Crude fat	30 ± 1.5	36 ± 4.0
Crude fiber	72 ± 5.8	48 ± 3.7
Crude ash	53 ± 1.4	52 ± 3.0
Calcium	7.2 ± 1.0	8.1 ± 1.0
Phosphorus	4.8 ± 0.4	6 ± 0.2
Energy (MJ ME/kg)	11.7 ± 0.3	13 ± 0.3

The feed was designed in accordance with the recommendations of the Society for Nutritional Physiology (GfE) (47).

on each side). The four farrowing units were arranged behind each other. Sows were housed in farrowing crates. Antibiotic treatments were not administered to sows within at least seven days prior to farrowing. In compliance with the analysis, the nutritional content of the sows' diet is shown in Table 1. More detailed information has been previously described by Rosengart et al. (18).

Experimental procedure and sampling

Data acquisition from the sows and the piglets was performed as described by Rosengart et al. (18). Briefly, during the experimental period, a clinical examination and infrared thermography of the mammary gland were performed shortly after farrowing, ~14 days after farrowing and at weaning.

A skin score of the carpal joints of the piglets took place about 5 days after birth (Figure 1). The modified scoring system from the Board of Trustees for Technology and Construction in Agriculture (Kuratorium für Technik und Bauwesen in der Landwirtschaft (KTBL)) (48) was used. Briefly, a score of 0 meant no bloody or encrusted injuries on the carpal joints with a diameter of 0.5 cm or more; a score of 1 meant >50% of the litter with bloody or encrusted injuries on the carpal joints with a diameter of 0.5 cm or more; and score 2 meant more than 50% of the litter with bloody or encrusted injuries on the carpal joints with a diameter of 0.5 cm or more (18).

Readings

Sows' and piglets' performance

The piglets were individually marked and weighed within the first 24 h of farrowing. In addition, the piglets were weighed at weaning. Thus, the daily weight gain of each piglet could be determined during this period. Usually, cross-fostering took place after the first 24 h of farrowing. Furthermore, all sows were weighed twice. The first weighing took place when sows were moved to the farrowing pen and the second weighing occurred when they were removed from the farrowing pen (Figure 1). Sows were moved to the farrowing pen on approximately day

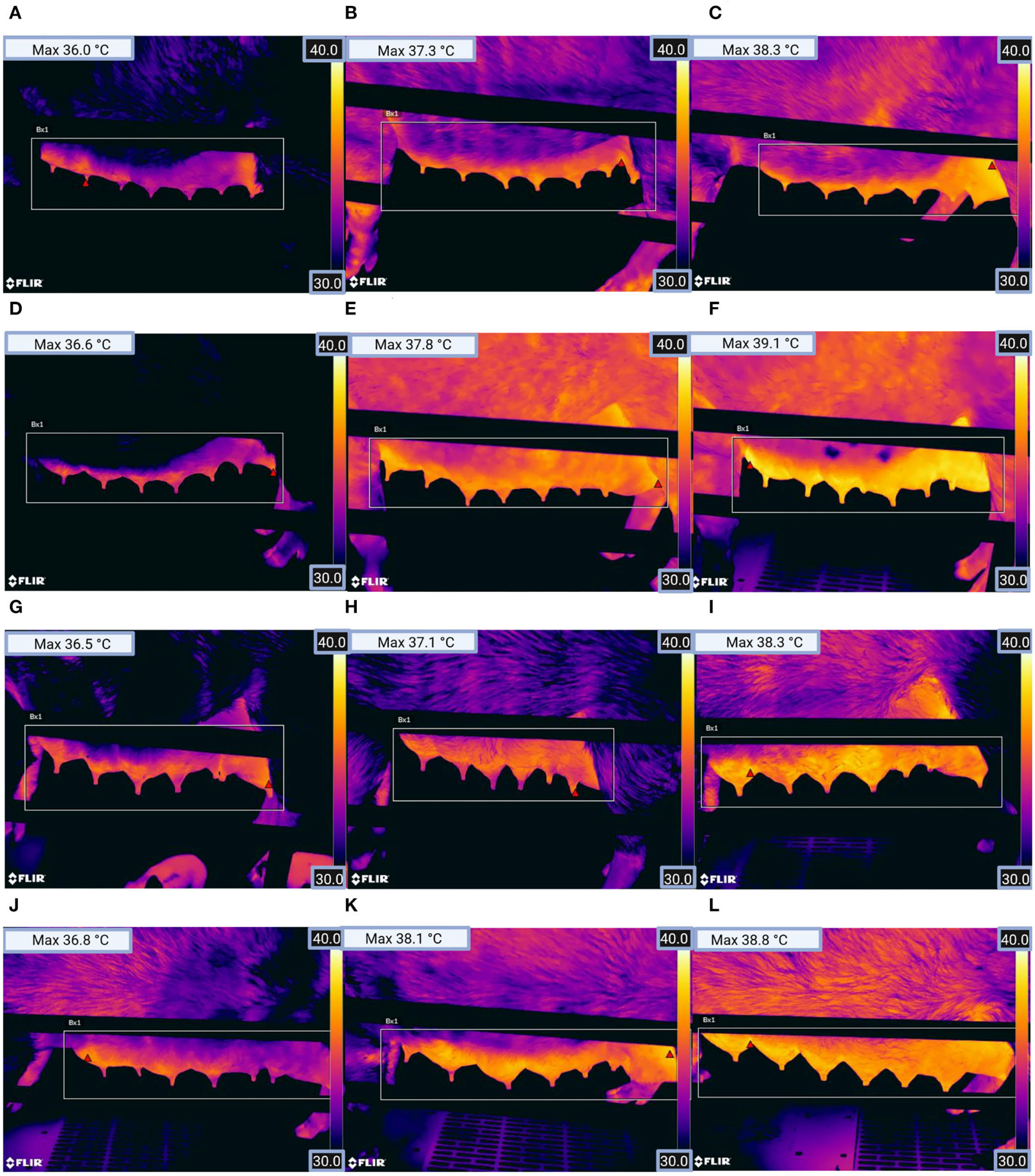

FIGURE 2
Thermographic images from the left mammary gland of sows for **(A)** parity group 1 (healthy), **(B)** parity group 1 (clinically suspicious), **(C)** parity group 1 (diseased), **(D)** parity group 2 (healthy), **(E)** parity group 2 (clinically suspicious), **(F)** parity group 2 (diseased), **(G)** parity group 3 (healthy), **(H)** parity group 3 (clinically suspicious), **(I)** parity group 3 (diseased), **(J)** parity group 4 (healthy), **(K)** parity group 4 (clinically suspicious), and **(L)** parity group 4 (diseased). The red triangle within the rectangle in the image shows the location of the pixel with the highest temperature (see Max temperature top left in the picture). (Figure was created with BioRender.com).

110 of gestation. Weighing was performed with a pair of low floor scales (Meier-Brakenberg GmbH & Co. KG, Extertal, Germany). The scales were designed for a weight range from 0 to 2000 kg and weighed in increments of 1 kg. Moreover, all collected data for the sow management software db. planer (Version 1906, BHZP GmbH, Dahlenburg-Ellringen, Germany) were available, i.e., sows' parity, a total of born piglets (TBP), number of stillborn piglets (NS), number of piglets born alive (NBA), number of piglets that had died before weaning (PWM), and number of weaned piglets (NWP) for each sow for that litter.

Thermal image capture and analysis and diagnosis of PDS

The ambient temperature in the farrowing unit was read and recorded as displayed by the barn equipment before entering and after leaving a farrowing unit. The temperature measurement of the barn equipment was verified by non-stop temperature measurement with TGP-4500 Tinytag Plus 2 temperature loggers. Humidity was measured by Tinytag Plus 2 temperature loggers.

Every mammary complex was inspected individually shortly after birth (0, 1, or 2 days afterwards), ~14 days after birth, and at weaning. Inspection and palpation of the mammary gland characterized the clinical examination. The scoring system from previous studies (18, 42) was used for this. In this way, the degree of formation, the degree of redness, the consistency, whether there were nodes in the parenchyma, and whether mammary complexes were painful were described. Score 0 meant that everything was physiological, score 2 meant the greatest deviation from normal, and score 1 was in the middle of both. Clinical findings were recorded. Moreover, sows with no food consumption, purulent discharge, or that were reluctant to stand up were recorded, too.

Following the clinical examination of the sows, a picture with infrared thermography was taken of the sows' mammary gland [on both sides of the mammary gland, Figure 2 (FLIR T540, FLIR Systems, Inc., Wilsonville, or, the USA)]. Infrared images of the sows, with a temperature scale, were taken in a standing position. In accordance with previous studies (42, 49), emissivity was set at 0.96. The spacing between the sow and the camera was about one meter and was measured by the camera before each image was captured. While capturing an image, an angle of <60° to the udder was avoided (42). The farrowing pens were ProDromi farrowing pens, so the heating plates of the piglet nests were located in front of the sows' head and surrounded by plastic. That is why stray radiation from that heating was no problem. Lastly, with a Veterinary Thermometer VT 1831

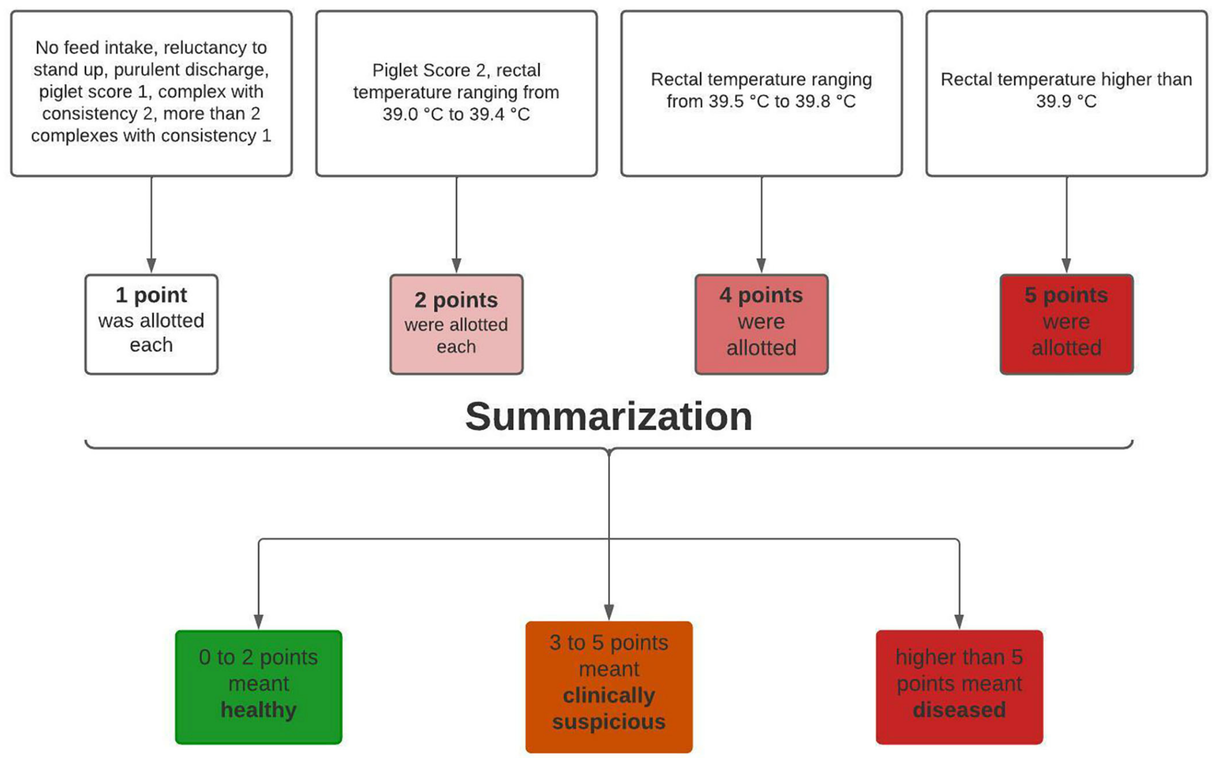

FIGURE 3
Schematic diagram about aggregation of clinical data. Adapted from Rosengart et al. (18).

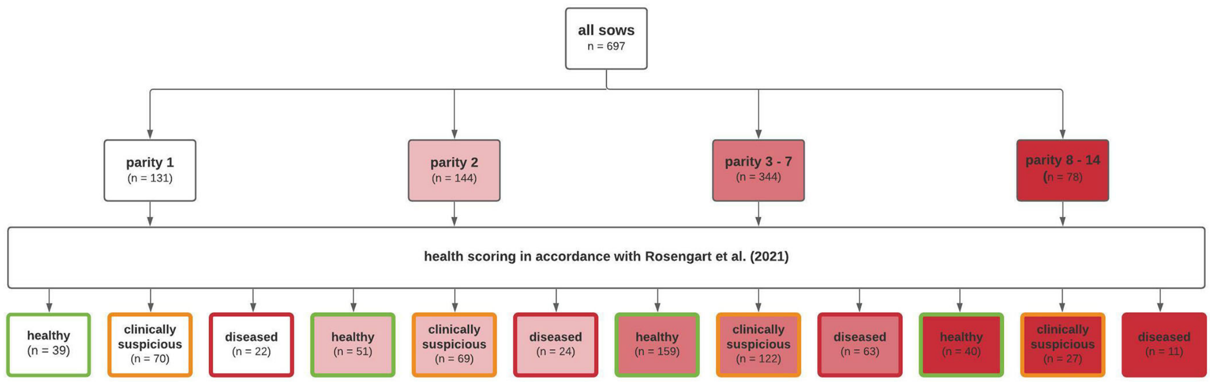

FIGURE 4
Schematic diagram showing group categorization of investigated sows.

(Microlife AG, Widnau, Switzerland), the rectal temperature was measured and recorded.

The evaluation of the thermal images was performed with the FLIR Tools program (FLIR Systems, Inc.). Using this program, the highest skin temperature of each thermal image could be recorded. This way was gone following a previous study (43) that achieved excellent results with this analysis method. Subsequently, the mean value of the highest skin temperature on the right and left sides of the udder was calculated.

To detect PDS-affected sows, the clinical data were afterwards aggregated into a health score (18) depending on the extent of clinical findings, where the number of score points reflected a weighted number of clinical findings. No feed intake, reluctance to stand up, purulent discharge, piglet score 1, mammary complex with consistency two or more than two mammary complexes with consistency 1 meant one point was allotted. A piglet score of 2 or a rectal temperature ranging from 39 to 39.4°C meant two points were allotted. A rectal temperature ranging from 39.5 to 39.8°C meant a score of four points, and a rectal temperature higher than 39.8°C meant a score of five points (Figure 3).

A healthy group scored a total of zero to two points, a clinically suspicious group a total of three to five points, and a total of more than five given points meant classification into the diseased group. In addition, the sows were categorized according to parity. By categorizing according to parity and health status, all sows were divided into 12 groups (Figure 4). The first group categorization was made at parity. First parity sows were allocated to parity group 1 (n = 131), second parity sows to parity group 2 (n = 144), third to seventh parity sows to parity group 3 (n = 344), and sows with eighth or higher parity to parity group 4 (n = 78). Moreover, every parity group was divided into three health classes (healthy, clinically suspicious, and diseased) (Figures 3, 4).

Statistical methods

Data were statistically analyzed using the SAS Enterprise Guide (version 7.1, Fa. SAS Institute Inc., Cary, NC, USA). Mean values and the standard deviation were calculated for all parameters shown in Tables 1, 2, 7. The Kolmogorov-Smirnov test was used to test for normal data distribution. Interactions between health categories and parity groups were tested with a two-way analysis of variance (ANOVA). Differences between health categories and parity groups were examined using a one-way analysis of variance (ANOVA). For multiple pairwise means comparison between the three groups, the Ryan-Einot-Gabriel-Welsch multiple-range test (REGWQ) was used. The data for which the correlations are given in Tables 3–6 were all normally distributed. Therefore, the correlation coefficient was determined in accordance with Pearson. In accordance with Akoglu (50), 0.00–0.29 meant poor correlation, 0.3–0.59 fair correlation, 0.6–0.79 moderate correlation, 0.8–0.99 very strong correlation, and one perfect correlation.

Results

In general, there were no interactions between health categories and parity groups, except for the daily weight gain of the piglets (DWG), the rectal temperature shortly after birth (RT0), and the rectal temperature 14 days after birth (RT14).

Infrared thermography and rectal temperature

Moreover, at three different time points (shortly after farrowing, 14-d postpartum, and at weaning), rectal temperature (RT) and the highest skin temperature of the sow's mammary

TABLE 2 Thermographic skin temperature and rectal temperature (°C) compared between healthy, clinically suspicious and diseased sows of different parities.

Item	Parity	*N*	Healthy	*N*	Clinically suspicious	*N*	Diseased
TH0	1	39	$37.0^{Aa} \pm 0.63$	70	$37.5^{Ab} \pm 0.58$	22	$38.1^{c} \pm 0.52$
TH0	2	51	$37.3^{Ba} \pm 0.49$	69	$37.6^{ABb} \pm 0.48$	24	$38.5^{c} \pm 0.59$
TH0	3-7	159	$37.2^{ABa} \pm 0.53$	122	$37.7^{Bb} \pm 0.54$	63	$38.4^{c} \pm 0.57$
TH0	8+	40	$37.1^{ABa} \pm 0.52$	27	$37.5^{ABb} \pm 0.53$	11	$38.3^{c} \pm 0.39$
RT0	1	39	$38.9^{Aa} \pm 0.24$	70	$39.2^{b} \pm 0.22$	22	$39.7^{Ac} \pm 0.36$
RT0	2	51	$38.9^{Aa} \pm 0.29$	69	$39.3^{b} \pm 0.23$	24	$40.2^{Bc} \pm 0.38$
RT0	3-7	159	$38.7^{Ba} \pm 0.28$	122	$39.2^{b} \pm 0.22$	63	$39.9^{ABc} \pm 0.45$
RT0	8+	40	$38.7^{Ba} \pm 0.31$	27	$39.2^{b} \pm 0.27$	11	$39.8^{Ac} \pm 0.26$
TH14	1	37	$37.8^{A} \pm 0.60$	68	$38.0^{A} \pm 0.50$	21	$38.0^{A} \pm 0.50$
TH14	2	48	$37.6^{ABa} \pm 0.54$	63	$37.8^{ABa} \pm 0.47$	23	$38.0^{Ab} \pm 0.58$
TH14	3-7	149	$37.6^{ABa} \pm 0.62$	115	$37.7^{BCab} \pm 0.64$	62	$37.8^{Ab} \pm 0.66$
TH14	8+	36	$37.4^{B} \pm 0.61$	26	$37.5^{C} \pm 0.66$	8	$37.0^{B} \pm 0.83$
RT14	1	38	$39.3^{A} \pm 0.33$	68	$39.3^{A} \pm 0.38$	21	$39.4^{A} \pm 0.22$
RT14	2	48	$39.0^{B} \pm 0.35$	63	$39.1^{B} \pm 0.37$	23	$39.1^{B} \pm 0.35$
RT14	3-7	150	$38.9^{BC} \pm 0.32$	115	$38.9^{C} \pm 0.31$	62	$39.0^{B} \pm 0.3$
RT14	8+	36	$38.8C^{a} \pm 0.29$	26	$38.9^{Ca} \pm 0.44$	9	$38.5^{Cb} \pm 0.22$
TH21	1	36	$37.5^{Aa} \pm 0.61$	66	$37.8^{Aab} \pm 0.52$	21	$37.9^{Ab} \pm 0.62$
TH21	2	48	$37.3^{ABa} \pm 0.60$	65	$37.4^{Ba} \pm 0.46$	23	$37.7^{ABb} \pm 0.51$
TH21	3-7	144	$37.2^{Ba} \pm 0.62$	113	$37.3^{BCab} \pm 0.62$	58	$37.4^{BCb} \pm 0.63$
TH21	8+	36	$36.8^{C} \pm 0.59$	24	$37.0^{C} \pm 0.83$	9	$37.1^{C} \pm 0.71$
RT21	1	36	$38.9^{Aa} \pm 0.38$	66	$39.0^{Aab} \pm 0.41$	21	$39.2^{Ab} \pm 0.63$
RT21	2	48	$38.6^{B} \pm 0.31$	65	$38.7^{B} \pm 0.33$	23	$38.8^{B} \pm 0.25$
RT21	3-7	144	$38.4^{Ca} \pm 0.35$	113	$38.5^{Cb} \pm 0.36$	58	$38.6^{Bb} \pm 0.35$
RT21	8+	36	$38.2^{D} \pm 0.38$	24	$38.5^{C} \pm 0.52$	9	$38.5^{B} \pm 0.28$

Mean value of the highest skin temperature of the udder (infrared thermography of the right and left side) shortly after farrowing (TH0), around 14 days after farrowing (TH14) and at weaning (TH21). Rectal temperature shortly after farrowing (RT0), around 14 days after farrowing (RT14) and at weaning (RT21).

^{a,b,c}Values within a row with different superscripts differ significantly ($p < 0.05$).

^{A,B,C,D}Values within a column with different superscripts differ significantly ($p < 0.05$).

TABLE 3 Pearson correlation coefficient between thermographic skin temperature, rectal temperature, stillborn piglets, number of weaned piglets and daily weight gain of suckling piglets of first parity sows.

***p*-value (*N*)**	**Pearson correlation coefficient**								
	TH0.1	**RT0.1**	**TH14.1**	**RT14.1**	**TH21.1**	**RT21.1**	**NS1**	**NWP1**	**DWG1**
TH0.1		**0.64**	**0.42**	0.15	**0.39**	**0.30**	−0.003	0.14	**−0.25**
RT0.1	**<0.0001, *n* = 131**		0.21	0.13	**0.24**	0.15	−0.05	0.02	−0.13
TH14.1	**<0.0001, *n* = 126**	<0.05, *n* = 126		**0.61**	**0.64**	**0.41**	−0.06	0.13	−0.18
RT14.1	0.09, *n* = 127	0.16, *n* = 127	**<0.0001, *n* = 126**		**0.39**	**0.43**	−0.13	0.09	−0.14
TH21.1	**<0.0001, *n* = 123**	**<0.01, *n* = 123**	**<0.0001, *n* = 122**	**<0.0001, *n* = 123**		**0.71**	−0.06	0.06	**−0.19**
RT21.1	**<0.01, *n* = 123**	0.09, *n* = 123	**<0.0001, *n* = 122**	**<0.0001, *n* = 123**	**<0.0001, *n* = 123**		−0.01	0.12	**−0.28**
NS1	0.98, *n* = 131	0.54, *n* = 131	0.49, *n* = 126	0.13, *n* = 127	0.53, *n* = 123	0.90, *n* = 123		0.09	0.02
NWP1	0.12, *n* = 131	0.80, *n* = 131	0.15, *n* = 126	0.33, *n* = 127	0.51, *n* = 123	0.17, *n* = 123	0.32, *n* = 131		**−0.32**
DWG1	**<0.01, *n* = 121**	0.14, *n* = 121	0.05, *n* = 116	0.12, *n* = 117	**<0.05, *n* = 113**	**<0.01, *n* = 113**	0.79, *n* = 121	**<0.01, *n* = 121**	

Mean value of the highest skin temperature of the udder (infrared thermography of the right and the left side) of first parity sows shortly after farrowing (TH0.1), around 14 days after farrowing (TH14.1) and at weaning (TH21.1); rectal temperature of first parity sows shortly after farrowing (RT0.1), around 14 days after farrowing (RT14.1) and at weaning (RT21.1); NS1 (n); stillborn piglets of first parity sows, NWP1 (n); weaned piglets of first parity sows, DWG1 in g; daily weight gain of the piglets from farrowing to the 2nd weighing suckled by first parity sows. Values in bold indicate significant values with $p < 0.05$.

TABLE 4 Pearson correlation coefficient between thermographic skin temperature, rectal temperature, stillborn piglets, number of weaned piglets and daily weight gain of suckling piglets of second parity sows.

p-value (*N*)	Pearson correlation coefficient								
	TH0.2	**RT0.2**	**TH14.2**	**RT14.2**	**TH21.2**	**RT21.2**	**NS2**	**NWP2**	**DWG2**
TH0.2		**0.67**	**0.47**	**0.23**	**0.47**	**0.31**	−0.01	0.11	−0.16
RT0.2	**<0.0001, $n = 144$**		0.20	0.17	0.21	0.21	0	0.06	0
TH14.2	**<0.0001, $n = 134$**	<0.05, $n = 134$		**0.53**	**0.77**	**0.43**	−0.16	**0.20**	−0.17
RT14.2	**<0.01, $n = 134$**	<0.05, $n = 134$	**<0.0001, $n = 134$**		**0.40**	**0.42**	0.02	**0.25**	−0.16
TH21.2	**<0.0001, $n = 136$**	<0.05, $n = 136$	**<0.0001, $n = 133$**	**<0.0001, $n = 133$**		**0.59**	**−0.18**	**0.22**	−0.05
RT21.2	**<0.01, $n = 136$**	<0.05, $n = 136$	**<0.0001, $n = 133$**	**<0.0001, $n = 133$**	**<0.0001, $n = 136$**		**−0.21**	0.17	−0.15
NS2	0.90, $n = 144$	1, $n = 144$	0.07, $n = 134$	0.78, $n = 134$	**<0.05, $n = 136$**	**<0.05, $n = 136$**		−0.15	0.18
NWP2	0.21, $n = 144$	0.45, $n = 144$	**<0.05, $n = 134$**	**<0.01, $n = 134$**	**<0.05, $n = 136$**	0.05, $n = 136$	0.08, $n = 144$		**−0.30**
DWG2	0.10, $n = 100$	0.97, $n = 100$	0.10, $n = 94$	0.13, $n = 94$	0.63, $n = 95$	0.14, $n = 95$	0.07, $n = 100$	**<0.01, $n = 100$**	

Mean value of the highest skin temperature of the udder (infrared thermography of the right and left side) of second parity sows shortly after farrowing (TH0.2), around 14 days after farrowing (TH14.2) and at weaning (TH21.2); rectal temperature of second parity sows shortly after farrowing (RT0.2), around 14 days after farrowing (RT14.2) and at weaning (RT21.2); NS2 (n); stillborn piglets of second parity sows, NWP2 (n); weaned piglets of second parity sows, DWG2 in g; daily weight gain of the piglets from farrowing to the 2nd weighing suckled by second parity sows. Values in bold indicate significant values with $p < 0.05$.

TABLE 5 Pearson correlation coefficient between thermographic skin temperature, rectal temperature, stillborn piglets, number of weaned piglets and daily weight gain of suckling piglets of third to seventh parity sows.

p-value (*N*)	Pearson correlation coefficient								
	TH0.3	**RT0.3**	**TH14.3**	**RT14.3**	**TH21.3**	**RT21.3**	**NS3**	**NWP3**	**DWG3**
TH0.3-7		**0.73**	**0.46**	**0.22**	**0.42**	**0.30**	−0.03	0	−0.09
RT0.3-7	**<0.0001, $n = 344$**		**0.17**	0.18	0.14	**0.19**	−0.06	−0.03	−0.04
TH14.3-7	**<0.0001, $n = 326$**	**<0.01, $n = 325$**		**0.49**	**0.75**	**0.40**	−0.03	0.05	0.02
RT14.3-7	**<0.0001, $n = 327$**	<0.05, $n = 326$	<0.0001, $n = 326$		**0.33**	**0.44**	**−0.15**	0.02	−0.07
TH21.3-7	**<0.0001, $n = 315$**	<0.05, $n = 314$	**<0.0001, $n = 310$**	**<0.0001, $n = 311$**		**0.62**	−0.03	0.08	0
RT21.3-7	**<0.0001, $n = 315$**	**<0.01, $n = 314$**	**<0.0001, $n = 310$**	**<0.0001, $n = 311$**	**<0.0001, $n = 315$**		−0.03	0.07	**−0.13**
NS3-7	0.52, $n = 345$	0.27, $n = 344$	0.58, $n = 326$	**<0.01, $n = 327$**	0.65, $n = 315$	0.57, $n = 315$		0.01	0.03
NWP3-7	0.96, $n = 345$	0.61, $n = 344$	0.40, $n = 326$	0.73, $n = 327$	0.17, $n = 315$	0.25, $n = 315$	0.86, $n = 345$		−0.06
DWG3-7	0.16, $n = 265$	0.57, $n = 264$	0.71, $n = 252$	0.25, $n = 253$	0.91, $n = 244$	**<0.05, $n = 244$**	0.68, $n = 265$	0.29, $n = 265$	

Mean value of the highest skin temperature of the udder (infrared thermography of the right and left side) of third to seventh parity sows shortly after farrowing (TH0.3-7), around 14 days after farrowing (TH14.3-7) and at weaning (TH21.3-7); rectal temperature of third to seventh parity sows shortly after farrowing (RT0.3-7), around 14 days after farrowing (RT14.3-7) and at weaning (RT21.3-7); NS3-7 (n); stillborn piglets of third to seventh parity sows, NWP3-7 (n); weaned piglets of third to seventh parity sows, DWG3-7 in g; daily weight gain of the piglets from farrowing to the 2nd weighing suckled by third to seventh parity sows. Values in bold indicate significant values with $p < 0.05$.

gland were measured using thermography (TH) from the right and left sides of the udder. The mean value of the two measured values was calculated. Results with regard to parity and the three health condition groups of sows are shown in Table 2.

Readings shortly after farrowing

Shortly after farrowing, the diseased animals always showed the highest values in infrared thermography (TH0) and rectal temperature (RT0). Differences between health categories (healthy, clinically suspicious, and diseased) were statistically significant in every parity group (Table 2, $p < 0.05$). Age-dependent differences were marginal at this particular time (Table 2). The highest values were found in the diseased group of second parity sows (RT0.2, Table 2).

Readings 14 days postpartum and at weaning

On day 14, postpartum, diseased sows showed no differences or slightly elevated temperatures (parity 2 and 3–7; $p < 0.05$) in infrared thermography (TH14) in comparison to suspicious or healthy sows. With regard to rectal temperature (RT14), diseased sows showed no differences or lower values (parity 8+; $p < 0.05$) in rectal temperatures in comparison to suspicious or healthy sows. At weaning, the same tendency was seen in diseased sows, these animals showing no differences or slightly elevated TH21

TABLE 6 Pearson correlation coefficient between thermographic skin temperature, rectal temperature, stillborn piglets, number of weaned piglets and daily weight gain of suckling piglets of eighth and higher parity sows.

p-value (*N*)	Pearson correlation coefficient								
	TH0.8+	**RT0.8+**	**TH14.8+**	**RT14.8+**	**TH21.8+**	**RT21.8+**	**NS8+**	**NWP8+**	**DWG8+**
TH0.8+		**0.71**	0.17	0	**0.38**	**0.33**	0.03	−0.11	**−0.32**
RT0.8+	**<0.0001, $n = 78$**		−0.04	−0.06	0.16	0.25	0.01	−0.08	**−0.30**
TH14.8+	0.16, $n = 70$	0.72, $n = 70$		**0.64**	**0.71**	**0.39**	**−0.26**	0.08	0.18
RT14.8+	1.00, $n = 71$	0.62, $n = 71$	**<0.0001, $n = 70$**		**0.50**	**0.45**	−0.21	0.23	0.03
TH21.8+	**<0.01, $n = 69$**	0.18, $n = 69$	**<0.0001, $n = 68$**	**<0.0001, $n = 68$**		**0.75**	−0.07	−0.03	0.08
RT21.8+	**<0.01, $n = 69$**	<0.05, $n = 69$	**<0.01, $n = 68$**	**0.0001, $n = 68$**	**<0.0001, $n = 69$**		0.04	0.11	−0.15
NS8+	0.81, $n = 78$	0.90, $n = 65$	**<0.05, $n = 70$**	0.08, $n = 71$	0.59, $n = 69$	0.73, $n = 69$		−0.12	**−0.28**
NWP8+	0.35, $n = 78$	0.50, $n = 78$	0.53, $n = 70$	0.05, $n = 71$	0.79, $n = 69$	0.37, $n = 69$	0.29, $n = 78$		−0.15
DWG8+	**<0.01, $n = 72$**	**<0.05, $n = 72$**	0.15, $n = 65$	0.83, $n = 65$	0.54, $n = 64$	0.22, $n = 64$	**<0.05, $n = 72$**	0.21, $n = 72$	

Mean value of the highest skin temperature of the udder (infrared thermography of the right and left side) of eighth and higher parity sows shortly after farrowing (TH0.8+), around 14 days after farrowing (TH14.8+) and at weaning (TH21.8+); rectal temperature of eighth and higher parity sows shortly after farrowing (RT0.8+), around 14 days after farrowing (RT14.8+) and at weaning (RT21.8+); NS8+ (n); stillborn piglets of eighth and higher parity sows, NWP8+ (n); weaned piglets of eighth and higher parity sows, DWG8+ in g; daily weight gain of the piglets from farrowing to the 2nd weighing suckled by eighth and higher parity sows. Values in bold indicate significant values with $p < 0.05$.

(parity 1; 2; 3–7; $p < 0.05$) and RT21 values (parity 1; 3–7; $p < 0.05$) mainly in comparison to the healthy group (Table 2).

On day 14 after farrowing and weaning, sows showed an age-dependent regular decrease in TH and RT from first parity sows to the oldest ones ($p < 0.05$). This proved the case for all three different health status groups.

Correlations

Table 3 shows the Pearson correlation coefficient between two variables from nine investigated parameters of first parity sows. Moderate correlations occurred between the TH21.1 and the RT21.1, the TH14.1 and the TH21.1, the TH0.1 and the RT0.1 and the TH14.1 and the RT14.1 ($p < 0.0001$).

Table 4 shows the Pearson correlation coefficient between two variables from nine investigated parameters of second parity sows. A moderate correlation occurred between the TH14.2 and the TH21.2 and the TH0.2 and the RT0.2 $p < 0.0001$).

Table 5 shows the Pearson correlation coefficient between two variables from nine investigated parameters of third to seventh parity sows. A moderate correlation occurred between the TH14.3-7 and the TH21.3-7, the TH0.3-7 and the RT0.3-7, and the TH21.3-7 and the RT21.3-7 ($p < 0.0001$).

Table 6 shows the Pearson correlation coefficient between two variables from nine investigated parameters, from eighth and higher parity sows. Moderate correlations occurred between the TH21.8 + and the RT21.8 +, the TH0.8 + and the RT0.8 +, the TH14.8 + and the TH21.8 + and the TH14.8 + and the RT14.8 + ($p < 0.0001$).

Sows' and piglets' performance

Differences between the parity groups

Healthy sows

The results of the evaluations of the performance parameters of sows in relation to health and parity and their piglets are presented in Table 7. The parity group with sows from third to seventh parity in the diseased group showed a significantly lower number of total born piglets (TBP3-7, $p < 0.05$) as well as a significantly lower number of piglets born alive (NBA3-7, $p < 0.05$). The parity group with sows equaling or more than eight parities (≥8) was most often characterized by significant differences. This group showed significant differences between the three health categories in the number of piglets born alive (NBA8+, $p < 0.05$). In addition, the same parity group showed a significantly higher weaning weight of the sows (SW2.8+, $p < 0.05$) in the diseased group compared with the healthy group. However, weight loss during lactation did not differ. Piglets suckled by old sows in the diseased group had a significantly lower daily weight gain (DWG8+, $p < 0.05$). Additionally, piglets suckled by diseased first parity sows also showed a significantly lower daily weight gain (DWG1, $p < 0.05$).

Clinically suspicious sows

Clinically suspicious sows in the parity group with sows equaling or more than eight parities (≥8) stood out again with significant differences. Likewise, the clinically suspicious sows showed a significantly lower number of piglets born alive in the group of highest parity sows (NBA8+, Table 7, $p < 0.05$). Moreover, the same sows had significantly more stillborn piglets (NS8+, $p < 0.05$) compared to first parity sows (NS1, Table 7). Again, the weight of sows entering the farrowing room (SW1) and also the weaning weight of sows (SW2) were lower for the

TABLE 7 Comparison of performance parameters between healthy, clinically suspicious and diseased sows of different parities.

Item	Parity	*N*	Healthy	*N*	Clinically suspicious	*N*	Diseased
TBP	1	39	$17.6^{A} \pm 2.8$	70	17.2 ± 3.54	22	16.4 ± 3.79
TBP	2	51	$17.4^{A} \pm 2.88$	69	17.3 ± 3.20	24	16.6 ± 3.34
TBP	3–7	159	$17.8^{Aa} \pm 3.36$	122	$18.0^{a} \pm 3.25$	64	$16.6^{b} \pm 3.73$
TBP	8+	40	$14.9^{B} \pm 3.16$	27	16.8 ± 2.78	11	14.5 ± 4.23
NBA	1	39	$17.3^{A} \pm 2.93$	70	$16.9^{A} \pm 3.36$	22	$16.1^{A} \pm 3.7$
NBA	2	51	$16.9^{A} \pm 2.92$	69	$16.9^{A} \pm 3.08$	24	$16.2^{A} \pm 3.47$
NBA	3–7	159	$16.7^{Aa} \pm 3.3$	122	$16.9^{Aa} \pm 3.4$	64	$15.4^{Ab} \pm 3.89$
NBA	8+	40	$12.7^{Ba} \pm 2.93$	27	$14.8^{Bb} \pm 2.73$	11	$10.6^{Bc} \pm 3.17$
NS	1	39	$0.33^{A} \pm 0.74$	70	$0.3^{A} \pm 0.67$	22	$0.23^{A} \pm 0.69$
NS	2	51	$0.51^{AB} \pm 0.92$	69	$0.33^{A} \pm 0.72$	24	$0.42^{A} \pm 0.97$
NS	3–7	159	$1.12^{B} \pm 1.49$	122	$1.09^{B} \pm 2.15$	64	$1.2^{A} \pm 2.27$
NS	8+	40	$2.2^{C} \pm 2.39$	27	$2.0^{C} \pm 1.62$	11	$3.91^{B} \pm 4.93$
PWM	1	37	$3.28^{A} \pm 2.73$	70	2.99 ± 2.16	22	$3.23^{A} \pm 2.31$
PWM	2	51	$2.59^{AB} \pm 2.69$	69	2.62 ± 2.18	24	$2.04^{AB} \pm 1.94$
PWM	3–7	159	$1.84^{BC} \pm 2.02$	122	2.05 ± 1.97	64	$1.72^{AB} \pm 2.11$
PWM	8+	40	$1.33^{C} \pm 1.21$	27	1.96 ± 2.89	11	$0.82^{B} \pm 1.08$
NWP	1	39	$12.4^{A} \pm 1.51$	70	11.9 ± 2.84	22	$13.0^{A} \pm 2.36$
NWP	2	51	$12.6^{A} \pm 3.4$	69	12.6 ± 1.89	24	$13.1^{A} \pm 2.85$
NWP	3–7	159	$12.1^{AB} \pm 1.33$	122	12 ± 1.75	64	$12.1^{A} \pm 1.73$
NWP	8+	40	$11.4^{B} \pm 1.42$	27	11.6 ± 1.78	11	$10.5^{B} \pm 2.66$
SW1	1	38	$236^{A} \pm 19.3$	70	$233^{A} \pm 17.5$	22	$237^{A} \pm 16.3$
SW1	2	50	$241^{A} \pm 15.5$	66	$244^{B} \pm 21.8$	24	$242^{A} \pm 13.7$
SW1	3–7	152	$274^{B} \pm 26.1$	121	$276^{C} \pm 26.5$	63	$275^{B} \pm 19.5$
SW1	8+	40	$304^{C} \pm 21.7$	27	$320^{D} \pm 28.9$	10	$320^{C} \pm 24$
SW2	1	37	$181^{A} \pm 18.1$	70	$181^{A} \pm 14.2$	22	$185^{A} \pm 15.8$
SW2	2	50	$199^{B} \pm 20.3$	66	$202^{B} \pm 18.8$	24	$201^{B} \pm 15.6$
SW2	3–7	152	$229^{C} \pm 24.3$	120	$229^{C} \pm 26.8$	63	$230^{C} \pm 19.7$
SW2	8+	39	$265^{Da} \pm 16.9$	27	$277^{Dab} \pm 22.2$	10	$284^{Db} \pm 23.4$
SWD	1	37	$55.7^{A} \pm 13.4$	70	$51.9^{A} \pm 13.2$	22	$52.0^{A} \pm 17.0$
SWD	2	50	$42.5^{B} \pm 16.6$	66	$42.4^{B} \pm 14.4$	24	$40.6^{AB} \pm 13.1$
SWD	3–7	152	$45.0^{B} \pm 17.5$	120	$46.7^{AB} \pm 18.3$	63	$45.0^{AB} \pm 16.2$
SWD	8+	39	$38.4^{B} \pm 13.6$	27	$43.1^{B} \pm 17.8$	10	$36.9^{B} \pm 12.0$
DWG	1	35	$197^{a} \pm 22.9$	64	$187^{a} \pm 29.3$	22	$171^{ABb} \pm 33.8$
DWG	2	38	185 ± 35.3	46	187 ± 33.7	16	$191^{A} \pm 27.2$
DWG	3–7	116	197 ± 33.4	100	194 ± 35.6	49	$193^{A} \pm 35.5$
DWG	8+	37	$192^{a} \pm 31.2$	25	$191^{a} \pm 31.3$	10	$148^{Bb} \pm 50.3$

TBP (n); total number of born piglets, NBA (n); piglets born alive, NS (n); stillborn piglets, PWM (n); pre-weaning mortality, NWP (n); weaned piglets, SW1 (kg); sow weight when entering the farrowing house, SW2 (kg); sow weight at weaning, SWD (kg); weight loss during lactation (SW1-SW2), DWG (g); daily weight gain of the piglets from farrowing to the 2nd weighing.

^{a,b,c}Values within a row with different superscripts differ significantly ($p < 0.05$).

^{A,B,C,D}Values within a column with different superscripts differ significantly ($p < 0.05$).

first parity and the highest for sows in the eighth or higher parity (Table 7, $p < 0.05$). However, first parity sows again had the greatest weight loss during lactation (SWD1, Table 7).

Diseased sows

Diseased sows in the parity group with sows equaling or more than eight parities (8+) stood out with significant differences. Likewise, the diseased sows showed a significantly lower number of piglets born alive in the group with the highest parity sows (NBA8 +, Table 7, $p < 0.05$)). In addition, the same sows weaned significantly fewer piglets (NWP) than the other sows (Table 7, $p < 0.05$). Moreover, the highest parity group sows had a significantly higher number of stillborn piglets (NS8 +, Table 7, $p < 0.05$). Pre-weaning mortality (PWM) was highest in litters of sows in first parity and the lowest number of piglets that died was documented in the litters of eighth or higher parity sows (Table 7, $p < 0.05$). Again, the weight of sows entering the farrowing room (SW1) and the weight at the weaning of sows (SW2) were lowest for first parity sows and highest for eighth and higher parity sows (Table 7, $p < 0.05$). However, first parity sows again had the greatest weight loss during lactation, while eight and higher parity sows had the lowest (SWD1, Table 7, $p < 0.05$). Moreover, piglets suckled by diseased sows with equaling or more than eight parities showed a significantly lower daily weight gain (DWG) compared with piglets suckled by a diseased third to seventh parity sows and diseased second parity sows (Table 7, $p < 0.05$).

Differences between health categories

The parity group with sows from third to seventh parity in the diseased group showed a significantly lower number of total born piglets (TBP3-7, $p < 0.05$) as well as a significantly lower number of piglets born alive (NBA3-7, $p < 0.05$). The parity group with sows of eighth or higher parity (8+) was most often characterized by significant differences. This group showed significant differences between the three health categories in the number of piglets born alive (NBA8+, $p < 0.05$). In addition, the same parity group showed a significantly higher weaning weight of the sows (SW2.8+, $p < 0.05$) in the diseased group compared with the healthy group. However, weight loss during lactation did not differ. Piglets suckled by sows in the diseased group had a significantly lower daily weight gain (DWG8+, $p < 0.05$). Additionally, first parity sows showed a significantly lower daily weight gain for the piglets suckled by sows in the diseased group, too (DWG1, $p < 0.05$).

Discussion

The primary purpose of precision livestock farming (PLF) is to provide guaranteed, affordable, and straightforward solutions to severe problems (17). Animal welfare and farm sustainability are major concerns for future agriculture, especially pig production. Therefore, it is necessary to use more advanced methodologies such as precision livestock farming (PLF) to assist traditional farming methods (1). The NWP per sow mainly determines the profitability of piglet production. Increased litter sizes are associated with lower birth weights, lower growth rates of many light piglets (51), and lower piglet survival (31, 52, 53). Decreased survival rates and more piglets with lower performance potential make the monitoring of diseases and infections, like PDS in sows, within pig production even more crucial (23, 36, 54). It is known that infrared thermography, in general, can help to find diseased sows early on (18, 42, 43). This would allow for timely treatment. Previous studies have showed that the parity of sows influences the sows' performance (44–46). Nonetheless, little is known concerning how the parity influences the information from infrared thermography of the mammary gland. Moreover, it is largely unknown how PDS influences different parity sows. However, this knowledge is important to gain a more differentiated evaluation of the infrared thermography of the mammary gland and of its management.

Infrared thermography and rectal temperature

Differences between the parity groups

Gilts showed significantly the highest temperatures on day 14 (TH14.1 and RT14.1) and at weaning (TH21.1 and RT21.1). On the other hand, the oldest sows had significantly lower temperatures (TH14.8+, RT14.8+, TH21.8+, and RT21.8+) in every health class. All temperatures (TH14, RT14, TH21, and RT21) decreased continuously from the first parity groups to the highest parity groups in every health class. Unlike shortly after farrowing, 14 days after farrowing, and at weaning, nearly all sows were without clinical disease symptoms. This is probably the explanation for the fact that temperatures 14 days after farrowing and at weaning are more age-dependent than directly after farrowing. Together with the knowledge from this study that the sows' weight increased at least up to eighth parity, this could be why the basal metabolic rate per kg body weight decreases in heavier sows. Furthermore, the relative proportion of metabolically active organs in the total body mass decreases (55). In addition, a previous study reports from heat production in sows of 400kJ/kg$^{0.75}$ body weight (56). In relation to a 200 kg sow and a 300 kg sow, this means that the 300 kg sow has 50% more body mass, but only 35 % more heat production. This statement probably applies even more strongly today because, due to increased fertility performance, feed intake has increased in recent decades, especially during lactation (57). For this reason, large animals must consume less feed per kg of body mass than small animals; therefore, perhaps less metabolic heat is produced from feed intake. This can be an advantage when outside temperatures are high.

Differences between health categories

In our study, the skin temperature of the udder and rectal temperature shortly after farrowing were mainly influenced by the health status of the sows and not by age. All three health groups differed significantly with regard to TH0 and RT0. This is not dependent on parity. In addition, TH0 and RT0 were moderately correlated (see below). The second highest value, with 0.71, was detected in the group of sows with eighth and higher parity. This is in accordance with previous studies. Schmitt and O'Driscoll (19) found moderate and very strong correlations between thermographic images of the back and the ear base, as well as the rectal temperature of piglets. One study investigated sows' mammary glands by infrared thermography at 21 d, 7 d, and 1 d ante partum and 1 d, 3–4 d, and 14 d post-partum. This former study found significant correlations between the mean temperature of the first six mammary complex pairs and simultaneously measured rectal temperature (42). Another publication showed a moderate correlation between mammary skin temperature and rectal temperature (43). In our study, the rectal temperature was not affected by room temperature. For that reason, we assumed that it did not affect the animal welfare of the animals in our study.

In summary, our study and other studies confirmed a close relationship between the skin temperature of the mammary gland and other regions measured by infrared thermography and rectal temperature. Our study clearly shows that this applies to the skin temperature of the mammary gland regardless of parity. This becomes clear because the diseased groups always had the highest temperature in the thermographic image and the highest rectal temperature in our study. This means that the infrared thermography of sows' mammary glands of all parities contains clues that can help identify diseased sows, especially among old sows (correlation coefficient of 0.73 and 0.71, respectively). This is very important to know because piglets of sows with eighth and higher parity had a normal daily weight gain unless the sows became ill. When these old sows became ill, the piglets' daily weight gain was 23 % lower. Thus, if old diseased sows are identified early on, it becomes apparent which sows need to get fewer piglets by cross-fostering and which sows have to be treated. The other old sows can get as many piglets as the other parity groups. This results in increased sustainability in sow herds and, above all, increased animal welfare. For this selection, infrared thermography coupled with AI can be a helpful management tool in the future. On the other hand, it must also be said that so far, a relatively small proportion of sows on farms are needed for more than seven lactations, on the farm where the data collection for this study took place, 11%. However, this may change in the future if there are PLF-tools for the farmer so that it is easier to manage old sows.

Correlations

TH0 and RT0 were correlated moderately. Similar moderate correlations could be seen for TH14 and RT14 in first and eighth and higher parity sows, and a fair correlation was demonstrated in second and third to seventh parity sows. Again, moderate correlations were observed between TH21 and RT21, except for TH21.2 and RT21.2. This predictability of the rectal temperature by measuring the skin temperature is in accordance with the findings of the previous studies discussed above (19, 42, 43). Otherwise, the study by Wendt et al. (58) contradicts this. However, it must be realized that they investigated the base of the ear, the back, and the anus region with infrared thermography—not the mammary gland. In addition, a more recent study contradicts this (59). The reason for this is probably the change in pig genetics over the last 20 years. Another study reported high predictability of rectal temperature by surface temperature on the snout and around the eye (21). In our study, TH14 and TH21 correlated moderately. In comparison, RT14 and RT21 correlated only fairly. Without considering the influence of parity, a previous study described similar results (18). In summary, it can be said that the surface temperature of the mammary gland is more constant from 14 days after farrowing to weaning than the rectal temperature. This is not dependent on parity. Thus, a thermal image of the mammary gland seems to provide very similar information at the two time points.

Moreover, TH0.8+ and DWG8+ correlated fairly negatively. This value supports the conclusion from chapter above. In addition, it underlines that infrared thermography of the mammary gland shortly after farrowing can indicate the expected lactation performance, especially of old sows. NWP1 and DWG1, as well as NWP2 and DWG2 correlated fairly negatively, too. This means that in litters with few piglets, higher daily weight gains tend to be expected. Therefore, it seems to be problematic to infer the lactation performance of the sows at these parities based on the daily weight gain of the piglets alone. Regarding the methodology of using only the warmest pixel of a thermal image for the evaluation, it can be said that this offers a certain risk of inaccuracies. Otherwise, a previous study (43) compared messages from the warmest pixel, from the warmest 10 and 25% of pixels from thermal images of the mammary gland and found the best message about the rectal temperature in the warmest pixel.

Sows' and piglets' performance

Differences between the parity groups

In our study, the NBA of sows with parity 8–14 was significantly lower in every health class. Lavery et al. (60) compared NBA between first to sixth parity sows and found the lowest number of piglets born alive (10.9) in sixth parity sows. Higher parity sows were not investigated. Moreover, Koketsu et al. (46) reported the lowest NBA in first, eighth, ninth, and tenth parity (NBA (n): 10). Older sows were not investigated.

We found that NS8+ was significantly higher in every health class and that old diseased sows had more stillborn piglets than old sows in healthy and clinically suspicious groups. However, this difference was not significant. The reason for this probably the small number of investigated diseased sows ($n = 11$). Due to good management on the farm, there were few diseased old sows. For organic herds, Rangstrup-Christensen et al. (61) reported an increased risk for stillborn piglets for thin sows (BCS = 2) with a parity higher than four. The reason for this could be a higher risk of uterine inertia with increasing age. In addition, the documented number of stillborn piglets is partially in accordance with a previous study (62) that found an increased risk for stillborn piglets for higher parity sows when stillbirth occurrence at previous farrowing was taken into account.

NWP8+ was significantly lower in healthy and diseased sows in our study in comparison to the other parity groups. This is not in accordance with Knecht et al. (63), but the latter compared first, second, third, fourth, and fifth parity sows in winter, spring, summer, and autumn. They reported a significantly lower number of weaned piglets in autumn for first parity crossbreed sows compared with fifth parity crossbreed sows [parity 1; 9.51 ± 2.09, parity 5; 10.48 ± 1.76 ($p < 0.05$)]. Higher parity sows were not investigated. Otherwise, Lavery et al. (60) compared first to six parity sows with each other and reported that fifth and sixth parity sows weaned with ∼9.9, the lowest number of piglets. The differences in the number of weaned piglets in different age groups are probably due to differences in cross-fostering. The differences, in general, are probably because we investigated modern German hyper-prolific sows that were kept under good management. Thus, in total, about three piglets more were weaned per litter in our study compared with the above mentioned studies (60, 63). As there was a high NBA in our study already in young sows, cross-fostering did not affect the NWP.

SW1.1 and SW2.1 (parity 1) were the lowest in every health class. The weight increased continuously from the first parity groups to the eighth and higher parity groups. SW1.8+ and SW2.8+ (parity ≥8) were the highest in every health class. This is not in accordance with a previous study (64) that found an increasing bodyweight for Danish sows up to fifth parity. After the fifth parity, a constant weight up to the ninth parity was observed. In the previous study, sows were weighed at the end of gestation. Higher parity sows were not investigated. Another publication (65) reported increasing weights of sows antepartum up to seventh parity. In addition, SWD1 was the highest, and SWD8+ was the lowest in every health class. This is not in accordance with the previous study (66) that compared first to fourth parity sows with each other and reported the lowest weight loss in first and fourth parity sows. However, that study is more than 20 years old, and the sows were housed in Thailand under other climatic conditions. In addition, Landrace and Yorkshire sows were investigated, but not German hyper-prolific hybrid sows.

In summary, the results of our study and previous findings show that old sows have a lower number of piglets born alive and a higher number of stillborn piglets. In our study, this was especially true for old and PDS-affected sows. The statement regarding the number of weaned piglets is not so clear, the reason for this most likely being a difference in cross-fostering. In contrast to the literature, our data show that the weight of the investigated sows increased at least up to the eighth parity, and the highest weight loss could be seen in first parity sows.

Differences between health categories

We found that TBP3-7, NBA3-7, NBA8+, and SW2.8+ were significantly lower in diseased groups. In addition, DWG8+ was significantly lower in the diseased group (−23%). The same could be shown with DWG1 (−13%). The two other parity groups (parity 2–7) showed no significant differences in DWG between the health classes. To our knowledge, this all has not been differentiated according to parity and health status in modern genetics. Irrespective of parity, previous studies reported similar findings but not to this quantitative extent. Two earlier studies reported that piglets that suckled by PDS-affected sows had about 5% less daily weight gain until weaning than piglets that suckled by non-diseased sows (18, 67). Patra et al. (36) showed significant differences in DWG in piglets between PDS-affected and non-diseased sows in winter (PDS-affected; 97.78 g ± 23.76, healthy; 132.25 g ± 36.1) and in summer (PDS-affected; 118.63 g ± 18.73, healthy; 141.56 g ± 30.03). Worthy of mention, this previous study focused on crossbred sows (Hampshire × Ghungroo) kept in the tropics (India). This explains the overall large difference between the DWG in piglets in our study compared with a previous study (36). We investigated German hyper-prolific sows kept under good management (about 190 g ± 30 in piglets suckled by non-diseased sows and 148–193 g ± 40 in piglets suckled by PDS-affected sows) and Patra et al. (36) investigated other crossbred sows (Hampshire × Ghungroo) in the tropics. Yu et al. (68) showed no significant differences in piglets' body weight at weaning when comparing piglets suckled by PDS-affected sows to piglets suckled by non-PDS-affected sows. However, a trend toward a negative influence of the PDS disease on the piglets' body weight at weaning could be shown.

In conclusion, old PDS-affected sows, first of all, those with eighth and higher parity, had especially lower NBA and litters with lower DWG. PDS-affected middle-aged sows (parity 3 to 7) had lower TBP and NBA and showed no significant differences in the DWG of piglets compared with healthy and clinically suspicious sows. This means that they were able to suckle as many piglets as first and second parity sows. Moreover, they were able to suckle as many piglets as those parity sows that were healthy or clinically suspicious. This knowledge is important for cross-fostering. It remains to be noted that subclinical conditions were not recognized and considered.

Conclusion

In summary, results are suggestive that sows of higher parities (≥8) indicate a nearly normal performance after farrowing, measured in the daily weight gain of the suckling piglets, unless they become diseased. Old sows suffering from PDS show a bad performance, the disease especially having negative consequences on the daily weight gain of the suckled piglets until weaning and probably even beyond that. Infrared thermography of the mammary gland provides similar information compared to rectal temperature and can help identify diseased sows. Thus, in the future, infrared thermography of the mammary gland coupled with precision livestock farming and smart farming innovations can provide the tool to detect old diseased sows even earlier. With this knowledge, more individualized cross-fostering and more targeted piglet feeding would be possible. In this way, animal welfare for the piglets is enhanced because of better feeding and the resulting improved health. Moreover, such technology could allow the longer use of sows so that their animal welfare could also be improved, making it possible for the farm to save on herd replacement costs.

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/s.

Ethics statement

The animal study was reviewed and approved by Animal Welfare Officer of the University of Veterinary Medicine Hannover, Foundation, Hannover, reference: TVO-2020-V-9. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

MW, HH, AD, JT, IT, and CV conceptualized the study and acquired funding for the project. SR, L-ST, MW, and CV designed the methodology. SR, MW, and CV validated the study. SR, BC, and CV performed the formal analysis. SR investigated the experiment and prepared the original manuscript draft. SR, HH, and CV performed the data curation. SR, BC, MW, and CV contributed to the writing—review and editing. SR and CV visualized the study. MW and CV supervised the project. AD administered the project. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

Author AD was employed by EVH Select GmbH, Meppen, Germany. Author HH was employed by BHZP GmbH, Dahlenburg-Ellringen, Germany.

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

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Effect of lameness on feeding behavior of zero grazed Jersey dairy cows

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The dairy industry faces major challenges with high levels of lameness, in parallel to an increased consumer focus on animal welfare. This encourages farmers to consider more robust breeds, such as Jersey cows. As little is known about the behavior of this breed under loose housing conditions, the present study sought to describe the feeding behavior of lame and non-lame Jersey cows in different parities. Such breed-specific information of behavioral changes is needed for breed-specific herd management decisions and may contribute to identifying animals that are susceptible to developing lameness in the future, thus reducing impacts on the welfare and production of cows. Feeding data from 116 Danish Jersey cows were collected using automatic feeders, and lameness status was assessed by technicians every second week. The cows were kept in a loose housing system, with cubicles, a slatted concrete floor, and automatic milking robots. Eating time per visit and per day, the number of visits per day, and intervals between meals were analyzed using generalized linear mixed effects models. The effect of lameness was not significant for any variable. Primiparous Jersey cows had significantly longer eating times per day, shorter meal intervals, and a lower number of visits per day than older Jersey cows. Week in lactation affected the eating time per visit and per day, the number of visits, and between-meal intervals. In conclusion, we found no differences between lame and non-lame Jersey cows but between parities, which disagree with previous research on other breeds, suggesting that Jersey cows not just differ in size and looks but also in their behavioral reaction when lame. Although data from only one herd of a research center were used, this study has demonstrated the need for further research about breed-specific differences and their implications for the health and welfare of the animals.

KEYWORDS

feeding behavior, dairy cattle, Jersey cows, lameness, breed difference

Introduction

Achieving good animal welfare of high-producing dairy cows in intensively managed systems remains a major challenge for the dairy industry while facing consumer demands and changing climate conditions (1, 2). One of the most important welfare problems in dairy cows is lameness, as it causes pain and thus may reduce animal welfare and productivity (3, 4).

Risk factors associated with lameness range from housing environment and herd management, to genetic factors and breed (4). Compared to purebred Holstein cows, the smaller framed Jersey breed is receiving increasing interest from dairy farmers and scientists (5–7). The Jersey breed differs from Holstein cows not only in size and looks but it also produces milk with higher nutrient density and has a higher reproductive performance and heat tolerance (8, 9). Even though mainly used for grazing systems (10) and crossbreeding, pure-bred Jersey cows are currently, in numbers, the second largest dairy breed in many countries (11, 12). Recently, it has been demonstrated that Jersey cows have a lower carbon footprint per kg of fat-and-protein corrected milk produced compared to Holstein cows (13).

Nevertheless, knowledge remains limited about the impact of lameness on the feeding behavior of zero-grazed Jersey cows over the entire period of lactation. Knowledge about the effects of lameness on feeding behavior is important as the feeding behavior has a significant impact on productivity, and it may improve the early detection of lameness and thus have the potential to reduce impacts on the well-being and performance of cows (14, 15).

At present, there are indications that changes in eating time may indicate changes to the health status of a cow (14, 16), and connections between dairy cow behavior and lameness have been studied (17, 18). Grimm et al. (19) showed that lame cows eat fewer and shorter meals and have a lower intake per meal. So, the feeding behavior could be a predictive tool for the early detection of lame cows. Yet, despite the growing numbers of housed Jersey cows in Europe (5), most studies about the effect of lameness on feeding behavior focus on Holstein cows or grazed Jersey cows.

Thus, here, we aimed to describe and compare the feeding behavior of lame and non-lame Jersey cows in a loose housing system. We hypothesized that the behavior of lame Jersey cows would be affected in a similar way as has been described for lame Holstein cows, with lame cows having shorter and fewer visits to the feeder. Additionally, we expected primiparous cows to be having more visits of shorter duration and, consequently, shorter eating time per day and between-meal intervals.

Materials and methods

Animals

Data from 116 individual Danish Jersey cows housed at the Danish Cattle Research Center (Foulum, Denmark) were collected between 4 January, 2018 and 30 April, 2019. Because feed composition affects the feeding behavior of cows (20, 21), and to keep environmental conditions as constant as possible, only animals fed the standard partially mixed ration (PMR) were included. The proportion of cows within first and later parities was 40 and 60%, respectively. Parity ranged from one to eight lactations. The group composition was dynamic, with cows entering and leaving the experiment, depending on their expected calving dates. Unless moved to a hospital pen, cows that received veterinary treatment during lactation were not excluded from the study. Over the whole study period, 158 treatments have been counted. Reasons for handling spanned from mastitis over heat induction to routine hoof trimming. Ethical approval for the study was not needed according to the European and Danish regulations and current guidelines for the ethical use of animals in research.

Housing and management

The cows were kept as one group in a loose housing system, with a slatted concrete floor and cubicles with mattresses (Comfi Cushion, Egtved, Denmark). The group of Jersey cows had free access to one automatic milking system (AMS) (DeLaval AB, Tumba, Sweden), water, and PMR, which was fed *ad libitum* using computerized feeding bins (Insentec Roughage Intake Control system; Insentec BV, Marknesse, Netherlands). Feed was delivered four times a day. Cows had access to 29 feed bins. The stocking density (animal to feed bin ratio) ranged from 1.8 to 2.3. Each feeder electronically identified individual cows, and cows were free to use any feeder.

Locomotion scoring (LS) of all cows was done while cows were walking freely along the aisle by experienced and calibrated technicians every second week using the scale described by Thomsen et al. (22) with LS1 = normal, LS2 = uneven gait, LS3 = mildly lame, LS4 = lame, and LS5 = severely lame. The distribution of scores by parity and assessment day is shown in Figure 1.

Feeding behavior

All cows were allowed to feed on PMR *ad libitum* and were fed up to 3 kg of concentrate per day in the AMS during milking. Silage and concentrate samples were collected every week. PMR

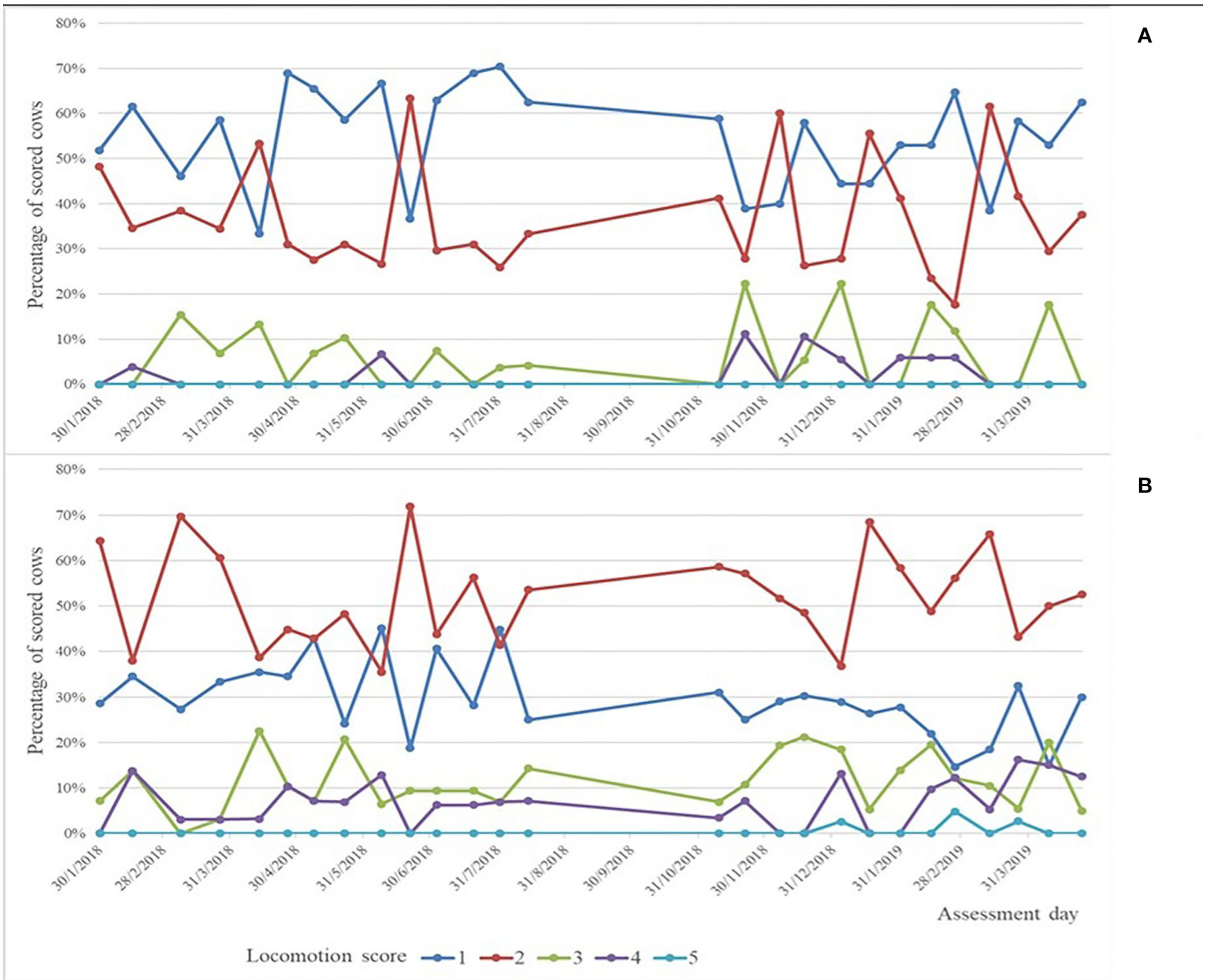

FIGURE 1
Percentage of cows assessed in each lameness score by assessment day for primiparous **(A)** and multiparous **(B)** cows.

samples were pooled over the course of the study to obtain the average. PMR was composed of a mean (± standard deviation) of 6.51 ± 0.04 MJ/kg dry matter, 35.5 ± 6.6% wheat and mineral mix, 28.7 ± 1.5% grass-clover silage, 26.8 ± 1.1% corn silage, 6.9 ± 6.0% barley, 0.6 ± 2.0% horse beans, and 0.5 ± 0.5% spring barley straw. The concentrate contained a mean of 18.2% crude protein and 10.2% crude fiber.

The number of visits and the duration of each visit to a feed bin were recorded using the automatized feeding bins. Individual cows were identified *via* a transponder attached to the ear. To calculate the daily eating time (min/d), the duration of each visit to a feeder (recorded by the Insentec Roughage Intake Control system) was summed over a day. Time intervals between visits were calculated for each cow from the stop time of the previous visit to the start time of the next visit. To determine if an interval was a part of a meal, we estimated a minimum interbout interval as follows. Time intervals measured in seconds were put in 1-min bins for the whole experimental period. Then, the average bin frequency was plotted against minutes. The x-axis was log-transformed to delineate the breakpoint clearly for this curve and, consequently, the threshold for meals (i.e., minimum interbout interval). The minimum interbout interval criterion was set at the breakpoint of 3 min, and time intervals shorter than this were deleted.

Data handling

To investigate the effect of lameness and parity on feeding behavior, recordings obtained from an average of 59 individual cows per day were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Due to data cleaning, however, data from six Jerseys cows (3 at first parity and 3 at a higher parity) were excluded from the analyses, as < 14 days of records were available within a parity. Additionally, data from 30 cows (14 at first parity, 16 at higher parities) were excluded from the analyses, as they had < 5 locomotion scorings within lactation. Additionally, data collected during the first 14 days of lactation were not included in the analyses. Similarly, any measurements exceeding 252 days (= 35 weeks) from calving were omitted from the analyses to exclude the effects of special handling of cows at the end of lactation. In addition, during a period of autumn of 2018, many cows were enrolled in other experiments and, therefore, fed differently. This led to exclusion of 63 dates to keep the numbers of cows similar across days. For the analyses, parity was dichotomized into first and second or higher lactation (primiparous and multiparous). Some cows who have more than one lactation were included.

After exclusions, data from 419 calendar dates from a total of 99 individual Jersey cows (contributing a mean of 235 days, range 70–390) remained available for the analyses. Cows could be lame and non-lame at different times during lactation. The total number of non-lame Jersey cows at first and later parities was 46, 71 and 20, and 51, respectively, were lame for at least one lactation week within the study period.

Statistical analysis

The effects of lameness and parity, as well as their interaction, on eating time per visit, eating time per day, and between-meal intervals were analyzed by the linear mixed-effects models using the MIXED procedure in SAS. The GLIMMIX procedure with Gamma distribution and log link function was used to analyze the number of visits per day.

For the analyses, weekly averages of daily recordings were calculated and lameness status was assigned for this period as either lame or non-lame: To account for consecutive locomotion scorings, an average lameness score (ALS) was calculated for each cow and week. The LS was carried forward and placed on each day. Then, a backward moving average over 7 days was calculated using the EXPAND procedure in SAS:

$$Y_{kt} = \frac{1}{m}\left(\sum_{j=1}^{m} x_{k(t-m+j)}\right), \quad (1)$$

where Y_{kt} is the ALS, x_{kt} is the LS at lactation day $t = 15, \ldots, 252$ for cow $k = 1, \ldots, n$, and $m = 7$ is the number of days to include in the time window.

The daily ALS was averaged over weeks, and cows with a weekly average ALS of ≥ 2.5 were assigned as lame for this week. The percentage of animals-assigned lame among weeks in milk is shown in Figure 2.

Time (week in milk), parity (primiparous, multiparous), and lameness (lame, non-lame) and their two-way interactions were included as explanatory variables (fixed effects). Non-significant variables ($p > 0.05$) were eliminated from the model by backward elimination, resulting in different final models. However, the main effects of parity and lameness were always kept to not remove the relevant information. The final models are shown in the Supplementary File, none of which included the two-way interaction between parity and lameness.

Cow within parity was considered the experimental unit, and a continuous-time first-order autoregressive covariance structure was applied to account for repeated measures over weeks. Distributional assumptions and homogeneity of variances were examined by the graphical analysis of residuals for each model. Weekly averages of eating time per visit and per day and between-meal intervals were log-transformed (natural logarithm) to fulfill the assumption of normally distributed residuals.

For clarity, the results are reported on the original scale as exponentially back-transformed least squares means with 95% confidence intervals. The p-values from the Type 3 test for fixed effects were considered statistically significant when the p-value is ≤ 0.05. Further descriptions of the statistical analyses can be found in the online Supplementary File.

Results

There was no significant difference between parities for eating time per visit (Figure 3A). Primiparous cows spend 2.84 min per visit compared to 2.70 min per visit for older cows ($p = 0.6090$). Eating time per visit changed throughout lactation ($p = 0.0065$) with similar trends between parities. Visual inspection showed that the eating time per visit decreased over the first 25 weeks and subsequently remained constant (Figure 4A). Eating time per visit did not differ between lame and non-lame cows ($p = 0.1504$) who were eating for 2.79 and 2.75 min per visit, respectively (Figure 3B).

Parity influenced the daily eating time ($p = 0.0090$). We found that older Jersey cows spent 156.20 min per day eating compared to 157.29 for primiparous cows (Figure 3C). Additionally, eating time per day changed throughout lactation differing with respect to parity ($p = 0.0007$; Figure 4B). Until the 10th week of lactation, the daily eating time of multiparous cows increased sharply and decreased again from week 16, after staying relatively constant in between. Primiparous cows exhibited a moderate increase in the daily duration of eating until the 20th week, before slowly declining toward the end of lactation.

The effect of lameness on eating time per day was not significant ($p = 0.2276$). Lame cows spent 157.68 min per day eating while non-lame ate for 155.81 min per day (Figure 3D).

Figure 3E shows the distribution of an average number of visits per day by parity. With 57.08 visits per day, primiparous cows visited the feeder significantly fewer times ($p = 0.0267$)

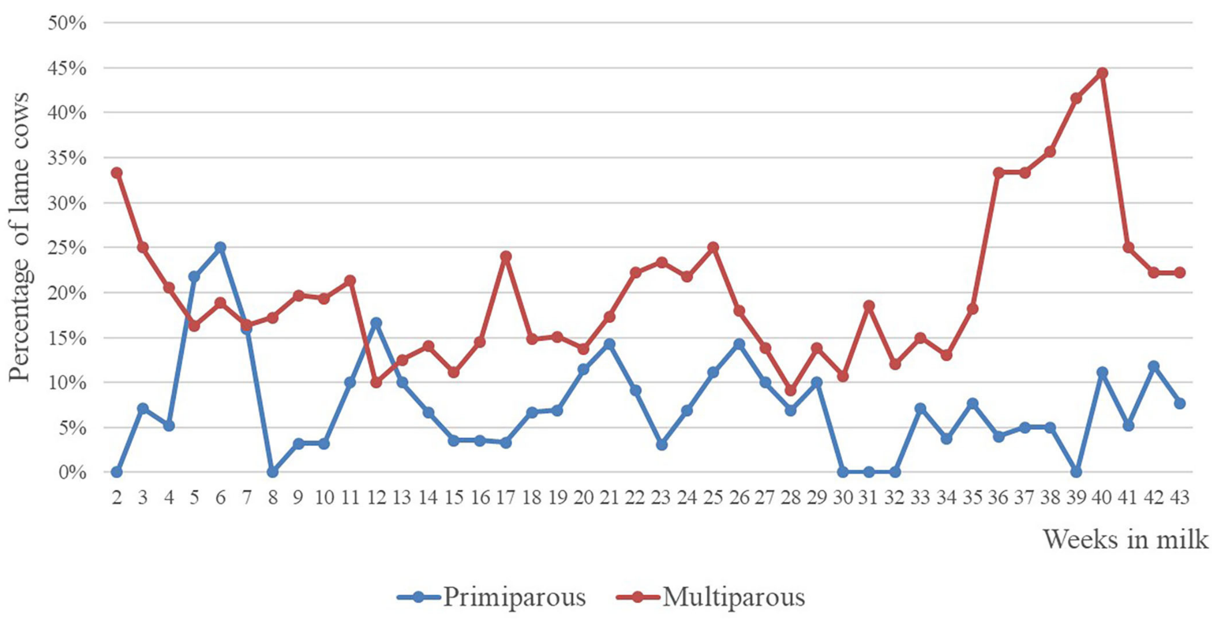

FIGURE 2
Percentage of cows assigned lame by parity and lactation week.

than multiparous cows having 76.23 visits per day. Week in lactation affected the number of visits significantly with the first order coefficient differing between parities (interaction $p = 0.0117$). The number of visits per day of primiparous cows increased steadily until the 29th week in lactation, whereas the number of visits of multiparous cows increased over the first 17 weeks of lactation and then decreased (Figure 4C). The effect of lameness on the number of visits per day was not significant ($p = 0.4735$). Lame cows had 65.65 visits per day while non-lame cows had 66.27 visits per day (Figure 3F).

The minimum between-meal interval was 10.71 min longer ($p < 0.0001$) for multiparous cows (85.61 min) compared to primiparous cows, spending at least 74.90 min between two meals, which can consist of multiple visits (Figure 3G). Time intervals between meals were not significantly different ($p = 0.2799$) for lame cows (80.54 min) compared to non-lame cows (79.61 min) with the distribution between meal intervals presented in Figure 3H.

Discussion

Lameness distribution

A wide variety of lameness definitions and different scoring systems that range from dichotomous scores of lame and non-lame up to the nine-point scales make it difficult to compare studies of lameness (3). Within the same scoring system, the lameness prevalence found in the present study is comparable to a previous Danish study using 1,340 cows from 42 dairy herds, of which 508 (38%), 437 (33%), 232 (17%), 121 (9%), and 42 (3%) have been scored with LS 1, 2, 3, 4, and 5, respectively. However, their study included Jersey and Holstein cows. A study of four farms with a total of 959 LS from 348 Danish Holstein cows had a slightly higher lameness prevalence with a score of 1, 2, 3, 4, and 5, respectively, for 325 (34%), 276 (29%), 194 (20%), 141 (15%), and 23 (2%) cows (17).

To our knowledge, there is no study with pure Jersey cows within Denmark using the described scoring system.

Duration of eating time per visit and per day

We hypothesized that the eating time per visit would be longer for lame cows than for non-lame cows. Interestingly, we found no differences in the time spent eating per visit between non-lame and lame cows. Our findings are in agreement with previous studies (18, 23) which showed no differences between lame and non-lame cows for eating time per visit.

With the cows being fed *ad libitum*, it is possible that lame cows were able to use other times of the day to avoid competition at the feeders and fulfill their needs without changing the duration of their visits. We suggest further research with a sufficiently large dataset investigating if lame cows feed at different times of the day or if differences in other feeding variables such as the feeding rate occur. Contrary to our results,

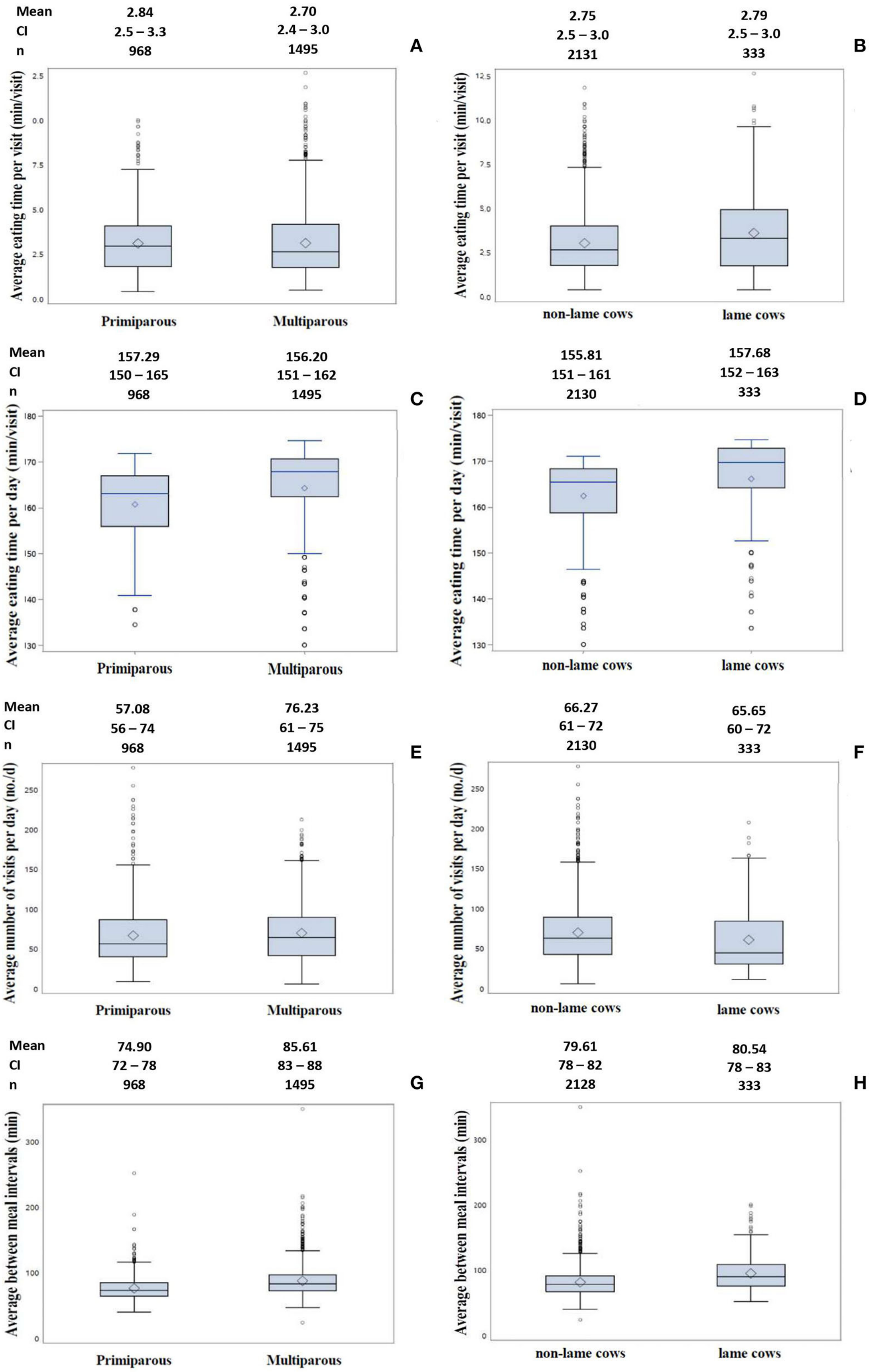

FIGURE 3
Distribution of eating time per visit, average number of visits per day, and between-meal intervals by parity **(A,C,E,G)** and lameness **(B,D,F,H)**, with the diamond inside the box indicating the mean value. The lower boundary of the box indicates the 25th percentile, the line inside the box is the median, and the upper boundary of the box INDICATES the 75th percentile. The whiskers represent the 10th and 90th percentiles, respectively. The bullet points are outliers, which are below or above the whiskers. The number of included measures as well as the back-transformed least squares mean with a 95% confidence interval is given above each boxplot.

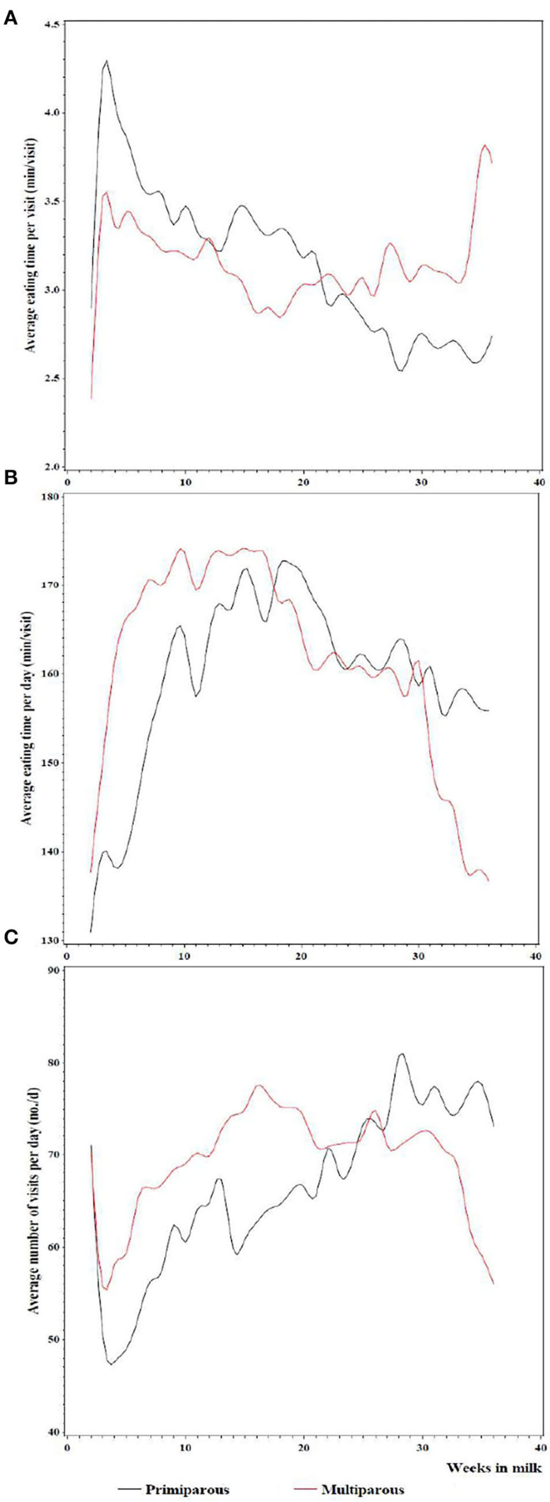

FIGURE 4
Average eating time per visit **(A)**, per day **(B)**, and the average number of visits per day **(C)** *vs*. weeks in milk for primiparous and multiparous Jersey cows. Daily records were averaged for each week in milk and each animal, and smoothed curves were drawn through the scatter points against weeks in milk.

previous studies found that lame cows fed fewer and shorter meals (16, 24). It is possible that lame cows try to reduce the frequency of activities that tend to be painful and, therefore, feed in fewer but longer visits. This is in agreement with the results of Thorup et al. (17), who found reduced activity in lame dairy cows. The number of visits between lame and non-lame cows was not significantly different.

Our expectation of increasing eating time per visit with parity was not confirmed. We observed no significant differences between parities. Val-Laillet et al. (25) assumed that the motivation or persistence of the animal plays a role in competitive success to gain access to feeders. If primiparous cows are more motivated to feed due to their high energy requirements for growth and milk production, they would be displaced less often by others and, therefore, have longer visit times. Further, older cows having a greater eating rate and spending more time lying and ruminating and, thus, less time eating (14) might explain this phenomenon. In our study, primiparous cows spent more time eating per day compared to multiparous cows. This result is in contrast to our observation for feeder visits per day; thus, older cows visited the feeder more often but spent less time eating per day but not per visit. This finding was unexpected, as dry matter intake, eating time, and feeder visits are correlated (26). Further, it should be noted that the difference, even though statistically significant, numerically is small and, therefore, might be biologically irrelevant. Some studies support the assumption of eating time increasing with parity (27, 28), whereas others found that younger cows spend more time eating than older cows (29, 30). These differences between studies might be attributed to different experimental conditions, such as feed composition or forage ratios affecting eating behavior (31).

Number of visits

Contrary to our hypothesis, we found no statistically significant difference in feeder visits between lame and non-lame Jersey cows.

The rather high stocking density in our study might have affected the number of visits to the feeder, increasing competition and causing animals to be more frequently displaced from feeders and, therefore, limiting differences between them. Further, it is well documented that restricting access to feed increases the frequency of displacement, especially for subordinate cows (32, 33). Therefore, we had expected that, with increasing lameness severity, lame cows might reduce the number of visits to limit confrontations and likely painful movements such as walking, getting up, and lying down. We suggest additional research with larger samples to further test the hypothesis that lameness impairs the number of visits per day in Jersey cows.

Jersey cows showed the pattern of multiparous cows visiting the feeder significantly more times compared to primiparous cows disagreeing with studies with Holstein cows (27, 34, 35). Further, it contradicts previous studies on Jerseys, which found no difference between parities (36) or reported primiparous cows to be having a higher frequency of feeder visits (35). Possibly due to their lower body weight, primiparous cows inherit a lower rank within the herd, forcing them to visit the feeder at less bustling times (37, 38), while, at the same time, first parity cows increased the time feeding per day and with that possibly compensating for the lesser visits. Further, older cows tend to lie down for longer and have higher milk yields and body weights (18, 39), increasing their motivation for feeding but shortening the time available. Thus, multiparous cows might optimize their active time by visiting the feeders more often within a meal, but having fewer meals per day and, therefore, longer intervals between meals.

The increase in feeder visits during early lactation in our study likely compensated for an increase in energy demand during early to peak lactation (26, 40).

Between meal intervals

An increase in lameness severity often leads to decreasing time devoted to eating (24). As most movements like getting up or lying down are painful for lame cows, affected cows are thought to limit their feeding bouts (3, 41) and, therefore, the time between meals would increase. Additionally, fewer visits to the feeder are likely to result in fewer confrontations with herd mates. Our findings do not support this, with intervals between meals not being different for lame cows. This is similar to findings by Blackie and Maclaurin (42), which found no statistically significant differences in the lying behavior of lame and non-lame zero-grazed Jersey cows. Together with our findings, this may raise concerns about the suitability of behavioral reactions to detect lameness occurrences in Jersey cows. It also highlights that comparing breed differences is an important issue for future research.

Compared to multiparous cows, primiparous Jersey cows had significantly shorter between-meal intervals, supporting our hypothesis. The between meal intervals increased with parity. This might be explained by older cows spending more time ruminating and thus having fewer meals over a day (27). Our findings of longer between meal intervals are in agreement with findings of higher parity cows visiting the feeder significantly fewer times compared to younger cows (34, 35), which consequently increases the time between visits. Yet, in our study, we found multiparous cows having more feeder visits compared to first parity cows. The reason for this result is not clear. It is possible that older Jersey cows will visit the feeders more often within a meal, while having fewer meals over a day and, therefore, longer between-meal intervals.

Conclusion

This study showed that, contrary to previous research in other breeds, no differences were found in the eating time per visit, the daily eating time, and feeding frequency for lame and non-lame Jersey cows.

Although the amount of data was limited as data from only one herd were used, this study provides a first indication that Jersey cows could react differently to lameness compared to other breeds, namely the predominant Holstein breed, and that feeding parameters might not be used as an early indicator of the onset of lameness in Jersey cows. However, further studies are needed to confirm the findings.

Data availability statement

The datasets presented in this article are not readily available because all data belongs to the Aarhus University. Requests to access the datasets should be directed to Department of Animal Science Aarhus University anis@au.dk.

Ethics statement

Ethical review and approval was not required for the animal study because the animals have not been directly affected according to European and Danish regulations, and current guidelines for the ethical use of animals in research.

Author contributions

SG and LF analyzed the data. SG, CL, LF, and PT interpreted data. SG drafted the initial manuscript. CL, LF, and PT provided critical revisions and improvements. All authors contributed to the article and approved the submitted version.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.980238/full#supplementary-material

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Discrepancies between farmers' perceptions and actual animal welfare conditions on commercial pig farms

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Animal welfare is a multiparameteral concept that encompasses the physical and mental health of animals and includes various aspects such as physical wellbeing, absence of hunger and thirst, and ability to express motivated behavior, to which farmers usually attach different importance. The objectives of this study were to evaluate animal welfare on Slovenian commercial pig farms, to determine whether farmers' perceived importance of animal welfare differ from actual animal welfare on farms and to determine, if farmer's age, gender, their level of education and participation in vocational training have an influence. For that purpose, we created an Animal Welfare Protocol/Questionnaire for Pig Farms (AWQ/P-P) that assessed several parameters of animal welfare: (1) general status, (2) animal behavior, (3) health status, (4) living conditions, and (5) environmental conditions. Each parameter included at least five observation points and was scored on a 5-point scale. The same observation points were used to measure farmers' perceived importance of animal welfare and for observational assessment. Consequently, we were able to compare both statistically. Farmers from 14 ($N = 14$) large Slovenian pig farms participated in the study. Results show that farmers rate all parameters of animal welfare very highly. For them, animal health status is the most important, and environmental conditions are the least important factors for animal welfare. Observational inspections yielded significantly lower scores for animal welfare conditions than those obtained from farmer ratings. The highest correlations between farmers' perceptions and observational inspections were found for the parameters of animal behavior and environmental conditions. The results of this study also suggest that vocational training is a significant variable in increasing levels of pig welfare. Age, gender, and education level are not significant variables, however, farms led by older male farmers with lower level of education but involved in vocational training from different sources had slightly better welfare on the farm. This should be further investigated before making conclusions, due to our small sample size. The significance of the study is to identify deficiencies in pig welfare as perceived by farmers and consequently improve pig welfare.

KEYWORDS

animal welfare, commercial pig farms, farmers' perceptions, human-animal relationship, education

Introduction

Slovenian pig farms are small and fragmented, agricultural land is limited, and natural conditions are not favorable for a larger scale of pig breeding (1). Pig farming makes up a small part of Slovenian agriculture, as the self-sufficiency rate for pork is only 20–25%. There are a total of 253,770 pigs in Slovenia, 22,262 of which are breeding sows. Pigs are bred on 12,843 farms, classified as commercial, non-commercial, and outdoor pig production. Only 22 of the farms are considered large with more than 1,000 pigs, the rest of the farms are small. Eleven thousand six hundred and thirty-one farms have 20 or fewer pigs (2).

Animal welfare is a broad term and can be defined in several ways, many of which are covered by the well-known five freedoms based on Brambell Commission's report to enquire into the welfare of animals kept under intensive livestock husbandry systems and created by the Farm Animal Welfare Council in 1979 (3, 4). World Organization of Animal Health declared in its Introduction to the recommendations for animal welfare, that "animals experience good welfare if they are healthy, comfortable, well-nourished, safe, are not suffering from unpleasant states such as pain, fear and distress, and can express behaviors that are important for their physical and mental state" (5).

Recently, three fundamental scientific concepts and approaches to the study of welfare have been developed worldwide. The first concept connects animal welfare to their natural environment (4, 6–8). Animals should live in an environment that allows them to behave in a natural way (9). Pigs are strongly motivated to express natural behaviors such as rooting, nesting and exploring, and in the impoverished environment they generally encounter in intensive breeding systems, they direct this natural need toward what is available to them—the equipment of the pen and other pigs (10, 11). The resulting behaviors, performed without an apparent function, are referred to as stereotypies and manifest as bar biting, sham chewing, and tongue rolling (4, 12). Stereotypies are therefore a clear indicator of impaired welfare (4, 13). Pigs are social animals that establish hierarchy in groups through aggression. However, aggression is absent in stable groups but does occur when pigs mix, encounter unfamiliar animals, or when resources are limited (1, 11).

The second concept of wellbeing links wellbeing to the biological functioning of animals. A central question in this concept is how an animal adapts to different environment (4, 8). Indicators of wellbeing regarding the environment primarily include an assessment of the animal health status, injuries, behavioral measurements, and quantitative measurements of physiological values such as cortisol levels (14). Production parameters have been considered an appropriate measure of welfare and low productivity an indicator of a lower welfare standard by scientists and farmers (15, 16). However, highly productive pigs can be mentally compromised (4), because they are often subjected to stress, which is a result of the desire for the greatest possible economic return (9), even though they may successfully adapt to such environment (14). From the pig's perspective, the environment in which it lives includes temperature, humidity, access to feed and water, and air quality (4). Among the environmental conditions, the temperature is the most important for the welfare of pigs, as they are highly susceptible to heat stress (1).

The third concept refers to the subjective feelings or affective states (4, 6–8). The feelings are negative with negative subjective states, such as hunger, thirst, pain, fear, and frustration, and positive with positive states, such as comfort and satisfaction with certain social interactions (4, 6). Reimert et al. conducted a study on pig behavior and cited tail wagging, play behavior and "play" bark vocalization as indicators of positive emotions in pigs (17). Many studies have demonstrated the importance of a positive human-animal relationship in reducing stress and enabling high productivity in farm animals (13, 18–22). Unpleasant handling, such as physical force, using electric shock and shouting negatively affects animals' health, productivity, behavior, welfare (4, 23, 24) and reduces meat quality (18). Zupan et al. examined the effects of early human handling on play and exploratory behavior in pigs and found that positive gestures prior to weaning, such as gentle petting on the back affected play behavior, object-oriented exploration, and the latency to approach a novel object or environment after weaning (22). Muns et al. discovered that positive human contact shortened the duration of piglet's escape behavior to tail

TABLE 1 Characterization of the visited pig farms ($n = 14$).

Farm characteristics		Number of farms
Type of production	Farrow to finish	11
	Rearing weaners up to 30 kg	3
Housing system	Indoor	6
	Indoor with outdoor access	7
	Outdoor	1
Number of pigs	<100	1
	101–500	7
	501–1,000	4
	>1,000	2
Breeding other farm animals	Pig farming only	7
	Poultry	5
	Wild ruminants	2

docking, reduced the pigs' fear of humans and modified the behavioral responses to stressors (20).

The objectives of this study were (i) to evaluate animal welfare on commercial pig farms, (ii) to determine whether farmers' perceptions of animal welfare differ from actual animal welfare on farms, (iii) to determine, if farmer's age, gender, their level of education and whether they participate in vocational training (informal training, i.e., conferences, reading professional literature in their field, etc.) have an effect on welfare on the farm or on farmer's perception of animal welfare.

Materials and methods

Farms and farmers

Fourteen commercial pig farms and farmers participated in the study (Table 1). Half of the participants were female. Four were under 40 years of age. Nine of them had completed high school, the others had higher education (higher vocational school or university). All participants were taking part in vocational training from different sources: reading professional journals and books, attending congresses and lectures, collaborating with the experts and their own projects on the farm (projects that contribute to better welfare such as building bigger nursery pens, modernizing feeding technology with electronic sow feeders, etc.). Farmers differed from each other in terms of the number of sources from which they receive vocational training (Table 2).

Protocol

For this study, an Animal Welfare Protocol/Questionnaire for Pig Farms (AWQ/P-P) that assessed several parameters of animal welfare was established: (1) general status—five parameters, (2) animal behavior—six parameters, (3) health status—eight parameters, (4) living conditions—five parameters, and (5) environmental conditions—six parameters. The animal welfare assessment protocol and the questionnaire of farmers' perceived importance of animal welfare on their farms (self-assessment) were identical in content and were used to compare farmers' perceived importance of animal welfare in pig farming with actual conditions on their farms. AWQ/P-P is included as Supplementary material S1. Observational assessment parameters were scored on a 5-point scale, while farmers' perceived importance was scored by 5-point Likert scale.

The welfare protocol was always assessed by two observing veterinarians. To minimize the differences between the two and to standardize the scores from the visits, observers received identical training prior to the assessment. The importance of the values in observational assessment is as follows: (1) major deficiencies (immediate action required), (2) deficiencies warranting a warning, (3) minor deficiencies (advice required), (4) no deficiencies (compliant with standards), and (5) no deficiencies (above-standard conditions). For each observation points, additional descriptions were provided (Supplementary material S1). The legal norm for setting up the points scale was "Rules on the protection of farm animals" from the Official Gazette of the Republic of Slovenia, Nos. 51/10 and 70/10 (25).

Questions in the questionnaire measuring farmers' perceived importance of animal welfare began with "In your opinion, how important is...?" (e.g., "How important do you think it is that lighting on the farm is not too strong or too weak, too short or too long?"). The scale represents the level of importance to farmers: (1) not important at all, (2) not important, (3) undecided, (4) it is important, (5) it is very important. In addition, several independent variables were included in the instrument: respondents' age and gender, their level of education, and whether they participate in vocational training.

First, the welfare of breeding sows, growers, and finishing pigs was assessed using the protocol, followed by an interview with the farm owner about his views on the welfare, using a questionnaire. Farms were visited during the period from July 9, 2021 to October 27, 2021.

Statistical analyses

All raw data were first transferred to MS Excel and transformed for use in SPSS (ver. 26). Mean values were calculated for each parameter of the questionnaire (general status, animal behavior, health status, living conditions, and environmental conditions) and compared using the independent variables—age, gender, level of education, and vocational training (Mann-Whitney test). In addition, the

TABLE 2 Characterization of farmers' education and vocational training.

Level of education	2 or less sources of vocational training	3 or more sources of vocational training	Total
Completed only high school	4	5	9
Completed or enrolled in a higher vocational school or university	1	4	5
Total	5	9	14

TABLE 3 Comparisons between observational assessment and self-assessed importance of animal welfare.

Parameters	Observational assessment			Self-assessed importance of welfare			Wilcoxon		Effect size
	M	*SE*	*SD*	*M*	*SE*	*SD*	*Z*	*p*	*r*
General status (A)	3.7	0.16	0.60	4.6	0.09	0.35	−3.084	0.002	−0.82
Animal behavior (B)	3.9	0.12	0.46	4.4	0.11	0.42	−2.947	0.003	−0.79
Health status (C)	3.8	0.08	0.30	4.8	0.07	0.25	−3.306	0.001	−0.88
Living conditions (D)	3.6	0.12	0.43	4.5	0.11	0.40	−3.188	0.001	−0.85
Environmental conditions (E)	3.7	0.09	0.32	4.3	0.13	0.50	−2.981	0.003	−0.80

O, observational assessment; S, self-assessed importance of animal welfare; M, Mean; SE, Standard error; SD, Standard deviation.

Wilcoxon test was applied to compare the observational results with farmers' importance of welfare ratings for individual parameters from the protocol and questionnaire. For each parameter, Spearman's correlation coefficients between observational results and farmers' perceived importance of animal welfare were calculated. Due to the small sample size, effect sizes were calculated to determine the strength of the statistical differences using the formula $r = Z/\sqrt{N}$. Values $<$ -0.2 or > 0.2 were treated as significant.

TABLE 4 Spearman's correlation coefficients between observational and self-assessed importance scores for individual parameter.

Parameter	r_S	*p*
General status	−0.015	0.480
Animal behavior	0.524	0.027
Health status	0.168	0.283
Living conditions	0.205	0.241
Environmental conditions	0.414	0.071

Results

Animal welfare on commercial farms

On observational assessment, the highest score was achieved for the parameter animal behavior and lowest for living conditions (Table 3). See Supplementary material S2 for full results with all the parameters and Wilcoxon test. For four out of thirty parameters from the observational assessment, the average score was below 3.5. On the other hand, there were almost no scores above the standards. Only for the parameters B—observing the animals and C—presence of umbilical or inguinal hernias did the average scores reach values above 4.0, indicating above standard conditions in some farms.

Comparisons between observational assessment and self-assessed importance of animal welfare

For all but one item (pigs' fear of humans), the experts' observations resulted in lower average scores than the participants' self-assessed data The highest self-assessed score was achieved for the parameter health status and the lowest for environmental conditions (Table 3; Supplementary material S2).

Correlations between observational and self-assessed scores for individual parameter show that there were significant correlations for animal behavior parameter only (Table 4). Medium correlations were also found for environmental conditions parameter, closing statistical significance. For both parameters, the higher the observational scores, the higher are scores from self-assessed importance values.

Effects of independent variables on observational assessment and self-assessed importance of animal welfare

In Table 5, there are effect sizes for individual independent variable presented. For full results with all the parameters and Mann–Whitney test see Supplementary material S3.

TABLE 5 Observational assessment and self-assessed importance of animal welfare effect sizes for gender, age, education level, and sources of vocational training.

Categories	Effect sizes			
	Gender	Age	Education status	Sources of vocational training
O_general status	0.00	−0.25	−0.23	−0.48
O_animal behavior	0.43	−0.10	−0.14	−0.27
O_health status	0.40	−0.26	−0.27	−0.13
O_living conditions	−0.03	−0.19	−0.20	−0.59
O_environmental conditions	−0.21	−0.21	−0.27	−0.22
S_general status	0.39	−0.08	−0.02	−0.09
S_animal behavior	−0.19	−0.40	−0.32	−0.41
S_health status	−0.39	−0.02	−0.51	−0.28
S_living conditions	−0.05	−0.73	−0.14	−0.31
S_environmental conditions	−0.24	0.10	0.00	−0.70

The results from the observational assessment show that female farmers scored lower on animal behavior and health status parameter and higher on environmental conditions parameter compared to male participants. There were no evident differences between the genders on general status and living conditions parameters. On the self-assessed importance of animal welfare, female farmers scored lower on health status and environmental conditions parameter and higher on the general status parameter than male farmers. There were no evident differences between the genders on living conditions parameter.

On farms where interviewed participants were older than 40 years, the scores from the observational assessment were higher in the parameters of general status and environmental conditions, while they were lower in the parameter of health status than in farms where younger participants were interviewed. There were no evident differences in other parameters. On the self-assessed part, older participants scored higher in the parameters of animal behavior and living conditions than younger participants. In the latter parameter, a large difference was found between age groups. There were no evident differences in other parameters.

Depending on education level, differences were found in four out of five parameters of observational assessment. Participants with lower educational level scored higher on general status, living conditions and environmental conditions parameters. In contrast, higher education level participants scored higher on the health status parameter. On the self-assessed part, it is evident that participants with higher education level rate health status higher while they rate animal behavior lower than the participants with lower education level.

Participation in various sources of vocational training affected four out of five parameters of observational assessment. Namely, participants who train from more sources scored higher than participants who train in fewer sources of vocational education. The same was true for four out of five parameters of the assessed importance of animal welfare.

Discussion with conclusions

Compared with preceding studies addressing farmers' perceptions of animal welfare this study also presents general information about commercial pig farms in Slovenia and the effect of different variables on animal welfare. The observers' evaluation showed that animal welfare in commercial pig farms in Slovenia can generally be scored as positive. As mentioned earlier, in only 4 out of 30 observation points, the average score was below 3.5, which means that advice should be given on these issues to improve animal welfare conditions. These observation points were: biosecurity on farms, lack of appropriate enrichment materials, no separation of pigs by different categories, and the lack of thermometers and hygrometers on farms. However, the farms reflected only compliance with the minimum requirements. Only in two observation points (pigs' fear of humans and the presence of hernias), farms reached above the average score. Farms had the highest welfare status regarding animal behavior (pigs not fearing humans, less aggression and fights among pigs, pigs showing curiosity, etc.) and lowest regarding living conditions of the pigs (stocking density, feeding space, enrichment material, etc.). Our results are similar to those from the study of Golinar Oven et al. on animal welfare in Slovenian conventional and alternative pig production systems using WQ® protocol (12). The conclusion was that growers and fatteners in Slovenian conventional farms were rated as acceptable, but Slovenian alternative farms were rated as enhanced.

The study shows that there are discrepancies between actual animal welfare on selected farms and farmers' self-assessed importance of animal welfare. With one exception (pigs' fear of humans and its importance), the experts' observations resulted in lower average scores than the participants' self-assessment. Slovenian farmers rate all parameters of animal welfare very highly. Many studies which include participants from different countries of the world reported similar results—people generally find animal welfare and the laws that protect animals important (26), most people want better welfare for animals (27), and find animal protection an important social issue (28). Animal health status is the most important, and environmental conditions are the least important factors for animal welfare, according to farmers in our study. Similar to our results, participating farmers in a study from Vigors et al. selected minimizing health issues as the most important factor for animal wellbeing (29). We also discovered that the farmers who rate animal behavior as the most important also have better actual welfare on the farms, regarding the parameter. Kiliç and Bozkurt conducted a similar study on the relationship between farmers' perceptions and animal welfare standards on sheep farms and found that farmers who rated the importance of welfare higher, had better actual welfare on their farms (30), similar results were reported by Munoz et al. who studied the relationship between farmer attitudes, management behavior and sheep welfare (31). Albernaz-Gonçalves et al. identified numerous management and animal indicators of poor welfare on the farms, included in their study. However, most farmers surveyed were satisfied with animal welfare standards at their farms and were not willing to improve the status (15). Kauppinen et al. reported that farmers included in their study who considered improving animal welfare more important had higher productivity on their farms (19).

There are numerous studies examining farmers' motives and willingness to improve animal welfare (15, 19, 32–37). For many farmers worldwide, cost and investment are important motivators (15, 33, 35, 37–39). Additional welfare improvements on the farms in our study would mean greater expenditures that are not covered or subsidized by the government, so any additional costs fall on the shoulders of farmers. For instance, in the year 2021, the Decree on the animal welfare measure from the Rural Development Program of the Republic of Slovenia for the period 2014–2020 supported farms that met animal welfare requirements that went beyond minimum conditions and normal husbandry practices. Farms that had 10% more unobstructed floor space per animal in group pens according to minimum standards were supported by funding (40). This implies significant investments, especially if major infrastructure changes are required. Costs could be the reason farmers identify health status as the most important parameter of animal welfare, as health problems produce great expenses (41). Another farmers' important motivator for improving animal welfare is increasing productivity of the pigs, which is again related to higher income (4, 15, 33, 41).

The results of our study varied according to the independent variables. The results were clearest for the vocational training variable, where farmers who continue their education from multiple sources score higher on both actual welfare and farmers' perceived importance of welfare, on 4 of 5 parameters (general status, living conditions, and environmental conditions). This implies that vocational training contributes to better actual and self-assessed animal welfare. Jo et al. conducted a study on broiler farmers' perceptions of animal welfare and concluded that as education levels increase, farm productivity and efficiency also increase (42). Improved education leads to higher job satisfaction among farmers and positively affects their perception of animal welfare (32). Coleman et al. trained farm workers to test whether behavior and attitude toward pigs on a commercial farm can be altered. Not only was there a decrease in negative behaviors toward pigs, but the change in attitude also had a positive effect on pig behavior (34).

Interestingly, we discovered that farmers with lower education level had better welfare at their farms compared to farmers with higher education level, on 3 out of 5 parameters (general status, living conditions and environmental conditions). That indicates that the level of education is not as important as vocational training, especially engaging in different types of training. This contrasts with other researchers' studies, which have found a significant influence of farmers' higher education level on improving animal welfare (30, 31, 42). No significant relationship was found between educational level and self-assessed importance of welfare, as all the participants rated welfare highly. Participants with higher education levels found health status more important and animal behavior less important than the participants with lower education levels. As our study sample is small, we believe further investigation is necessary to determine the effect of education on pigs' welfare before making any conclusions.

The results suggest that age of the farmer has a slight impact on animal welfare. Older farmers' farms had pigs with better general status and the environmental conditions were better taken care of (dust, humidity, odors, ventilation, and heating). Younger farmers had better general health status of pigs (less problems with trotters, diarrhea, hernias, conjunctivitis etc.). Older farmers also find animal behavior and living conditions more important than younger participants which is interesting, as the actual welfare regarding those parameters did not differ from younger farmers. Studies that consider age as a variable for attitudes toward animal welfare are inconsistent. Some studies report, that older farmers had higher empathy scores and were more likely to intervene in pig fights than young farmers (32). Others did not find significant relationship between age and welfare (21, 30). Some studies concluded that younger farmers have better welfare status on their farms (36, 43). Jo et al. found that an increase in farmers' age decreases farming efficiency and production level by up to 0.16% (42).

Males achieved slightly better results than females in our study. If the farmer was male, pigs had better health status and pigs' behavior was better compared to female farmers. Females had better environmental conditions. Male farmers also find animal behavior, health status and environmental conditions more important than females. Females think general status is more important than males. This contrasts with previous studies which prove that female farmers, veterinarians, and veterinary students, on average, show higher levels of positive behavior and empathy toward animals (29, 32, 36, 44–49). However, the study by Kauppinen et al. did not find strong correlations between gender and welfare (19).

The small sample size is a major limitation of this study and presumably the reason why we found only one significant association. To make more relevant conclusions, we intend to broaden the sample through our project. On the other hand, we visited the majority of larger Slovenian farms considering that most of the Slovenian farms are small. We also intend to perform the same test on other farm animals (horses, poultry, and cattle) and compare the results to this study.

In conclusion, the pig farmers in Slovenia consider animal welfare very important, but their farms follow only minimal statutory requirements. The welfare on Slovenian farms is adequate, but there is room for improvement, especially regarding biosecurity on farms, lack of appropriate enrichment materials, no separation of pigs by different categories, and the lack of thermometers and hygrometers on farms. The results of this study also suggest that vocational training is a significant variable in increasing levels of pig welfare. Age, gender, and education level are not significant variables, however, we found slightly better welfare on farms led by older male farmers with a lower level of education, who enroll in vocational training from many sources. This should be further investigated before making conclusions, due to our small sample size.

To our knowledge, similar studies of discrepancies between farmers' perceptions and actual animal welfare conditions on any kind of pig farms have not yet been conducted. We believe that with this research we have opened a discussion in an important field that should be investigated further. This study was carried out within the framework of the Slovenian Target Research Program. The goal of the program is to adjust the welfare guidelines in Slovenia and to educate farmers on topics where we found irregularities on the farms and, as a result, to raise the level of welfare in Slovenian pig farms.

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 evaluation of pig welfare in Slovenia was carried out within the framework of the Slovenian Target Research Program (Breeding of domestic animals by upgrading animal welfare in accordance with social requirements, No. V4-2024).

Author contributions

MŠ, IT, and AD conceived and designed the study. EN, IGO, MŠ, and JP conducted the study. IT analyzed the data. EN wrote the manuscript. All authors reviewed and approved the final manuscript.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.1010791/full#supplementary-material

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The relationship between common data-based indicators and the welfare of Swiss dairy herds

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The assessment of dairy cow welfare has become increasingly important in recent years. Welfare assessments that use animal-based indicators, which are considered the most direct indicators, are time consuming and therefore not feasible for assessments on a large number of farms. One approach to reducing this effort is the use of data-based indicators (DBIs) calculated from routine herd data. The aim of this study was to explore the relationship between common DBIs and the welfare of 35 dairy herds to evaluate the feasibility of a data-based welfare prediction method. For this purpose, the WelfareQuality® (WQ) protocol was used to assess the welfare of dairy cows on 35 Swiss farms, for each of which 10 commonly used DBIs were calculated from herd data. Spearman's rank correlations were used to investigate the relationship between DBIs and WQ criteria and measurements. The study found only a few statistically weak associations between DBIs and animal welfare, with no associations for measurements or criteria of resting comfort and appropriate behavior. Thus, the multidimensional welfare definition is insufficiently covered, and the present publication does not support the approach of a purely data-based prediction of dairy welfare status at the farm level. Instead, the regular calculation of DBIs that are indicative of isolated animal welfare problems or metrics of animal health could allow monitoring of these specific areas of animal welfare.

KEYWORDS

herd records, welfare assessment, routine herd data, national database, herd health, animal based measures

Introduction

In recent years, the valid assessment of farm animal welfare has become increasingly important for a growing number of stakeholders. Farmers benefit from animal welfare assessments for self-control, benchmarking their performance, or as advisory tools (1, 2). Market organizations, retailers, and organic farming associations employ welfare assessments by external auditors to ensure compliance with their welfare standards (3, 4) and federal institutions assess the fulfillment of legal welfare requirements and minimum welfare standards (1, 5). However, animal welfare is a complex, multidimensional concept that includes biological functions, animal behavior, and affective state (6). Because of its complexity, animal welfare itself cannot be measured directly but must be reflected by a variety of indicators that represent its multidimensionality (7). The indicators used for on-farm welfare assessments can be divided into two main types: input- and outcome-based indicators. Resource- and management-based indicators are used to survey the environment and the management that affect the animals (input-based). In contrast, animal-based indicators (ABIs) are collected directly from the animal and can thus indicate how the animal copes with the influencing factors (outcome-based) (1, 8). Therefore, ABIs are considered to reflect welfare more directly, leading to an increasing preference for ABIs over input-based indicators (9). Probably the most comprehensive welfare assessment protocol for various livestock species is the approach developed by the WelfareQuality® (WQ) project (10, 11). The WQ protocol for dairy cows includes 27 indicators that are mostly animal-based and complemented by input-based indicators only in areas where no suitable ABIs are available (12). However, conducting on-farm surveys, especially the assessment of animal-based indicators, is very time-consuming. Approximately 6 h are required to survey a herd of 60 cows using the WQ protocol (13). To survey larger numbers of farms, such as for monitoring animal welfare at the population level, alternatives must be found to allow a quick and cost-effective assessment (14).

Given the challenge of alternative approaches to assessing animal welfare, various attempts have been made to make animal welfare assessments on large numbers of farms more feasible or to shorten the duration of surveys. One way to accomplish this is to integrate routinely collected herd data into animal welfare monitoring, which could replace ABIs (8) or enable data-driven risk screening that could reduce the number of farm visits (15). Herd data collected directly from animals, such as somatic cell counts in milk, can be considered indirect animal-based indicators (16) or data-based welfare indicators (DBIs). Whereas traditional welfare assessments, such as the WQ protocol, usually include only a few DBIs, some research has aimed to predict farm-level welfare status based solely on DBIs (14, 15, 17–19). In this way, a data-based screening should be created that could limit on-farm surveys to high-risk farms.

A precondition for a wide use of DBIs, for example within the framework of national monitoring, is the availability of data of sufficient quality from as many farms as possible (20). This approach seems particularly suitable for dairy farms, as, due to European legislation, a large amount of routine herd data are available. For example, cattle must be individually identifiable, and data on birth, movement, and death must be collected and stored in national databases (21). Furthermore, analyzes of bulk milk delivered for food production must be carried out on a regular basis (22). These data are supplemented by milking records of individual animals obtained monthly by breeding or producer organizations in many countries (23).

In addition to the availability of the data, it is necessary that the DBI is related to the animal welfare of the farm to use a DBI to predict animal welfare (20). Based on the results of previous work, the aim of this study was to investigate the relationship between common data-based indicators and the welfare of Swiss dairy herds in order to assess the potential of data-based indicators to estimate the animal welfare of Swiss dairy herds.. For this purpose, an on-farm survey was conducted on 35 Swiss dairy farms. The animal welfare status was assessed using the WQ's criteria and measurements and subsequently examined for its association with 10 selected DBIs calculated from herd data.

Materials and methods

Farms and animals

Farm visits were conducted on Swiss dairy farms from January 2020 to March 2021. To recruit farms, farmers interested in previous studies or recommended by other farmers were contacted by telephone. Thirty-seven farmers agreed to participate and fulfilled the condition of having at least 16 lactating dairy cows at the time of the planned farm visit. The farms were visited once during the winter housing period between January and March (22 farms in 2020, 15 farms in 2021), after the cows had been mainly housed indoors for a minimum of 2 months. Of the 37 farms visited, 35 farms with a complete on-farm welfare assessment delivered valid values and were included in the analyzes.

The mean annual herd size was 47 dairy cows (range 16–136). Twenty farms had a free stall barn, and on 15 farms cows were kept in tie-stalls. Seven farms were run according to certified organic standards (Bio Suisse). All tie-stall farms participated in the Swiss animal welfare program RAUS (24), which requires regular outdoor exercise during winter and pasturing in summer. All loose housing farms participated in the Swiss animal welfare program BTS (24), which requires a comfortable lying area separated from the feeding area. In addition, 18 of the 20 loose housing farms participated in the RAUS program.

Assessment of farm animal welfare status using the WQ protocol

The welfare status of the herds was surveyed by conducting the entire WQ protocol for dairy cattle (12). All assessments were carried out by the first author, who had previous experience

TABLE 1 Overview of WelfareQuality® principles, criteria, and measurements as well as the expressions at herd level used for the analysis [Table modified from (12)].

	Criteria	WQ indicators	Measurements/herd-level expressions used for analysis
Principle: Good feeding	1. Absence of prolonged hunger	Body condition score	% BCS very lean
			% BCS fat†
	2. Absence of prolonged thirst	Water provision (number, length of water troughs/bowls)	
		Cleanliness of water points	*
		Water flow	*
		Functioning of water points	*
Principle: Good housing	3. Comfort around resting	Time needed to lie down	Mean time to lie down
		Animals colliding with housing equipment during lying down	% Collisions with stalls
		Animals lying outside the lying area	% Lying outside lying area
		Cleanliness of udder	% Dirty udders
		Cleanliness of flank/upper legs	% Dirty hindquarters
		Cleanliness of lower legs	% Dirty legs
	4. Thermal comfort	No measure developed yet	
	5. Ease of movement	Presence of tethering	*
		Access to outdoor loafing area or pasture	*
	6. Absence of injuries	Lameness	% Not lame†
			% Moderately lame
			% Severely lame
		Integument alterations	% Cows without skin alterations†
			% Cows with hairless patches
			% Cows with severe skin alterations
Principle: Good health	7. Absence of disease	Coughing	Frequency of coughing (coughs/cow/15 min)
		Nasal discharge	% Nasal discharge
		Ocular discharge	% Ocular discharge
		Hampered respiration	% Hampered respiration
		Diarrhea	% Diarrhea
		Vulvar discharge	% Vulvar discharge
		Milk somatic cell count	% Mastitis
		Mortality	% Mortality
		Dystocia	% Dystocia
		Downer cows	% Downer cows
	8. Absence of pain induced by management procedures	Disbudding/dehorning	*
		Tail docking	*
Principle: Appropriate behavior	9. Expression of social behaviors	Agonistic behaviors	Frequency of head butts (head butts/cow/h)
			Frequency of displacements and other agonistic interactions (interactions/cow/h)

(Continued)

TABLE 1 (Continued)

Criteria	WQ indicators	Measurements/herd-level expressions used for analysis
10. Expression of other behaviors	Access to pasture	*
11. Good human-animal relationship	Avoidance distance	*
12. Positive emotional state	Qualitative behavior assessment	*

† WQ protocol foresees survey and evaluation at herd level, but not inclusion in calculation of indicators and criteria.
* Exclusively considered as criteria score, individual measurements not taken into account for analyzes.

in dairy farming and the handling of cows. The proper application of the WQ was trained in a 3-day course given by an official trainer of the WQ consortium and routinized on three test farms, which were not included in the data analysis.

Farm visits started at the end of morning milking or, alternatively, at morning feeding, when the cows were at the feeding table. All measurements were collected following the guidelines of the WQ protocol. This involved direct observations of the herd, examinations of individual animals and husbandry conditions, and an interview with the farm manager. Additional information was derived from the farm records.

According to the guidelines of the WQ protocol (12), the on-farm measurements were expressed as herd-level prevalence or frequencies on a continuous scale and aggregated into WQ criteria scores ranging from 0 to 100. As the WQ does not currently include a measurement of thermal comfort, this criterion was not considered (see Table 1).

Calculation of data-based indicators used as animal welfare indicators

For the present study, DBIs were investigated that have already been used as animal welfare indicators or that are considered to be relevant for this purpose. In addition, the DBIs had to be calculable using data routinely available in Switzerland. To identify the DBIs, results from previous scientific literature (25) were used. These DBIs were supplemented with DBIs that are currently being used in other animal welfare projects or assessments, such as Q-check (26) or AssureWel (27), even if no peer-reviewed reports have yet been published for these projects or assessments. All DBIs fitting the criteria (routinely available, identified in scientific literature, or used in other projects) are listed in Table 2.

All farm-specific data were obtained with the consent of the farm managers. For each farm, data on cattle identification and registration were obtained from the Swiss animal movement database. Where available, data on bulk milk analysis were obtained from the national milk quality database dbmilch, and data on cow-individual milk analysis were retrieved from the breeding associations. From the data sets, the 10 selected DBIs were calculated for an annual period prior to the farm visit (see Table 2). As two farms did not supply milk for human consumption and were therefore not subjected to mandatory bulk milk analysis, the variable mean number of somatic cells in bulk milk (BMSCC) was calculated for 33 farms. Three farms did not participate in the monthly milk recording of individual cows; thus, DBIs derived from the monthly milk analyzes could only be calculated for 32 farms [cows with SCC $<$ 1,00,000 cells/ml (%), cows with SCC $>$ 2,00,000 cells/ml (%), cows with SCC $>$ 4,00,000 cells/ml (%), cows with milk fat-to-protein ratio $<$ 1.0 in 0–60 days in milk (%), cows with milk fat-to-protein ratio $>$ 1.5 in 0–60 days in milk (%)].

Statistical methods

All analyzes were performed in R version 3.6.3 (32). Descriptive analyzes included the scores of the criteria except for the criterion *thermal comfort*. Furthermore, for criteria with more than one animal-based measurement specified in the WQ, the individual measurements, aggregated at the herd level were included in the analyzes (in particular for the criteria *absence of prolonged hunger, comfort around resting, absence of injuries, absence of disease*, and *expression of social behaviors)*.This included also measurements that were collected and evaluated at the herd level as specified in the WQ but are not intended to be used in the calculations of indicators and scores (e.g., % fat cows, etc.) (see Table 1). The distribution of WQ measurements, WQ criteria, and calculated DBIs was described using minima, maxima, upper and lower quartiles, means, and medians.

To assess potential associations between DBIs and WQ measurements or criteria, we used Spearman rank correlations on each pair of DBI and either the WQ measurements or criteria. Spearman ranks were chosen because the farm results were not normally distributed in the criteria and measurements. The Spearman rank correlations were corrected for tied values. For the criteria *absence of prolonged thirst, ease of movement, and absence of pain induced by management procedures*, farm results were each grouped in three ranks. These three criteria differ from the other criteria in that their assessment in the WQ is not obtained on a continuous scale. Instead, decision trees were

TABLE 2 Data-based indicators, and their calculations and data sources used for the present study and reasons for the inclusion in the analyzes.

Data-based indicators	Definition and description	Data source	Reason for inclusion in the analyses
Cow mortality (%)	$\frac{Dead\ and\ euthanized\ cows}{Herd\ size\ (total\ number\ of\ cows)} \times 100$	Data on identification and registration, Swiss animal movement database	Use as a data-based animal welfare indicator (12, 26–29)
Culled cows in 0–60 DIM (%)	$\frac{Culled\ cows\ in\ 0-60\ DIM}{Total\ culled\ cows} \times 100$		Potential indicator of health problems in early lactation (30)
Stillbirths (%)	$\frac{Stillborn,\ euthanized\ and\ dead\ calves\ up\ to\ 48\ h}{Total\ number\ of\ calves\ born} \times 100$		Use of similar data-based animal welfare indicators (26, 27)
Mean productive lifespan (months)	*Mean timespan between the day of first calving and day of culling of all the cows culled during the 1-year period*		Use as a data-based animal welfare indicator (26, 28)
Cows with SCC $<$ 1,00,000 cells/ml (%)	$\frac{Cows\ with\ SCC<1,00,000\ cells/ml}{Total\ number\ of\ cows\ sampled} \times 100$	Monthly milk testing, breeding organizations	Recommended indicator for veterinary herd management (31)
Cows with SCC $>$ 2,00,000 cells/ml (%)	$\frac{Cows\ with\ SCC>2,00,000\ cells/ml}{Total\ number\ of\ cows\ sampled} \times 100$		
Cows with SCC $>$ 4,00,000 cells/ml (%)	$\frac{Cows\ with\ SCC>4,00,000\ cells/ml}{Total\ number\ of\ cows\ sampled} \times 100$		Use as a data-based animal welfare indicator (12, 26, 28)
Milk FPR $<$ 1. 0 in 0–60 DIM (%)	$\frac{Cows\ with\ FPR<1.0\ in\ 0-60\ DIM}{Total\ number\ of\ cows\ in\ 0-60\ DIM} \times 100$		Use of comparable data-based animal welfare indicators (26, 28)
Milk FPR $>$ 1. 5 in 0–60 DIM (%)	$\frac{Cows\ with\ FPR>1.5\ in\ 0-60\ DIM}{Total\ number\ of\ cows\ in\ 0-60\ DIM} \times 100$		
Mean BMSCC (cells/ml)	*Arithmetic mean of BMSCC*	Routine milk analyses of milk delivered for food production	Use as a data-based animal welfare indicator (29), availability for all milk-supplying farms.

All indicators were calculated for a 1-year period in advance of the welfare assessment.
DIM, days in milk; SCC, milk somatic cell count; FPR, fat-to-protein ratio; BMSCC, bulk milk somatic cell count.

used to compile the measurements into a limited number of possible scores. The limited number of ranks achieved led to the exclusion of the criteria *absence of prolonged thirst*, *ease of movement*, *and absence of pain induced by management procedures* from the subsequent analyzes. Furthermore, the farm results for the measurements *% hampered respiration*, *% nasal discharge*, *and % collisions with stalls* were grouped on a limited number of different ranks, which led to their exclusion from further analyzes. In total, the relationship between the DBIs and eight criteria scores and 23 measurements were analyzed.

As the number of pairwise comparisons increases the risk of false positive results, the obtained p-values were adjusted using Benjamini and Hochberg's false discovery rate adjustment (33). Because adjusting for false positives inadvertently increases the risk for false negatives, we carefully assessed all associations with an unadjusted p-value $\leq$ 0.05 based on plausibility, the correlation coefficient, and the unadjusted and adjusted p-value.

Results

Results of the welfare assessment and the calculation of data-based indicators

Descriptive results for farm animal welfare expressed as criteria of the WQ protocol are displayed in Figure 1, while results for the evaluated WQ measurements can be found in Supplementary Table 1. Descriptive statistics for the DBIs as calculated from herd data are shown in Table 3.

Associations between animal welfare and data-based indicators

The initial Spearman rank analysis revealed associations based on uncorrected p-values between the DBIs and 7 of 23 analyzed WQ measurements, as well as 2 of 8 analyzed WQ criteria (Table 4). WQ measurements found to be associated with DBIs were predominantly indicators of animal health, as was the criterion *freedom from disease*. Furthermore, the criterion *absence of hunger* and the corresponding measurement *% cows very lean* were associated with the DBI cows with a milk fat-to-protein ratio $>$ 1. 5 in 0–60 DIM (%). Out of the five analyzed measurements of the criterion *comfort around resting*, the *percentage of cows with dirty udders* was associated with the mean productive lifespan. No association could be shown for measurements or criteria related to the principle of *appropriate behavior*.

Correction of the p-values to multiple analyzes confirmed five associations with a p-value $<$ 0.05 at a high level of confidence. After correction, the associations between the

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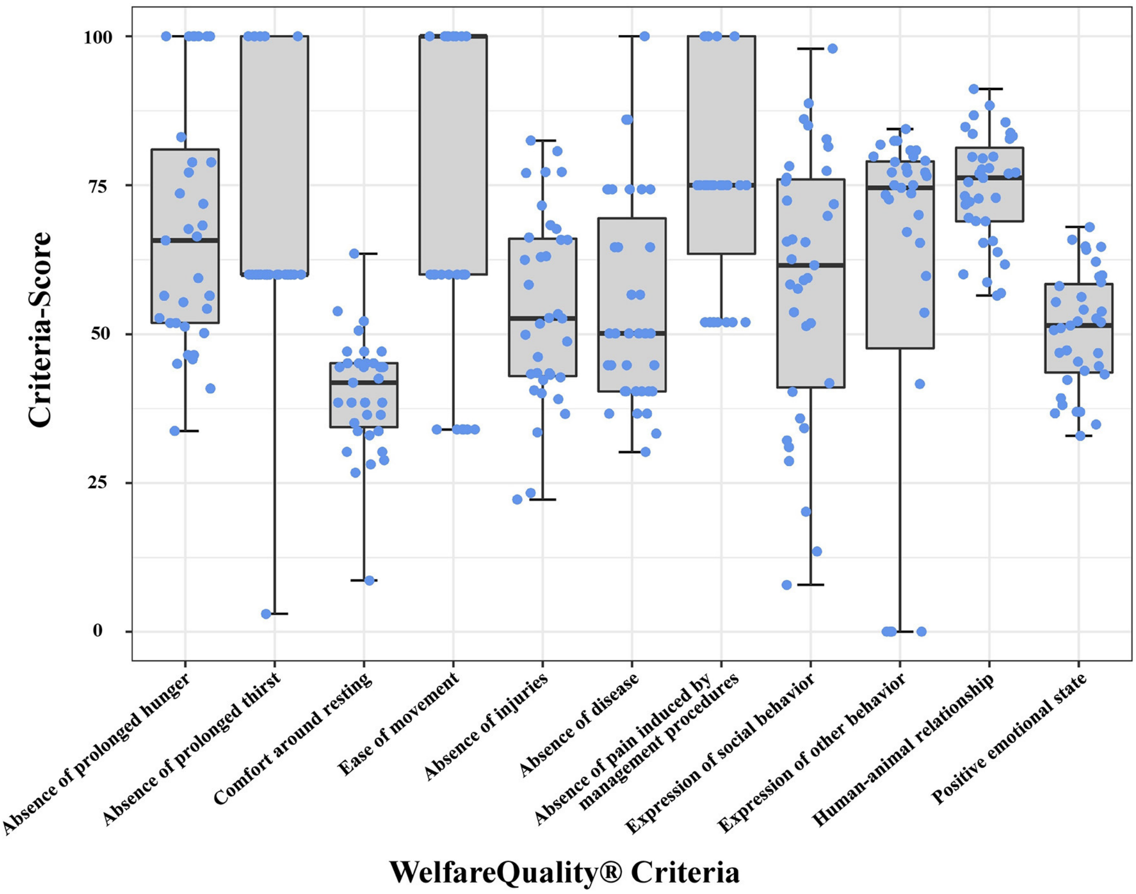

FIGURE 1
Boxplots and jitter plots representing the WQ scores of 35 analyzed Swiss dairy farms in 11 calculable WQ criteria. Boxes represent the distribution of farm scores, whiskers display the range between the lowest and the highest criteria score observed and blue jitters show the individual farm scores. For the criterion *thermal comfort,* no measurement has yet been assigned in the WQ protocol; for reasons of clarity, a presentation has been omitted.

TABLE 3 Descriptive statistics from 10 common data-based indicators calculated from 35 Swiss dairy farms for a 1-year period in advance of the welfare assessments.

Data-based indicators	Min	1st Quartile	Median	Mean	3rd Quartile	Max	Farms with lacking data
Cow mortality (%)	0.00	0.00	2.65	2.51	3.85	10.70	
Culled cows in 0–60 DIM (%)	0.00	0.00	2.65	4.12	4.51	18.94	
Stillbirths (%)	0.00	3.30	6.45	6.43	8.33	18.18	
Mean productive lifespan (months)	791.00	1136.00	1492.00	1498.00	1724.00	2876.00	
Cows with SCC < 1,00,000 cells/ml (%)	30.57	52.13	61.22	58.43	63.40	80.80	3
Cows with SCC > 2,00,000 cells/ml (%)	8.00	14.73	19.23	19.68	24.39	32.18	3
Cows with SCC > 4,00,000 cells/ml (%)	3.09	5.43	7.95	8.49	11.55	16.06	3
Milk FPR < 1.0 in 0–60 DIM (%)	0.00	6.27	11.06	11.93	16.58	29.66	3
Milk FPR > 1.5 in 0–60 DIM (%)	1.30	4.91	11.46	13.51	20.03	35.67	3
Mean BMSCC (cells/ml)	64640.00	108292.00	133792.00	138645.00	161500.00	229815.00	2

DIM, days in milk; SCC, milk somatic cell count; FPR, fat-to-protein ratio; BMSCC, bulk milk somatic cell count.

TABLE 4 Associations between measurements and criteria of the WelfareQuality® -Protocol and data-based indicators. Associations with an adjusted $p<0.05$ are highligted in gray.

WelfareQuality criteria or measurements	Data-based indicators	*r*	*p*-Value	*p*.adj
Absence of prolonged hunger	Milk FPR > 1. 5 in 0–60 DIM (%)	−0.38	0.030	0.638
Absence of disease	Stillbirths (%)	−0.37	0.029	0.638
% BCS very lean	Cows with FPR > 1. 5 in 0–60 DIM (%)	0.38	0.030	0.638
% Dirty udders	Mean productive lifespan (months)	0.38	0.024	0.638
% Not lame	Culled cows in 0–60 DIM (%)	−0.39	0.020	0.638
% Mastitis	Cows with SCC < 1,00,000 cells/ml (%)	−0.57	0.001	0.039
	Cows with SCC > 2,00,000 cells/ml (%)	0.79	< 0.001	< 0.001
	Cows with SCC > 4,00,000 cells/ml (%)	0.75	< 0.001	< 0.001
	Mean BMSCC (cells/ml)	0.40	0.022	0.638
% Mortality	Cow mortality (%)	0.57	< 0.001	0.032
	Cows with SCC < 1,00,000 cells/ml (%)	0.42	0.017	0.638
	Cows with SCC > 2,00,000 cells/ml (%)	−0.36	0.046	0.835
% Dystocia	Cow mortality (%)	0.55	0.001	0.039
% Downer cows	Cows with SCC > 2,00,000 cells/ml (%)	0.37	0.039	0.761
	Cows with SCC > 4,00,000 cells/ml (%)	0.50	0.004	0.190
	Mean BMSCC (cells/ml)	0.38	0.031	0.638
	Stillbirths (%)	0.39	0.020	0.638

DIM, days in milk; SCC, milk somatic cell count; FPR, fat-to-protein ratio; BMSCC, bulk milk somatic cell count.

measurement *% mastitis* and the DBIs based on the cow-specific SCC as well as the associations between the WQ measurements *% mortality* and *% dystocia* and the DBI cow mortality % yielded a p-value < 0.05.

Discussion

To identify the potential benefits of DBIs for monitoring herd-level welfare, the aim of the present study was to determine the relationship between 10 commonly used DBIs and animal welfare, expressed in terms of WQ measures and criteria. Overall, the results of the present study suggest that only a few criteria or indicators measured with the WQ are associated with the tested DBIs. The analyzes revealed statistically reliable and at the same time strong associations of the DBIs for only the WQ measurements *% mastitis*, *% mortality and % dystocia*. The two WQ measurements % mastitis and % mortality are both collected from herd data and are thus already data-based indicators. Therefore, these associations are of limited use for the monitoring of dairy welfare.

In addition, associations shown in the initial Spearman-Rank analyzes could also be valuable for predicting animal welfare status, although most of these associations were eliminated after correction for multiple associations. This comes from the fact that the correction used to adjust for multiple analyzes inadvertently increases the risk of false-negative associations. Hence, associations found in the initial analysis that were not significant after correction of the p-values may also be worth further investigation. Among these associations, most were found between DBIs and WQ measurements used as indicators of animal health. Only one association with a measurement of the criterion *comfort around resting* was shown, whereas associations with measurements or criteria of the principle *appropriate behavior* were lacking completely.

One might wonder why the present study showed only a few relationships between DBIs and animal based measurements in comparison to previous work (14, 15, 18, 34). One reason could be the number and selection of DBIs included. For our study, a reduced approach that did not include fertility or milk yield data was used, although those DBIs were found to be associated with animal welfare in other studies (14, 15, 18, 34). These data were omitted, as only the DBIs that were calculable for most Swiss dairy farms and allowing for comparisons between farms were included. The milk yield could have limited the comparability between farms due to the diverse intensity levels of Swiss milk production (e.g., localization of the farm in valley or mountain regions, conventional or organic production, production for drink milk or cheese, the use of dual-purpose or high-yield breeds).

In addition, for both reproduction data and milk yield, the relationship to herd welfare is unclear (35, 36), with a direct link strongly questioned (37). Furthermore, even previous studies examining broad sets of DBIs were unable to predict all criteria of animal welfare. In 2011, a review observed that only a few studies reported relationships between DBIs and measurements of resting comfort and animal behavior (38). On one hand, this was explained by a general lack of studies examining correlations between DBIs and behavior or resting comfort, as was also reported recently (25). On the other hand, the potential of herd data to detect problems in resting comfort and behavior was questioned (38). Indeed, even among the studies that included behavioral parameters, the number of associations between DBIs and animal welfare was low compared to other parameters (14, 15, 34). Hence, the lack of relationships between DBIs and measurements of behavior or comfort of resting might be due to the nature of the DBIs. With the exception of the mean productive lifespan, the DBIs included in the present study are closely related to animal health or describe risk factors for impaired animal health. In contrast, none of the DBIs has a strong direct relationship with animal behavior or resting comfort. Whereas the considered DBIs and the health-related measurements could be linked by common factors (e.g., health management), resting comfort and animal behavior were likely to have no common link with selected DBIs.

Our study was intended as a preliminary investigation to determine relationships between DBIs and animal welfare to estimate the predictive potential of DBIs for animal welfare at the herd level. Regarding the methodology used, one may question why criteria and herd-level measurements were used to express the welfare status of herds rather than the overall score. The overall score was omitted as it results from a multi-step weighted aggregation of measurements, which partly allows the compensation of different welfare aspects (39). However, the weighted and compensating aggregation is questioned in animal welfare research, as it has been shown that the overall score is strongly influenced by only few measurements (11, 40, 41). Furthermore, the weightings, which were determined partly based on expert opinion (39), have not been adjusted to reflect changes in agriculture and changing attitudes toward animal welfare.

Concerning the statistical methodology, the present study investigated univariate relationships between DBIs and welfare measurements and criteria. This approach derives from current efforts to routinely evaluate a range of DBIs that are not aggregated into predictive models (26). Furthermore, the approach was chosen to facilitate comparison with previous studies on DBIs that also initially analyzed univariate associations between DBIs and animal-based measures (14, 15, 18, 34). It should be noted that, based on the information provided, none of these studies adjusted the univariate associations for the presence of multiple analyzes. The results of the present study suggest that univariate relationships between DBIs and welfare measurements and criteria obtained without correction for multiple testing should be interpreted with caution.

It is clear that replicating the present study with more farms, possibly targeting farms with suspected good or poorer animal welfare status or a random selection of farms, would increase the reliability of the results. Nonetheless, in connection with the results from the literature, conclusions can be drawn for the predictive potential of DBIs. The predominantly statistically weak associations of the tested DBIs with only a few measurements of WQ indicate that the tested DBIs are not sufficient to comprehensively predict animal welfare. Given the inadequate coverage of behavioral measurements and indicators of resting comfort, it is questionable whether additional DBIs could complete the predictability of dairy herd welfare status as described by the WQ. This is in line with previous studies which—despite finding a number of associations between DBI and welfare measurements—concluded that associations found were limited (34) and DBIs could only identify problem herds with moderate accuracy rather than estimate the welfare status on the farms (14, 15, 18). Since both the currently used animal welfare definitions (6) and the Swiss Animal Welfare Act (42) require a multidimensional definition including species-appropriate behavior and adequate husbandry, we doubt the applicability of DBIs to predict animal welfare in its multidimensionality in the near future.

Nevertheless, DBIs should not be generally considered inappropriate for the monitoring of dairy cow welfare at the herd level. All 10 variables investigated in the present study are used in veterinary medicine or herd monitoring to gain insights into herd-level animal health status (43, 44). Moreover, increased cow mortality (45) or a high stillbirth rate (46) can themselves be considered animal welfare issues. For example, applying current alarm thresholds to the DBI % cow mortality [4–5% cows (47, 48)] would classify about 14% of the study farms as at-risk for the welfare problem of high cow mortality. Thus, the more welfare issues that can be captured using data, the more direct DBIs could be applied to identify farms at risk. However, since only a limited number of animal welfare issues can currently be monitored directly by data screening, it must be clear that good performance in these parameters does not necessarily reflect a sufficient herd welfare status.

Conclusions

This study demonstrated few associations between DBIs and animal welfare as measured by the WQ protocol. The associations shown for DBIs were predominantly statistically weak and emerged for a limited number of criteria and herd-level measurements of the WQ, with no associations identified with resting comfort or appropriate behavior. Thus, as DBIs were not able to adequately reflect the multidimensionality of animal welfare, the study suggests that the potential of DBIs is to provide information on specific welfare aspects rather than to provide a comprehensive predictive tool for dairy welfare status at the herd level.

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 reviewed and approved by Cantonal Veterinary Office Thurgau and the Ethical committee for animal experiments of the Cantonal Veterinary Office Zurich. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

BT and J-BB: conceptualization and funding acquisition and funding acquisition. BL: investigation and writing—original draft preparation. BL and DS: formal analysis. BT, J-BB, DS, and SZ: review and editing. J-BB: supervision. All authors have read and agreed to the submitted version of the manuscript.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.991363/full#supplementary-material

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Treatment protocols for claw horn lesions and their impact on lameness recovery, pain sensitivity, and lesion severity in moderately lame primiparous dairy cows

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This study aims to investigate the effects of routine treatment protocols for claw horn disruptive lesions (CHDL) on lameness recovery rates, pain sensitivity, and lesion severity in moderately lame primiparous cows. A cohort of first parity cows was recruited from a single commercial dairy herd and randomly allocated to five treatments, comprising four lame groups (LTNB, LTN, LTB, and LT) and a single group non-lame group. Eligibility criteria for the lame cows included a first lameness score (score 3/5), presence of CHDL on a single foot, good body condition score of 3.0 to 3.5, and no history of previous lameness. LTNB received a combination of therapeutic trim, administration of a non-steroidal anti-inflammatory drug (NSAID; Ketoprofen) for 3 days, and hoof block on the healthy claw. Both LTN and LTB received the same treatment as LTNB without hoof block and NSAID, respectively. LT received only a therapeutic trim, whereas non-LT (negative control) received either a therapeutic or preventive trim. Pain sensitivity was assessed using the limb withdrawal reflex while lesion severity was recorded using the International Committee Animal Records (ICAR) Atlas guide. The enrolled cows were observed at weekly intervals, and the primary outcomes were assessed 28 days after treatment. The number (%) of recovered cows was 15 of 20 (75%), 13 of 21 (61.9%), 6 of 14 (42.9%), and 6 of 15 (40%) for LTNB, LTN, LTB, and LT, respectively. LTNB had significantly higher odds of successful treatment (OR = 4.5; 95% 1.1–19.1) compared to LT. Pain sensitivity based on limb withdrawal reflex was absent in a significantly higher number of cows (15/20; 75.0%) in LTNB compared to LTB and LT. LTB had a significantly lower lesion severity score in comparison to LTN. Overall, cows with limb withdrawal at day 28 after treatment were less likely (OR = 0.06; 95% CI 0.01–0.24) to develop a non-lame score. In conclusion, the treatment with therapeutic trim, hoof block, and NSAID led to better recovery and reduced pain sensitivity in moderately lame primiparous cows with good BCS compared to those that received only therapeutic trim. Further research on the changes within the hoof capsule following various treatment protocols is needed to elucidate the clinical benefits observed in this study.

KEYWORDS

lameness, claw lesions, hoof trimming, dairy cows, treatment, animal welfare

Introduction

Lameness is one of the most common health issues in dairy cattle, resulting in economic losses and severe impacts on animal welfare (1, 2). Claw horn lesions account for the majority of lameness events in dairy cows (3, 4). Lame cows are categorized either as mild, moderate, or severe depending on the extent of gait changes and postural defects (5), which may be associated with behavioral and production changes (6). For instance, severely lame cows spent less time at the feeding bunk (7), exhibited lower rumination time (8), loss in body condition (9), and produced less milk (2) compared to non-lame cows. Similar behavioral alterations were observed in newly and moderately lame cows (10), thereby highlighting the need for prompt detection and proper treatment. However, there is data paucity on the management of moderately lame cows affected with CHDL with a higher likelihood of dairy farmers underestimating lameness prevalence in such groups of cows (11, 12).

Claw horn disruptive lesions (CHDL) such as sole ulcer, sole hemorrhage, and white line disease are the most common conditions causing lameness in dairy cows (4, 13). Nevertheless, there is a deficit of information regarding the management of CHDL (14) with recent studies recommending further research to develop effective treatment protocols for these lesions (15, 16). Moreover, reports from previous studies highlighted that most information on the treatment of CHDL is based on experience and knowledge gained by field experts rather than evidence-based clinical trials (1, 14, 15).

A few studies have reported promising outcomes in the treatment of CHDL by a combination of therapeutic hoof trimming, application of hoof block to relieve pressure on the affected claw, and pain management using a non-steroidal anti-inflammatory drug (NSAID), such as Ketoprofen (15, 17). Higher recovery rates were observed when lame cows were promptly treated, whereas chronically lame cows failed to respond positively to the same treatment protocol (18). To elucidate the effects of various treatment protocols for CHDL, it is important to consider diverse lameness recovery measures, such as gait changes, lesion progression, and pain sensitivity.

Cow-level factors, such as parity, body condition score (BCS), previous lameness history, and lesion severity have also been demonstrated to influence the recovery rates of lame cows after treatment (3, 19). These factors need to be considered when evaluating the impact of a treatment protocol. For instance, parity has been widely reported as a risk factor for CHDL in dairy cows (19, 20). The sudden introduction to a new environment and housing systems may contribute to the onset of CHDL in first parity cows immediately after calving (21). These events are important on farms that frequently purchase young stock and lack routine hoof care management. This study aims to evaluate treatment options for moderate lameness caused by CHDL and their impacts on lameness recovery measures in first parity cows with good BCS. It was hypothesized that lameness recovery rates, gait changes and locomotion scores, pain sensitivity and lesion progression would vary with the treatment provided against CHDL in moderately lame cows.

Materials and methods

Study design

A randomized clinical trial (RCT) was applied in this study. Specifically, a positive controlled trial was conducted since all the enrolled animals were assigned to specific treatment groups. The protocol was reviewed and approved by the Institutional Committee for Animal Use and Care, University of Putra Malaysia (Ref: UPM/IACUC/ AUP-R010/2019) and this manuscript was prepared based on the guidelines outlined in the REFLECT statement for reporting RCT in livestock (22).

Study herd (animal housing, feeding, and management)

This study was conducted on a dairy farm located in Linggi, Negeri Sembilan, Malaysia. The farm was one of the herds enrolled in a cross-sectional study to investigate the prevalence of lameness and associated risk factors in dairy farms in Peninsular Malaysia. The farm was considered suitable for the RCT based on its relatively large herd size (> 300 lactating cows), moderate lameness prevalence (20–29%), availability of trim chute and hoof care unit, and computerized health and production recording system. The farm manager's contact and email address were retrieved from the registry list provided by the Department of Veterinary Services, Putrajaya, Malaysia.

Thereafter, the farm manager was briefed about the study objectives, inclusion criteria, and consent to participate.

The farm had a total of 452 Holstein Friesian and Friesian Sahiwal primiparous cows with an average milk yield of 4,575 L that were housed all through the year. The animals were kept in free stalls divided into four pens. Each stall has in-placed rubber mats, which were installed 3 years prior to this study. The floor at the walkway and feed alley was made up of concrete, whereas rubber mats were placed in the holding barn and milking parlor. A herringbone was used in milking the cows twice daily (6.00 am and 5:30 pm). Routine hoof trimming was performed weekly for cows in early (within 100 days in milk; DIM) and late lactation (above 200 DIM) by two farm staff using the five-step Dutch method. Hoof health data on the type and severity of claw lesions, claws affected, and the procedure performed was recorded electronically for each cow. Lame cows were identified by weekly locomotion scoring as per routine management and treated immediately upon detection. Other lameness management includes a footbath containing copper sulfate located at the exit of the milking parlor. The footbath was changed weekly by dissolving 200 grams of copper sulfate in 2 l of water. A motorized scrapper and manual water pumping systems were used to clean the floors at the milking parlor, resting barn, walkway, and holding yards. The cows were fed a total mixed ration composed of alfalfa hay, soyabean cake, fish meal, grain corn, and other supplements, which were adjusted depending on the stages of lactation.

Animals selection and enrollment criteria

Cow selection and enrollment began in June 2020 until December 2020. Locomotion scoring was conducted for all the lactating primiparous cows in the herd by a trained observer twice a week as cows exited the milking parlor on a leveled and non-slippery surface. A five-point scoring (23) was applied and cows with locomotion scores of 1 (sound), 2 (mildly lame), and 3 (moderately lame) were selected. Acceptable intra-observer reliability was obtained ($Kp = 0.88$) upon comparing the locomotion scores of cows in two different pens on two occasions.

The inclusion criteria comprised cows presenting the first lameness event on a single hind or forelimb that were selected based on available farm records and a lame score (locomotion score equal to or >3) after two successive non-lame scores. Upon further screening, only those affected with CHDL (sole hemorrhage and ulcer; SHU and white line disease; WLD) on a single claw and having a good BCS (a score of 3.0–3.5 on a scale of 5.0) were enrolled. Meanwhile, cows with a history of treatment for lameness on any limb or had received parenteral antibiotics or NSAID within the previous 2 weeks, having low BCS (<3.0), and within 10 DIM were not enrolled. Specifically, BCS was recorded using the five-point scoring scale employed by Vasseur et al. (24). The eligible cows were then restrained in a hoof-trimming chute and their hooves were examined. Detection of the lame foot entailed information from visual observation during locomotion scoring and the presence of withdrawal reflex upon applying pressure to the claw zones using a hoof tester. For the non-lame group, cows were considered eligible if they exhibited sound locomotion scores for at least 2 weeks before enrollment. Since the freestall was partitioned into four pens, an approximately equal number of cows were allocated to the various experimental groups from each pen.

Table 1 depicts the classification and description of the CHDL and corresponding severity scores recorded during the hoof examination as described by Sogstad et al. (25). Claw lesions were diagnosed by the researcher, a trained veterinarian, by using the ICAR claw health classification as a guide. Intra-observer reliability was not performed for claw lesion classification. Cows were enrolled dynamically as they fulfiled the inclusion criteria.

Random allocation and treatments administered

Enrolled animals were randomly allocated to five experimental groups (Table 2). Randomization was blocked with lesion type to ensure the matching of an equal proportion of cows with each diagnosis. The standard treatment protocol involved therapeutic hoof trimming, placing a hoof block on the healthy claw, and administration of NSAID (Ketoprofen) for 3 days.

Therapeutic trim of the whole foot consisted of a standard application of the five-step Dutch method, trimming of the identified lesion, removal of the diseased horn, and ensuring a balance of the heel height and sole thickness for even weight distribution between the medial and lateral claws (15, 26). The hoof block comprised a natural wooden type (Vettec Animal Health, the Netherlands), approximately 110 mm long, 55 mm wide, and 23 mm deep, that was positioned on the healthy claw to replicate proper claw placement and weight distribution. An adhesive glue designed for bovine hooves (Bovi-BondTM 210 cc, Vettec Animal Health) was applied to facilitate the adhesion of the block to the claw. Meanwhile, the NSAID comprised a three-days course of ketoprofen (100 mg/mL) administered by deep intramuscular injection at 3 mg/kg.

LTNB received the standard treatment protocol; therapeutic trim, NSAID, and hoof block, LTN received therapeutic trim and hoof block, LTB received therapeutic trim and NSAID, and LT received only therapeutic trim. Meanwhile, the non-lame group (Non-LT) received either a preventive trim or therapeutic trim with an emphasis on reducing the overgrown claw, debriding the claw lesion, and ensuring a balanced sole surface between the medial and lateral claws as described in our previous study

TABLE 1 Definition and categories of severity scores for claw lesions.

Lesion	Score	Definition
Sole ulcer	1	Corium is exposed, but unaffected
	2	Presence of granulation tissue, purulent exudates, necrosis and separation of the sole horn
	3	Involvement of the deeper structures of the claw
Sole hemorrhage	1	Mild haemorrhagic discolouration
	2	Moderate hemorrhage on a single spot and covering >20% of the sole surface (circumscribed)
	3	Marked hemorrhage on a single spot or extensive haemorrhagic discolouration covering >50% of the sole (diffused)
White line fissure	1	A fissure that disappears with a deep cut beneath the normal trimming level
	2	Deep fissure perforating next to the corium of sole or wall
	3	Corium is affected by purulent exudates, eventually with necrosis, granulation tissue, and separation of the wall or sole
White line hemorrhage	1	Slight haemorrhagic discolouration
	2	Moderate hemorrhage on a single spot or several superficial hemorrhages covering >20% of the white line
	3	Profound hemorrhage on a single spot or extensive haemorrhagic discolouration covering >50% of the white line

Adopted from Sogstad et al. (25).

TABLE 2 Experimental groups and treatments administered in the randomized clinical trial.

Groups and animals enrolled	Treatment	Description
LTNB ($n = 24$)	Standard treatment protocol	The standard treatment protocol consists of corrective trim, administration of NSAID, and application of hoof block on the healthy claw
LTN ($n = 23$)	Therapeutic trim + hoof block	Therapeutic trim plus hoof block attached to the healthy claw and no Ketoprofen administered
LTB ($n = 16$)	Therapeutic trim + NSAID	Therapeutic trim plus Ketoprofen, without hoof block, applied to the healthy claw
LT ($n = 18$)	Therapeutic trim only	Only therapeutic trimming
Non-LT ($n = 15$)	Only preventive or therapeutic trimming	Only preventive or therapeutic trimming

NSAID, non-steroidal and anti-inflammatory drug; STP, standard treatment protocol.

(26). Preventive hoof trimming entailed the use of the five-step Dutch method without completing the final step, which is the removal of a loose horn. All treatments were performed by a trained veterinary surgeon (the first author of this manuscript) who is familiar with the management of lame cows. A farm staff assisted the researcher to restrain the cows during treatment and was responsible for the administration of NSAID when the researcher was absent. Restraint and treatment durations were recorded in minutes.

Enrolled animals were identified using tag numbers and body marking for easy identification from a distance. Cows were managed according to the farm management, and farm staff were informed to notify the researcher if any further treatment is necessary. Under such situations, the cows were retreated as per the treatment groups by the trained farm staff or the researcher if visiting the farm within 24 h of receiving the complaint. The farm staff was aware of the different treatment protocols used in the study and the specific cows treated in each group.

Limb withdrawal, body movements, and vocalization

Limb withdrawal, body movements, and vocalization were recorded during treatment. The cows were initially allowed to remain standing in the trim chute for 5 min after being restrained. Upon immobilizing the affected limb, limb withdrawal was assessed using a hoof tester by exerting mechanical pressure on the suspected lesion site and claw zones. Limb withdrawal was considered positive when the cow exhibited mild twitches or attempted to adduct the restrained limb. Agitation or body movement was defined as the movement of both the treated limb and other body parts (neck and body) during treatment. Vocalization was defined as oral sound by the treated cow as a sign of discomfort. The outcomes were considered dichotomous and recorded as either present or absent.

Treatment follow-up and outcome measures

A 28-days observation period was adopted in this study. Locomotion scores, lesion severity, and pain sensitivity were recorded for each cow during treatment (day 1) and on days 7, 14, 21, and 28. The first follow-up observation was on 7 days (±3 days) after treatment as cows were assessed for locomotion scores and inspection of the lesion sites. The same locomotion and lesion-scoring techniques described earlier were employed to monitor the lameness recovery rate and lesion progression, respectively. Pain sensitivity was recorded using limb withdrawal and recorded as a dichotomous outcome (present or absent). Both vocalization and agitation or body movements were also recorded during the follow-up periods.

For LTNB and LTN, the block was re-applied if absent at any observation points. Animals were re-treated if their locomotion score had deteriorated from the time of enrollment to 14 or 21 days (±3 days) post-treatment. No cow was given additional NSAID therapy beyond 3 days. The final examination was on 28 days (±3 days) after treatment. At this stage, the hoof block was removed using a hoof nipper and careful leverage. Cows that were sold, culled, or died before the measurement of the primary outcomes were identified and removed from the study. All study outcomes were observed by the trained veterinarian and blinding was not applied.

Study inclusions

Primiparous cows were selected and assessed from June 1, 2020, to November 30, 2020. Cows' enrollment was discontinued on December 1, 2020, due to the low number of animals available for recruitment, an increased workload and few available staff to assist the researchers due to the introduction of new pregnant heifers. During the study period, 26 lame animals were not enrolled due to the following reasons: eight had severe hock lesions; six were lame but showed no visible claw lesion, two had digital dermatitis, six had swelling of the coronet, and four had either mastitis and downer cow syndrome that affected their locomotion. Meanwhile, five sound cows from the non-LT were excluded due to treatment for other post-calving complications aside from lameness.

Overall, a total of 96 cows were eligible for enrollment upon fulfilling the inclusion criteria (LTNB = 24, LTN = 23, LTB = 16, LT = 18, and non-LT = 15) but 11 of them were withdrawn before completing the study period. Table 5 outlines the allocation of cows (n = 85) to each treatment according to claw lesion diagnosis. A total of 51 cows (50.5%) were treated for sole ulcer, 20 (19.8%) for white line disease, and 14 (13.8%) for sole hemorrhage (Table 3).

Excluded cows

A total of 11 cows were withdrawn or lost before the 28-days study period. Four cows were culled (LT = 2 and LTNB = 2), 2 died (LT = 1 and LTB = 1), three were treated for other illnesses aside from lameness (LTNB = 1 and LTN = 2), and two were withdrawn for non-compliance with the study protocol after enrollment (LTNB = 1 and LTB = 1). No cow was removed from the NL group. Of the 70 cows that completed the RCT, eight of them required re-treatment at the 7 d (±3) after enrollment. Specifically, three cows received additional therapeutic trimming (LTNB = 1 and LTB = 2), seven cows had removed their hoof block, which was re-applied (LTNB = 3 and LTN = 4), and four cows were treated for hock lesions by wound cleaning and application of oxytetracycline spray (Woundsarex®).

Data analysis

Data analysis was conducted using the Statistical Package for Social Science (SPSS, IBM Version 23.0). Descriptive statistics were used to summarize the characteristics of the enrolled cows. To determine the success of random allocation, a one-way analysis of variance (ANOVA) was applied to evaluate differences in DIM, milk yield, lesion severity, time spent to restrain the cows, and treatment time between the groups during enrollment. Cross-tabulation was used to compare the categorical variables, such as vocalization, agitation, and limb withdrawal between the groups during enrollment and other follow-up periods. The outcome was defined as non-lame (locomotion score <3) or lame (locomotion score 3 and above) at each observation point after treatment. Final comparisons between treatments were based on the primary outcomes recorded at 28-days post-treatment (recovery rate, pain sensitivity, and lesion severity).

The proportions of recovered cows were compared between the treatments using cross-tabulation. A non-parametric test, the Kruskal Wallis test, was utilized to compare the pain sensitivity and lesion severity between the treatments since the data were not normally distributed. Logistic regression models were built to test for the association between covariates and successful treatment at 28 days post-treatment. The variables considered were the location of treated limbs (left and right hind limb), breed, BCS during treatment [3 and 3.5], DIM during enrollment, lesion type; sole hemorrhage/ulcer and white line disease, lesion severity, re-treatment at any observation point [7 and 14 days after treatment; Yes or no], and treatments. A two-stage model building process: univariable and multivariable model. At the univariable level, $P < 0.1$ was considered for factors to be introduced into

TABLE 3 Number of cows allocated to each treatment based on claw lesion diagnosis and overall cows that completed the trial.

	Claw lesion diagnosis				
Groups	Overgrown hoof	Sole hemorrhage	Sole ulcer	White line disease	At 28-d post-enrollment
LTNB	3 (16)	2 (16)	14 (62.5)	5 (20.8)	20
LTN	4 (21.7)	2 (13.0)	13 (60.8)	4 (17.4)	21
LTB	2 (18.7)	2 (18.7)	10 (62.5)	3 (18.7)	14
LT	2 (16.7)	3 (16.7)	11 (61.1)	4 (22.2)	15
Non-LT	5 (33.3)	5 (33.3)	1 (6.7)	4 (26.7)	15
Total (%)	16 (15.8)	14 (13.8)	51 (50.5)	20 (19.8)	85

Some lame cows had both overgrown hooves and CHDL. All cows were enrolled for having a single claw horn lesion, either sole hemorrhage, sole ulcer, or white line disease. Overgrown hooves were not considered claw horn lesions.

TABLE 4 Descriptive statistics of primiparous cows enrolled in the five experimental groups.

Variables	LTNB	LTN	LTB	LT	Non-LT	Overall
No. of animals	20	21	14	15	15	85
Median locomotion score	3	3	3	3	1	3
Lesion severity (Mean ± SD)	3.2 ± 0.5	3.4 ± 0.6	2.9 ± 0.9	3.3 ± 0.8	2.1 ± 0.7	3.2 ± 0.7
DIM at enrolment (Mean ± SD)[a]	53.1 ± 8.89	50.0 ± 7.71	55.7 ± 15.7	83.5 ± 22.4	114 ± 15.6	68.9 ± 6.5
BCS at enrolment (Median [IQR])	3 (0)	3 (0)	3 (0)	3 (0)	3.5 (0)	3(0)
Last recorded milk yield/day (kg; Mean ± SD)	17.4 ± 0.5	18.2 ± 0.5	17.5 ± 0.6	12.9 ± 0.6	12.6 ± 0.6	15.7 ± 0.3
Restraint duration (min; Mean ± SD)	7.6 ± 3.2	6.0 ± 1.3	7.5 ± 3.1	7.3 ± 2.4	7.3 ± 2.6	7.1 ± 2.6
Treatment duration (min; Mean ± SD)[a]	12.9 ± 2.1	12.4 ± 3.2	11.7 ± 2.4	15.3 ± 2.7	8.4 ± 0.7	12.2 ± 3.4
Treated limb/location of the lesion						
Rear left (%)	8	10	6	5	6	35 (41.2)
Rear right (%)	10	8	5	8	9	40 (47.1)
Front left (%)	1	0	1	0	0	2 (2.3)
Front right (%)	1	3	2	2	0	8 (9.4)
Vocalization during treatment (%)	3	5	4	4	2	18 (21.1)
Agitation during treatment (%)	17	12	10	12	8	69 (81.1)
Limb withdrawal during treatment[a] (%)	18	19	12	15	1	66 (77.6)

[a]Difference between experimental groups was significant.

TABLE 5 Number of recovered cows at various follow-up periods in each treatment and the final recovery rate at 28 days after treatment.

	Follow-up period and number of recovered cows					
Groups	Day 7	Day 14	Day 21	Day 28	Recovered (%)	Still lame (%)
LTNB[ab]	2	6	6	1	15 (75.0)	5 (25.0)
LTN [ac]	1	8	3	1	13 (61.9)	8 (38.1)
LTB[c]	0	0	3	3	6 (42.9)	8 (57.1)
LT[c]	1	2	2	1	6 (40.0)	9 (60.0)
Total (%)	4 (5.7)	16 (22.9)	14 (20.0)	6 (8.6)	40 (51.2)	30 (42.8)

Cows with successive non-lame scores after treatment were considered to have recovered and such cows were not re-introduced to the study if they became lame afterwards. Groups with different superscript letters are significantly different.

the multivariable model. A forward conditional method was applied in the final model and $P < 0.05$ was considered for significant differences. Treatment was forced into the models as categorical fixed effects and model fit was evaluated based on the change in the Akaike information criterion upon removing a covariate.

Results

Descriptive results and univariate analysis

Table 4 presents the characteristics of the enrolled cows. Overall, the median (IQR) LS was 3 (0) while the mean (± SD) DIM and daily milk yield during enrollment were 68.9 (± 6.5) and 12.9 (± 2.4), respectively. The mean (± SD) time taken to restrain and treat the cows was 7.1 (± 2.6) and 12.2 (± 3.4) min, respectively. Most of the cows (88.3%) were treated for claw lesions on the rear foot. There was no significant difference in BCS, lesion severity and lameness scores at treatment, treated foot, and the mean milk yield between all the treatments. All cows were enrolled at early to mid-lactation (within 220 DIM), but the mean DIM for LT and non-LT were significantly higher ($P < 0.05$) compared to LTNB, LTN, and LTB. The time taken to restrain cows was not significantly different between the treatments; however, LT and non-LT recorded the highest and lowest mean treatment time ($P < 0.05$), respectively compared to LTNB, LTN, and LTB. Vocalization was absent in a higher proportion of cows during treatment (78.8%; 67/85), whereas 68.4% (59/85), and 76.6% (65/85) showed agitation and limb withdrawal, respectively. The proportion of cows with vocalization and agitation was not different between treatments, but Non-LT recorded a significantly lower number of cows ($P < 0.05$) with limb withdrawal compared to other treatments.

Recovery rate

Table 5 presents the locomotion scores of the enrolled cows on day 28 after treatment. The number (%) of recovered cows was 15 of 20 (75%) for LTNB, 13 of 21 (61.9%) for LTN, 6 of 14 (42.9%) for LTB, and 6 of 15 (40%) for LT at 28-days after treatment. The highest proportion of recovery rates was recorded at 14 (40.0%; 16/40) and 21 d (35.0%; 14/40) after treatment, with the majority in LTN (50%; 8/16) and LTNB (42.8%; 6/14) at 14 and 21-days post-treatment, respectively. LTNB had higher odds of successful treatment at 28 d after treatment (OR = 4.5; 95% 1.1–19.1) compared to LT, but no significant difference was detected between the latter and other groups.

Pain sensitivity and lesion severity

Pain sensitivity based on limb withdrawal reflex was absent in a significantly higher number of cows (15/20; 75.0%) in LTNB compared to LTB (35.7%) and LT (40.0%) on day 28 after treatment (Table 6). Meanwhile, no significant difference was detected in the number of cows with limb withdrawal reflexes between LTN, LTB, and non-LT. LTN had the highest lesion severity score, followed by LT, LTNB, and LTB. Statistical difference was only detected between LTN and LTB. We observed no correlation between limb withdrawal reflex and lesion severity score. Specifically, the lowest lesion severity score was recorded in the group (i.e., LTB) with the highest percentage of animals with limb withdrawal present.

TABLE 6 Lesion severity score (Mean ± SD) and number of cows with and without limb withdrawal reflex.

Groups	Limb withdrawal reflex on day 28		Lesion severity score at day 28 (Mean ± SD)
	Absent (%)	Present (%)	
LTNB	15 (75.0)[a]	5 (25.0%)	2.15 ± 0.48[ab]
LTN	11 (52.4)[ab]	10 (47.6%)	2.38 ± 0.49[a]
LTB	5 (35.7)[b]	9 (64.3)	1.86 ± 0.66[b]
LT	6 (40.0)[b]	9 (60.0)	2.20 ± 0.67[ab]
Total (%)	37 (52.9)	33 (47.1)	2.17 ± 0.58

The limb withdrawal reflex on day 28 after treatment was compared between the groups using cross-tabulation and Chi-square test. Non-LT was not included since claw horn lesions were not present in all the cows during enrollment. Groups with different superscripts are significantly different at p-value = 0.05. Comparisons are along the column for each variable.

Logistic regression for factors associated with recovery rate

Table 6 shows the results of the multivariable model. Cows with limb withdrawal at d 28 post-treatment were less likely to recover relative to those without limb withdrawal. Treated limb (rear left or rear right limb) and restrain time during enrollment were not associated with recovery rates. However, there was a trend for lower odds of recovery among cows with vocalization at d 28 after treatment compared to those without vocalization (Table 7).

Discussion

Depending on the treatment administered, the recovery rates in moderately lame primiparous cows with good BCS treated for CHDL on a single foot were recorded in the present study. Cows that received therapeutic trim, NSAID, and hoof block (LTNB) recorded a significantly higher recovery rate than cows treated with only therapeutic trim (LT), which is consistent with previous findings (15, 16). The treatment protocol received by LTNB has been advocated for the treatment of CHDL as it corresponded to a shorter time for the restoration of normal gait in lame cows (15, 27). Wilson et al. (27) in a recent RCT also revealed that cows treated as in LTNB were less likely to become lame or severely lame (OR = 0.66 and 0.28) compared to those that received therapeutic trim and hoof block only when deemed necessary. However, non-lame cows were recruited in the latter

TABLE 7 Final logistic regression model showing the odds ratios of lameness recovery between the treatment groups and other associated factors at day 28 after treatment.

	B	S.E.	Wald	*P*-value	OR	95% CI
Groups			5.51	0.04		
LTNB	1.50	0.73	4.15	0.02	4.50	1.09–19.1
LTN	0.89	0.69	1.65	0.19	2.43	0.62–9.47
LTB	0.11	0.75	0.02	0.87	1.12	0.25–4.93
LT					Ref	
Treated foot						
Rear right limb	-0.95	0.63	2.24	0.13	0.38	0.11–1.34
Rear left limb					Ref	
Limb withdrawal						
Present	-2.78	0.70	15.67	0.001	0.06	0.01–0.24
Absent						
Restrain duration	0.14	0.12	1.22	0.26	1.15	0.89–1.47
Vocalization						
Present	-1.31	0.78	2.79	0.09	0.26	0.05–1.25
Absent						

CI, confidence interval, OR, odds ratio, SE, standard error, Ref, reference group.

study and observed until the first lameness event either before or after calving.

The primary outcome measures in this study were lameness score, nociceptive response (pain sensitivity), and lesion severity score on day 28 after treatment. Apart from these events, the other mechanisms underlying the causal effect of the standard treatment protocol (therapeutic trim, hoof block, and three-day course of NSAID) could not be elucidated in this study. There are several mechanisms through which the positive effects could occur. The underlying events in the pathogenesis of CHDL are the compression of the sole corium and subsequent vascular dysfunction leading to ischemia and hemorrhage (19, 27). The blocking of the healthy claw reduces the load-bearing and compression of the corium in the affected claw, thereby promoting injury healing. Likewise, the administration of NSAIDs might elicit a direct effect on the corium by reducing systemic and local inflammation in the hoof capsule and promoting healing following reduced loading due to block application. These events might explain the higher cure rates in LTNB compared to LT which received only therapeutic trim.

The highest proportion of recovered cows was recorded at 14 (40.0%; 16/40) and 21 35.0%; 14/40) days after treatment, especially in LTN (50%; 8/16) and LTNB (42.8%; 6/14). The inflammation associated with CHDL has been found to stimulate exostosis development (20) and digital cushion adipose metabolism (19, 27), especially a few weeks after the onset of the primary lesion. The implementation of early detection and treatment in the present study might have prevented the aforementioned lesion progression while contributing to high cure rates as demonstrated in prior research (18, 28, 29). Overall, the recovery rates in this study ranged from 45 to 70% which is higher than the reports by Thomas et al. (15). Differences in lameness definition, lesion severity, and observation period may contribute to the disparity. The high cure rates in our study could be linked to the relatively smaller dataset and less diversity in farm management compared to those of Thomas et al. (15). The fact that all the enrolled cows were in first parity, moderately lame, and experiencing their first lameness events may also contribute to the higher cure rates in our study.

Higher proportions of recovered cows were observed in LTNB compared to LTN and LTB but the difference was not statistically significant. LTNB and LTN were treated with a combination of therapeutic trim and blocking the healthy claw, while the latter group was not administered NSAID. Likewise, the mall number of cows in each group may explain this finding. The result reflects that blocking the healthy claw might play a more critical role in facilitating a faster healing process and improving the locomotion score. Furthermore, the finding aligns with that of Thomas et al. (15), where marginal differences in cure rates occurred between cows treated with NSAID without block and vice versa. Given that blocking the healthy claw is vital in reducing the compression of the corium and pressure on the affected claw, the non-significant difference between LTN and LTB highlights the multifaceted events leading to pain and gait disturbance in moderately lame cows affected with CHDL.

Most of the enrolled cows were moderately lame with less severe claw lesions. Hence, a significant difference might not be reflected in the gait scores when either the hoof block or

NSAID is missing from any of the treatments employed in this study. On the other hand, severe lesions may respond differently to treatment involving block and NSAID as both stages of corium compression and end-stage inflammation are present. For instance, block application in lame cows led to improved gait properties but the difference in weight distribution across the limbs was smaller in cows with more severe lameness than in mildly lame cows (30). Further investigations comparing treatment protocols in lame cows affected with claw lesions of varying severity would assist in elucidating the underlying events.

Notably, a few cows in LTNB, LTN, LTB, and LT developed lameness on the contralateral hind limb on day 28 after treatment. Two cows from Non-LT also developed lameness on the trimmed foot and contralateral limb during the study period. Block application may provoke discomfort and behavioral alterations that may initiate redistribution of weight-bearing between the claws (30), thereby resulting in a lower healing rate of the affected claw (15). However, concurrent development of claw lesions might occur in both hind limbs and subsequent onset of lameness at different periods. Further work is required to elucidate these findings, especially the varying loadings on the contralateral hind limb following blocking on the healthy claw adjacent to the diseased counterpart.

Limb withdrawal reflex was also assessed in this study as an indicator of lameness recovery. A significantly higher number of cows in LTNB did not exhibit this behavior on day 28 after treatment compared to LTB and LT. Further analysis also revealed a correlation between limb withdrawal and locomotion scores at the end of the trial. Limb withdrawal employed in this study is similar to the assessment of pressure or mechanical nociceptive response (31–34) and leg movements (17) in previous related research involving CHDL. The result highlights that limb withdrawal can be used for the detection of CHDL, degree of lameness, pain assessment, and monitoring recovery after treatment. A similar result was reported by Passos et al. (33) in which positive associations were found between the nociceptive response and locomotion of cows affected with sole ulcers and white-line disease. Likewise, a prior study documented that severely lame cows exhibited significantly higher frequencies of limb withdrawal upon attempting to rotate or compress the affected claw with a hoof tester (35). The present findings add to the existing body of knowledge that such behavior could be employed to monitor the recovery of lame cows, especially those suffering from acute CHDL.

In terms of lesion severity score, animals in LTB that were treated with therapeutic trim and hoof block without NSAID recorded the lowest lesion severity score, which was significantly different relative to LTN. Although this result may support the earlier discussion that blocking has positive effects on the corium and promotes injury healing, the insignificant finding compared to LT contradicts such an event. Overall, this finding reflects that lesion severity score may not be a good indicator of lesion progression, particularly for moderately lame cows suffering from CHL, which is consistent with reports from previous studies (17, 35). Another important result is the lack of correlation between the presence of limb withdrawal and lesion severity scores 28 days after treatment. Notably, LTB recorded the lowest lesion severity score and the highest percentage of cows with limb withdrawal present. Reports from previous studies distinguishing between lameness and CHL may explain our finding. Groenevelt et al. (29) found that some cows with severe lesions such as sole ulcers and toe necrosis were only moderately lame. In another study, a positive correlation was observed between severe lesions and greater perturbed locomotion, but some cows with normal gait appeared to have severe lesions (35, 36). Thus, the visual appearance of CHL either during hoof examination (i.e., during diagnosis) or after treatment may not depict the actual pain responsible for gait changes in lame cows. Meanwhile, the limb withdrawal reflex correlates with changes in locomotion scores and a pointer of lameness recovery after treatment as observed in this study. Since lameness is indicated by abnormal locomotion, particularly derangements in gait symmetry, limb withdrawal is more likely to reflect such changes rather than those observed visually on the claw.

The difference in lameness recovery rates between LTNB and LT and the association with limb withdrawal was further confirmed in the logistic regression model. Specifically, LTNB had four times higher odds of successful treatment compared to LT, which is consistent with previous studies as discussed earlier (15, 30). There was also a tendency for lower recovery rates in cows that exhibited vocalization during the last observation period. Vocalization has not been widely studied in lame dairy cows. A recent study reported that both non-lame and acutely lame cows affected with CHDL expressed vocalization during restraining (17). This result requires further investigation, as it may be a sign of hyperalgesia in chronically lame cows or hyperactivity due to discomfort during restraint and lifting the foot for examination.

Our findings add to the existing literature on the treatment of CHDL in dairy cows. The randomization of the recruited cows and the primary outcomes considered also signify the strengths of this study. The presence of a non-lame group (Non-LT) allowed for the comparison with other treatments during the follow-up period. This study was designed using an RCT performed per best practice standards (REFLECT guidelines), indicating that outcomes were unlikely to be influenced by bias. RCT provide strong evidence of a causal effect (37) and the intervention had no negative effects on health and welfare parameters in the various treatments.

However, certain limitations in this RCT could be considered in future studies. For instance, only primiparous cows with good BCS and affected with CHDL were enrolled, hence the findings might not be generalisable to cows with dissimilar characteristics. Conducting this experiment on a single farm and the small number of cows in each treatment also reflects a weakness in this study. These limitations might have affected the power and the chances of detecting a significant difference in the primary outcomes. This study was time-consuming, and expensive and was conducted for 7 months, which denotes why only a few RCTs on the management of lame cows have been published. We did not consider the influence of season on the primary outcomes, since all the cows were enrolled between June and December 2020, which is regarded as the dry season in Malaysia.

Conclusion

In conclusion, our findings revealed that treatment with therapeutic trim, blocking, and NSAID led to better recovery and reduced pain sensitivity in moderately lame primiparous cows with good BCS compared to those that received only therapeutic trim. These positive effects might not be reflected in the subjective lesion severity assessment. Hence, there are welfare benefits when lame cows are promptly detected and treated using a combination of therapeutic trim, hoof block, and pain management. Further research on the changes within the hoof capsule following various treatment protocols is needed to elucidate the underlying mechanisms in the clinical benefits observed in this study.

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 reviewed and approved by the Institution of Animal Care and Use Committee, Universiti Putra Malaysia (Ref.UPM/IACUC/AUP-R010/2019). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

SR, WS, and MS contributed to the conception of the research and funding. SR and MS made substantial contributions to data acquisition, analysis, interpretation, drafted and revised the work, and wrote the final version. SS-H and RM made substantial contributions to revising the drafted manuscript. All authors approved the final version of the article and agreed to be accountable for all aspects of the work.

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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

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Use of qualitative behavioural assessment to investigate affective states of housed dairy cows under different environmental conditions

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In addition to the reduction of suboptimal welfare, there is now a need to provide farmed animals with positive opportunities to provide confidence that they have experienced a life worth living. Diversification of the environment through environmental enrichment strategies is one suggested avenue for providing animals with opportunities for positive experiences. The provision of more stimulating environmental conditions has been widely implemented in other animal production industries, based on evidenced welfare benefits. However, the implementation of enrichment on dairy farms is limited. In addition to this, the relationship between enrichment and dairy cows' affective states is an under-researched area. One specific welfare benefit of enrichment strategies which has been observed in a number of species, is increased affective wellbeing. This study investigated whether the provision of different forms of environmental enrichment resources would impact the affective states of housed dairy cows. This was measured by Qualitative Behavioural Assessment, currently a promising positive welfare indicator. Two groups of cows experienced three treatment periods; (i) access to an indoor novel object, (ii) access to an outdoor concrete yard and (iii) simultaneous access to both resources. Principal component analysis was used to analyse qualitative behavioural assessment scores, which yielded two principal components. The first principal component was most positively associated with the terms "content/relaxed/positively occupied" and had the most negative associations with the terms 'fearful/bored'. A second principal component was most positively associated with the terms "lively/inquisitive/playful" and was most negatively associated with the terms "apathetic/bored". Treatment period had a significant effect on both principal components, with cows being assessed as more content, relaxed and positively occupied and less fearful and bored, during periods of access to additional environmental resources. Similarly, cows were scored as livelier, more inquisitive and less bored and apathetic, during treatment periods compared to standard housing conditions. Concurrent with research in other species, these results suggest that the provision of additional environmental resources facilitates positive experiences and therefore enhanced affective states for housed dairy cows.

KEYWORDS

qualitative behavioural assessment, cow, affect, positive welfare, enrichment

Introduction

Affective experiences are an inherent component of the overall welfare state of an animal (1, 2). Recently, as research into the emotional experiences of animals has developed, there has been a shift in the focus of animal welfare to advance from simply the reduction of suffering, which avoids poor welfare, to also provide animals with positive experiences (3, 4). There is currently demand for the development and implementation of positive welfare opportunities for farm animals, to ensure that they have experienced an acceptable quality of life (5). This stems from ongoing societal concern regarding the quality of lives of intensively housed livestock (6, 7), including dairy cows (8, 9). Evaluation of animals' affective states is an ongoing complex challenge, with the lack of a gold standard assessment (10, 11). Yet to be able to assess the success of interventions aimed at offering opportunities for positive welfare, evaluation of affective states is imperative.

One avenue that has been suggested to offer confined animals with opportunities for positive experiences is diversification of the environment (11, 12). This may provide opportunities beyond that of solely meeting basic needs, such as the facilitation of exploration, agency or a greater repertoire of behaviours (11, 12). Therefore, enrichment interventions are often implemented with the strategic goal of enhancing animals' affective states. The relationship between animals' living conditions and their affective experiences have started to be explored. Indications of more positive affective states were found following either a period of environmental enrichment or in animals housed in more stimulus diverse compared to basic housing conditions in different species including, dairy cows (13, 14), dairy calves (15, 16), chickens (17, 18), pigs (19, 20) and rats (21, 22). The inverse effect has also been observed in starlings, through the use of judgement bias, which monitors animals' responses to ambiguous situations to infer affective valence (23). Starlings expressed a pessimistic bias indicative of poorer affective states, following removal of enriched conditions (24). Similarly, increased negative behavioural decision-making has been observed in pigs that had previously spent time in enriched housing and were then transferred to barren housing, compared to pigs that had only ever experienced barren housing (19). Crump et al. (25) investigated whether pasture access improved emotional states in dairy cows and reported that cows which had access to pasture approached a known food reward slower than cows that were fully housed. The authors proposed that the explanation was a reduced reward anticipation, generally shown when higher or more frequent rewards are experienced in day-to-day life (26), concluding that pasture access may facilitate more rewarding lives and therefore better welfare. Environmental enrichment has been widely implemented in several other industries (27, 28) based on its contribution to welfare. However, its implementation on dairy farms is limited and the development of enrichment methods specifically for housed cows is required (29).

One method that has emerged for assessment of affective states of animals is Qualitative Behavioural Assessment (QBA). The method uses observer evaluation and interpretation of animals' behavioural expressive demeanour and the nature of their interaction with the environment (30, 31). The qualitative assessment aims to describe the animals' experience within its setting, by evaluating not solely what an animal is doing but by how it behaves, which encapsulates its subjective affective states (31). This information is collated through descriptive terms, which are then used to formulate quantitative variables. QBA is regarded as assessing more than physical body language – it is assumed to assess a psychological dimension of behavioural expressivity allowing judgement to be made of the quality of an animal's experience (31). QBA has been described as one of the most promising positive welfare indicators currently available, based on the breadth of evidence regarding its validity and reliability (10, 32, 33). Alongside this, QBA is very feasible as it requires little time or resources (34, 35), in contrast to other behavioural or physiological positive welfare indicators. This is particularly practical for the assessment of farm animal welfare. QBA is currently the only measure of positive affective state to be practically incorporated into on farm animal welfare assessments in the UK and is currently being used by two independent welfare assurance bodies (36, 37). QBA results have been shown to be concurrent with some physical health indicators in different species (38–40) and other behavioural tests linked to affect (39, 41). This is however not always the case, with other studies finding no correlations between QBA results and physical health indicators (42–44) or wider farm assurance assessment protocols (45). The technique has identified biologically plausible differences in behavioural expression and associated affective states in dairy cows infected with mastitis (38), in both positive and negative social situations (46) and between cows from tethered and loose housing systems (47). The technique has previously been used to directly evaluate the affective states of animals in different housing conditions, with results conducive to enhanced emotional wellbeing, in extensive compared to intensive systems in pigs (48), enriched compared to unenriched housing in pigs (20) and dairy goats with access to pasture compared to without (49).

The purpose of this research was to explore the relationship between housing conditions and affective states of dairy cattle. Our specific aim was to evaluate whether QBA could be used to detect changes in cows' behavioural expression during periods of altered housing conditions, comprising of access to an outdoor exercise area and provision of an indoor novel object.

Materials and methods

Ethical approval

The study was granted ethical approval by The University of Nottingham, School of Veterinary Medicine and Science Ethical Review Committee, approval number 2697-190221. All methods were performed in accordance with the relevant guidelines and regulations.

Animals and housing

The study was conducted at the Center for Dairy Science Innovation, University of Nottingham, a continually housed 300-cow research dairy herd, producing milk commercially.

Cows in the experimental groups were continually housed in two identical 774.9 m^2 buildings, containing 51 sand-bedded cubicles with concrete slatted flooring, scraped automatically daily (Figure 1). Subjects received ad libitum access to fresh water *via* three water troughs and were fed a total mixed ration (TMR) ad libitum which was replenished daily at 09:00. Subjects were milked robotically *via* a Lely automatic milking system where they received additional concentrate feed. One automatic brush was available in each building.

We selected 96 cows and assigned them to two separate study groups to repeat one experimental trial. Cows were randomly selected and matched by parity and stage of lactation to create two virtually identical groups. Cows were also selected subject to their drying off date being later than the end of the study period, to avoid removal of the cows from the study group at dry off. During the 19-week study, twenty-one cows were removed from the study groups for veterinary intervention or due to being regrouped unexpectedly for drying off (Group 1: 9 cows, Group 2: 12 cows). Any cows that were removed from the study groups, remained absent for the remainder of the trial and were immediately replaced with an alternative cow (matched by parity and days in lactation), to maintain group size. Seventy-five of the originally selected cows remained present for the entirety of the trial (Group 1: 39 cows, Group 2: 36).

Group 1 consisted of 48 Holstein cows averaging (mean ± SD) 107.15 ± 57.42 days in milk (median: 106.50, IQR: 101.5, range: 25.00 – 232.00), producing on average 39.13 ± 10.78 L of milk/day, of parity 2.19 ± 1.21. The proportion of parity groups were parity 1: 0.38, parity 2: 0.25, parity 3: 0.25, parity 4+: 0.125. Group 2 consisted of 48 Holstein cows averaging 106.83 ± 56.79 days in milk (median: 102, IQR: 106.5, range: 26–215), producing on average 40.00 ± 10.67 L of milk/day, parity 2.19 ± 1.21. The proportion of parity groups were parity 1: 0.38, parity 2: 0.25, parity 3: 0.25, parity 4+: 0.13. Groups were moved into the study housing 1 week before the start of the trial for acclimatisation. The two groups of cows were managed simultaneously in adjacent pens within one building (Figure 1). Cows had been reared on the farm and all buildings on the farm housing adult cows had the same design as that of the experimental buildings used within the trial. Cows were managed in line with commercial care and management procedures at The University of Nottingham Center for Dairy Science Innovation.

Treatment and experimental setup

The intervention within this trial consisted of two different housing modifications to the standard living conditions of the cows. The first housing modification was the provision of a hanging novel object (inflated sailing buoy), suspended within an area of loafing space, situated at one end of the building (Figure 2). The specific novel object used was chosen because it had been deemed safe and practical in a preliminary study.

The second resource was access to an outdoor yard with a concrete floor (Figure 2). Both groups were provided with an identical outdoor yard. The yard boundaries were constructed from 5 mobile steel gates which were secured in place by interlocking chains between gates and drop bolts. The initial gate was fixed to the building wall whilst the other gate was secured to the access gate to the housing. The outdoor yards measured ∼55 m^2. The outdoor yards for Group 1 and Group 2 were situated opposite

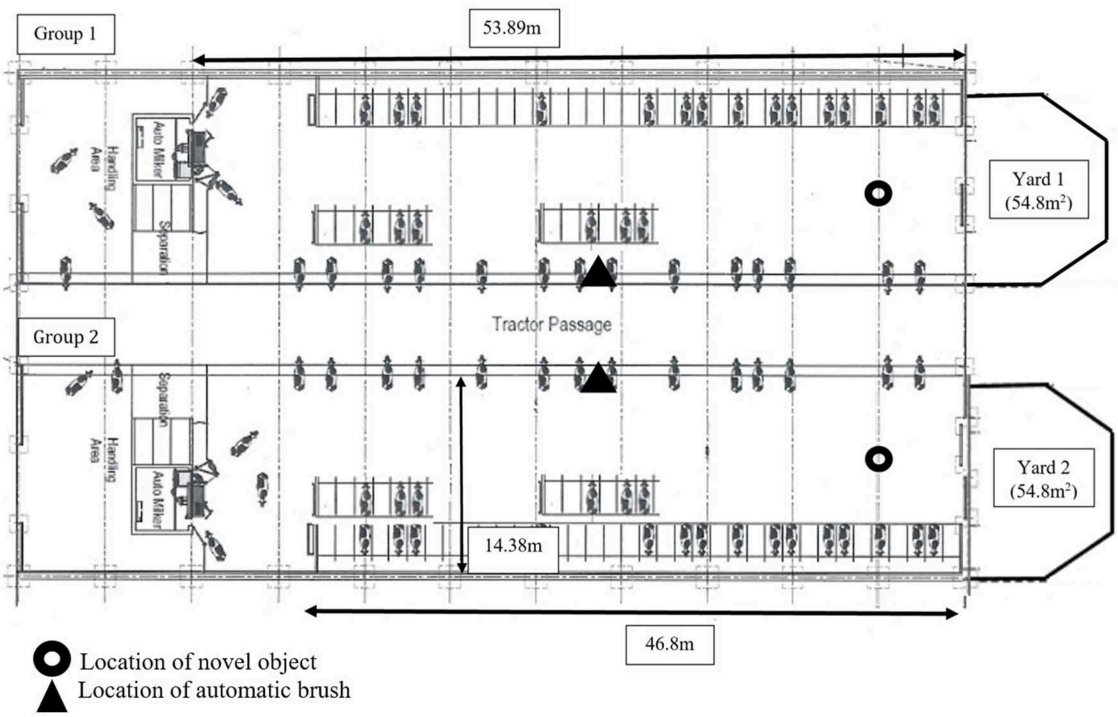

FIGURE 1
Schematic view of the experimental housing for both groups of cows within the study.

FIGURE 2
Images of the environmental resources provided as the intervention within the trial. **(A)** Displays the outdoor yard provided to Group 1. **(B)** Displays the indoor hanging novel object. Identical resources were provided to both groups of cows.

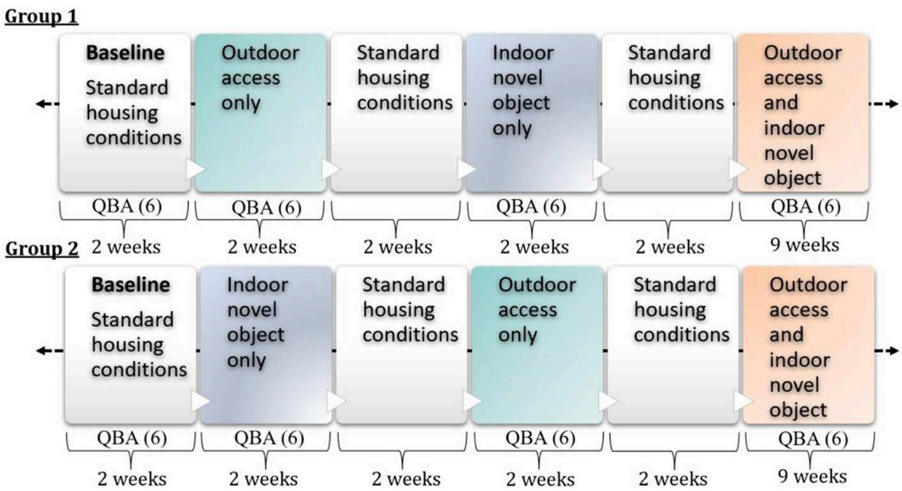

FIGURE 3
Timeline of the six consecutive treatment periods of the trial, displaying the length of time of each housing modification. Housing alterations were made on Mondays. The number of Qualitative Behavioural Assessments (QBA) that were conducted is displayed under each treatment period.

one another. Due to the close proximity, both yards provided almost identical outdoor views of the slurry collection area, an area of grassland used for storage and other farm buildings. A small covering of sand and grit was applied to the ground in icy weather conditions. Access to the outdoor yard was provided *via* an entry gate at the far end of the housing shed. All food, water and bedding areas were provided inside the building. During treatment periods when either one or both resources were made available, resources remained freely available 24 h a day for the entirety of that treatment period.

The trial ran for 19 weeks in total between the dates 22.11.2021 – 03.04.2022. The study timeline is illustrated in Figure 3. Groups were housed in standard housing conditions for 2 weeks to allow baseline observations to be taken. Standard housing conditions were as displayed in Figure 1, not including the outdoor yard or indoor novel object. Following this baseline period, both groups were given continuous 24-h access to a different enrichment resource for a period of 2 weeks. Group 1 were given access to the outdoor concrete yard and Group 2 were given access to a novel object within the building. Both resources were then removed and cows remained in standard housing conditions for 2 weeks. Following this period of standard housing, the initial treatment period was repeated but the resources were reversed, with Group 1 having access to the indoor novel object and Group 2 having access to an identical outdoor concrete yard. At the end of this two-week period, access to resources were removed and cows were housed in standard conditions for a further 2 weeks. Both groups of cows were then given continuous 24-h access to both resources for a period of 9 weeks.

Quantification of enrichment use

Video footage was collected using 4 fixed Axis M1065 IP cameras. Use of the outdoor concrete yard and the novel object, were recorded throughout five separate, continuous 24-h periods. One 24-h period was sampled during the first treatment period, when Group 1 cows had access to the outdoor area and Group 2 cows had access to the indoor novel object. The corresponding 24-h period was sampled during the second treatment period when the resources had been switched, with Group 1 cows having access to the novel object and Group 2 cows having access to the outdoor concrete yard only. Three 24-h periods were sampled during the choice phase of the study. The first 24-h period was taken on the 05.01.2022 during the first week of the choice phase. The next two sampled 24-h periods were taken on 23.02.2022 (choice week 4) and 23.03.2022 (choice week 8). The 24-h periods were recorded from 00:00 to 24:00 and chosen to avoid veterinary or husbandry intervention with the cows.

Physical interaction with the novel object was classed as any physical contact of the object with any part of a cows body. Cow ID and length of interaction were recorded for every contact made with the object throughout all 24-h recording periods. Use of the outdoor yard commenced when a cow put one hoof over the entry line to the outdoor yard. A cows' time outside then ended the moment its entire body crossed over the entry line back into the building. Cow ID and time spent outside were recorded for every visit made outside throughout all 24-h recording periods.

Qualitative behavioural assessment

One trained assessor completed one QBA for both groups of cows, three times per week, during every week of the trial (excluding non-treatment weeks when cows were in standard housing conditions). One QBA refers to one assessment, consisting of scoring the 20 descriptors, as outlined in the Welfare Quality Network protocol for dairy cows (36). The 20 terms used for every QBA were: active, relaxed, fearful, agitated, calm, content, indifferent, frustrated, friendly, bored, playful, positively occupied, lively, inquisitive, irritable, uneasy, sociable, apathetic, happy, distressed. The Assessments were made at the group level, which involved observation of all cows within the group. One QBA assessment was completed for both groups of cows, on Mondays, Wednesdays and Fridays, between 12:30 and 13:30. These days were chosen to avoid days where any form of human disturbance occurred, such as routine vet or foot trimming visits. The timeslot used to perform the QBA assessments was chosen to also avoid any routine management interference with the cows, such as feeding and cleaning. These days and times were therefore assumed to give the best indication of the herds undisturbed behaviour in these living conditions. The QBA assessment protocol and scoring sheet used was taken from the Welfare Quality Network Assessment Protocols for dairy cows (36) and was conducted by a trained assessor. The assessor observed the herd for 20 minutes in total, observing the expressive quality of group activity. If the cows were disturbed by the assessor's presence the assessment would be started a few minutes later when cows had resumed normal activity. This occurred infrequently due to the distance of the viewing platform from the living area of the cows. The assessor then moved away from the herd and scored the 20 descriptive terms manually on a visual analogue scale (VAS) on a paper form. Explanation of the VAS scoring system is provided by the Welfare Quality Network protocols (36). In brief, each VAS is defined by its left "minimum" and right "maximum" point, where minimum means the expressive quality indicated by the term is entirely absent in any of the animals and maximum means the expressive quality is dominant across all observed animals. It is possible to give more than one term a maximum score; animals could for example be both entirely calm and content. A score was then given for each term, by drawing a line on the assessment sheet on the visual analogue scale, at the point which best represented the level of that descriptive attribute to the herd. Each line point was manually measured in mm from the minimum mark to the given assessment line, resulting in a score between 0 and 125. Terms with positive connotations became more positive as the score increased and terms with negative connotations became more negative as the score became higher. To aid understanding of the terms used in the QBA assessment for dairy cattle from the Welfare Quality Assessment Protocols (36), definitions for each descriptor were checked *via* the Cambridge Dictionary online (50). The QBA assessor spent 2 weeks conducting QBA assessments on cows housed in the experimental buildings as part of training.

Weather

The temperature (°C) throughout the trial was recorded using an "Imonnit" weather sensor (Monnit Corporation, Utah, US). The sensor took a temperature recording every 2 h, throughout 24-h, providing 12 data points per day. The sensor was secured to the outside of the building, within the outdoor yard for Group 2. Given the close proximity of the outdoor yards, this sensor was accepted to provide weather details for the overall outdoor area used by both groups of cows.

Statistical analysis

All statistical analyses were performed using packages readr (51), dplyr (52), tidyverse (53), stats (54) and FactoExtra (55) in RStudio version 4.1.2 (56). The raw QBA linear measurements were centred and standardised to create a normal distribution for further analysis. QBA data were analysed using a principal component analysis (PCA), a multivariate technique of particular value to assess data consisting of correlated quantitative dependent variables. The procedure leads to the production of "principal components"; new variables which summarise information from the correlated variables (57). Descriptive assessment of QBA was conducted graphically to facilitate visualisation of the important variables contributing to the key principal components. The first two principal components, explaining the highest percentage of the variance of the data and with eigen values >1.0, were used for additional inferential analysis in line with standard procedure (57). A conventional linear model was constructed to test the effect of treatment period on principal component scores. Explanatory variables were retained in the models when $P < 0.05$. QBA results were evaluated separately for Group 1 and Group 2 – as well as in combination. Quantity of QBA assessments was matched, with 6 QBA assessments per group per treatment period. To achieve 6 QBA assessments for the final treatment period which lasted 9 weeks rather than the 2 weeks of other periods, we performed 3 QBAs in the first 2 weeks of this period and another 3 in the last 2 weeks.

Quantification of enrichment use is reported as the mean (±SD) time per cow spent using enrichment resources per treatment period. Treatment period refers to the single continuous 24-h period of footage from which results were obtained. Results are reported as the mean ± standard deviation. The percentage of the group that used the resource refers to the percentage of cows that used it during a specific 24-h period.

Results

Quantification of enrichment use

When cows were provided access to the indoor novel object only, they spent 6.34 ± 4.62 (Group 1) and 10.13 ± 8.66 (Group 2) min per day interacting with it. The percentage of the group that used the novel object was 94.87% (Group 1) and 100% (Group 2). When cows were provided with access to the outdoor yard only, they spent 55.67 ± 32.11 (Group 1) and 102.26 ± 59.92 (Group 2) min per day outside. The percentage of the group that used the outdoor yard was 94.87% (Group 1) and 100% (Group 2).

During the early choice phase, when cows had simultaneous access to both resources, Group 1 cows spent 4.91 ± 5.41 min per day using the indoor novel object and 98.37 ± 57.57 min per day outside. The percentage of Group 1 using the indoor novel object during this period was 94.87% compared to 97.44% using the outdoor yard. During the early choice phase, Group 2 cows spent 9.6 ± 7.58 min per day using the indoor novel object and 114.38 ± 55.28 min per day using the outdoor yard. The percentage of Group 2 using the indoor novel object during this phase was 94.44% compared to 97.22% using the outdoor yard.

During the late choice phase, Group 1 cows spent 3.12 ± 3.27 min per day using the indoor novel object and 55.06 ± 31.32 min per day using the outdoor yard. The percentage of Group 1 using the resources during this phase was 87.18% (indoor novel object) and 94.87% (outdoor yard). During the late choice phase, Group 2 cows spent 2.3 ± 2.66 min per day using the indoor novel object and 91.46 ± 47.02 min per day using the outdoor yard. The percentage of the Group 2 using the resources during this phase was 86.11% (indoor novel object) and 97.22% (outdoor yard).

QBA results Group 1

PCA of the QBA scores for Group 1 cows identified 5 principal components with eigen values >1. However, the first two principal components explained the majority of the variance in the data and were therefore retained for analysis. The first principal component (PC1) accounted for 38.45% of the variance and displayed the most positive correlating adjectives of "content"/"relaxed", with the most negative correlating adjectives of "fearful/bored". The second component (PC2) explained 15.76% of the variance and comprised of the most positive correlating adjectives of "lively/playful" and the most negative correlating adjectives of "apathetic/bored". Table 1 displays the full list of adjectives for both components with associated correlation value. Figure 4 displays the relationship between all variables in PC1 and PC2.

Results of the mixed effect linear model for cows in Group 1 are presented in Table 2. Treatment period had a significant effect on PC1, with cows scoring higher values on this component during the choice period (when cows had access to both the outdoor yard and the novel object, both at the beginning and at the end of this phase) compared to the baseline weeks. Cows also scored significantly higher on this component when they solely had access to the indoor novel object, compared to the baseline week. Higher scores on PC1 reflected cows being assessed as more content, relaxed and positively occupied compared to fearful, bored and indifferent. The effect of treatment period on PC2 was non-significant. The difference in PC1 and PC2 between treatment period are presented graphically in Figure 5.

TABLE 1 Group 1: Principal components 1 and 2, displaying associated correlations between variables and principal components for each behavioural descriptor.

Descriptor	PC1	PC2
Active	−0.54	0.59
Relaxed	**0.87**	0.10
Fearful	**−0.88**	−0.02
Agitated	−0.56	0.41
Calm	0.52	−0.18
Content	**0.88**	0.26
Indifferent	−0.73	−0.36
Frustrated	−0.67	0.27
Friendly	0.23	−0.07
Bored	**−0.81**	**−0.39**
Playful	−0.16	**0.67**
Positively occupied	0.81	0.01
Lively	−0.37	**0.70**
Inquisitive	−0.39	0.51
Irritable	−0.32	0.46
Uneasy	−0.67	−0.04
Sociable	−0.26	0.52
Apathetic	−0.62	**−0.51**
Happy	0.77	0.43
Distressed	−0.47	−0.19

The bold values indicate the two most positive and two most negative correlating adjectives.

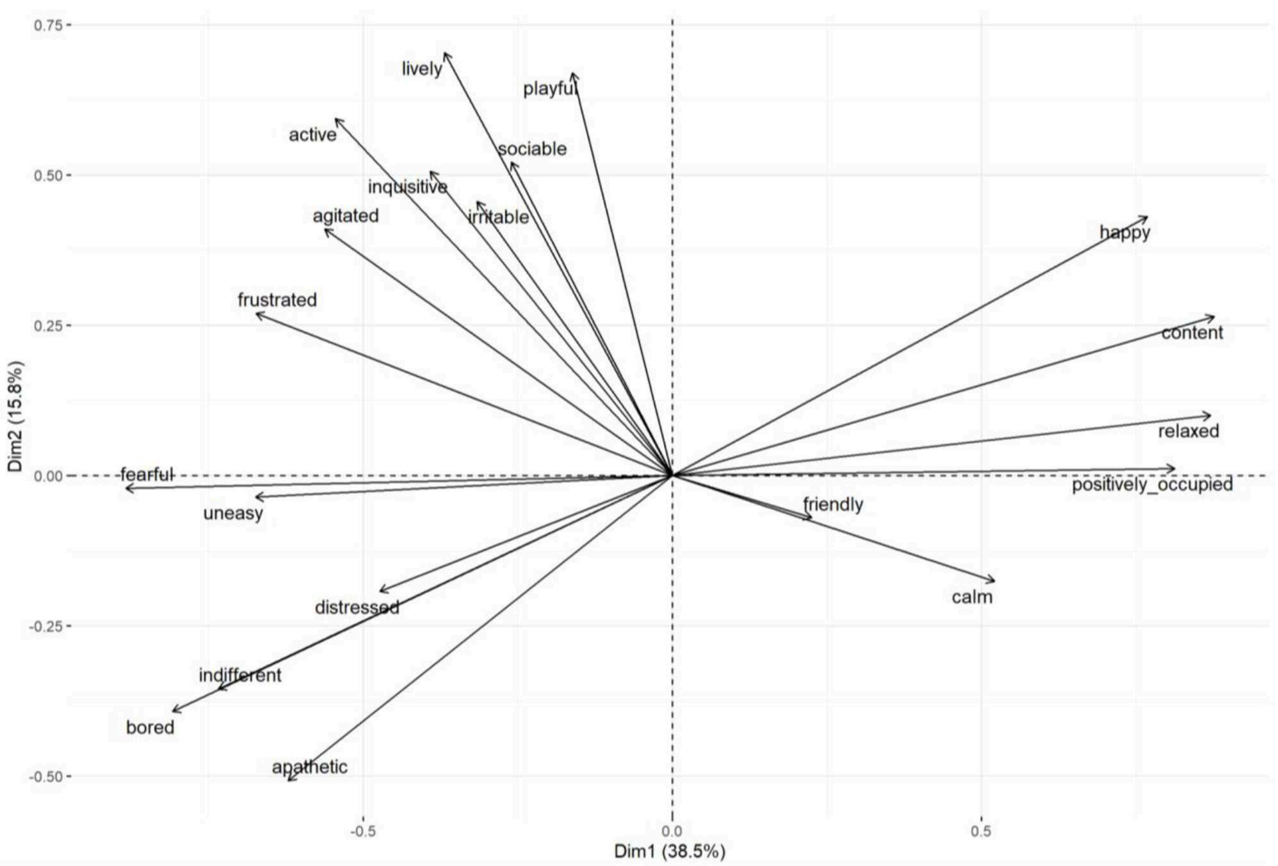

FIGURE 4
Group 1: Variable correlation plot displaying the relationship between all variables in PC1 (Dim1) and PC2 (Dim2) and each terms value figure in contribution to the principal component.

TABLE 2 Group 1: Results of the linear model assessing PC1 and PC2 scores attained during the different treatment periods.

Coefficients of the model	Estimate	Confidence interval (95%)	P-value
Reference: Baseline housing conditions Intercept	−3.04		
Choice phase (early)	4.94	2.7–7.11	<0.01
Choice phase (late)	6.00	3.82–8.17	<0.01
Indoor novel object	2.33	0.16–4.51	0.04
Outside	1.95	−0.22–4.13	0.07

Choice phase (both resources simultaneously available), indoor novel object only and outside (access to the outdoor yard only).

QBA results Group 2

PCA of the QBA scores for Group 2 cows identified 5 principal components with eigen values >1. The first two principal components explained the majority of the variance in the data and were therefore retained for analysis. The first principal component (PC1) accounted for 40.03% of the variance and displayed the most positive correlating adjectives of "bored/fearful", with the most negative correlating adjectives of "content/relaxed". The second component (PC2) explained 17.39% of the variance and comprised of the most positive correlating adjectives of "lively/inquisitive" and most negative correlating adjectives of "bored/apathetic". Table 3 displays the full list of adjectives for both components with associated correlation value. Figure 6 displays the relationship between all variables in PC1 and PC2.

Results of the mixed effect linear model for cows in Group 2 are presented in Table 4. Treatment period had a significant effect on PC1, with cows scoring lower values on this component during the late stage of the choice period (when cows had access to both the outdoor yard and the novel object) compared to the baseline weeks. Lower scores on PC1 reflected cows being assessed as more content, calm and relaxed, compared to bored and fearful. Treatment period also had an effect on PC2, with cows scoring higher values on this component during all treatment periods compared to baseline. Higher scores on PC2 reflected cows being assessed as more lively, inquisitive and active compared to bored, apathetic and indifferent. The difference in PC1 and PC2 between treatment periods are presented graphically in Figure 7.

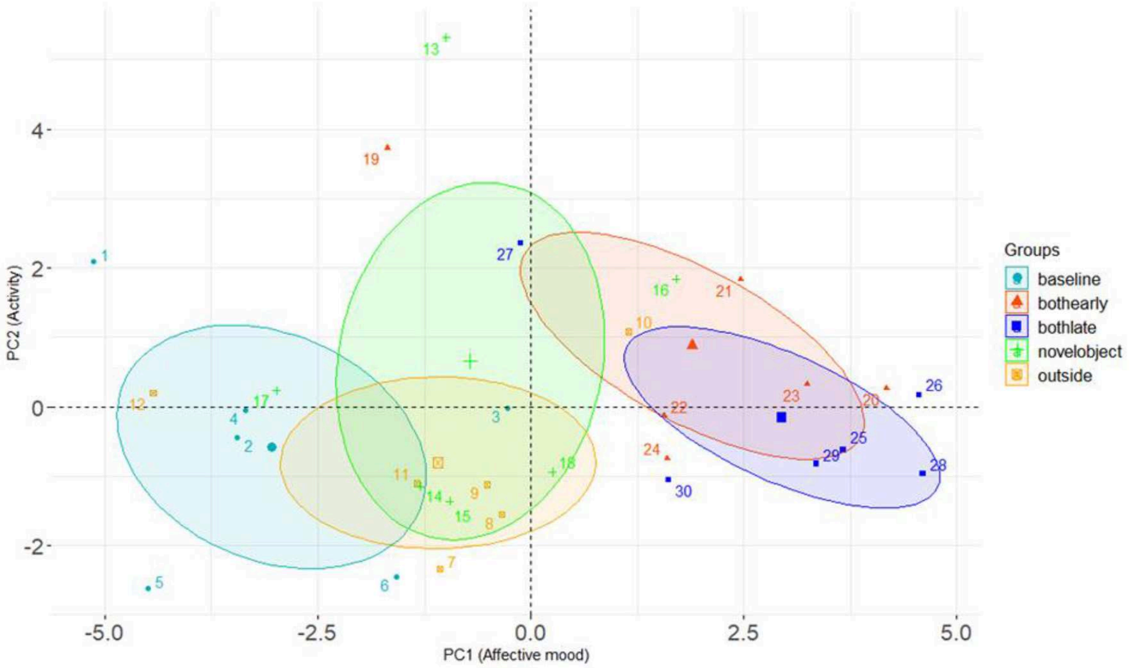

FIGURE 5
Group 1: Biplot displaying all QBA assessment scores in terms of PC1 and PC2. Each separate point displays one assessment date. Points are coded per treatment period [baseline, novel object, outside, both resources (early) and both (late)] as indicated on the plot. Group means are in bold and ellipses indicate the 95% confidence intervals of the group mean.

QBA results Group 1 and 2 combined

PCA of the QBA scores for Group 1 and 2 combined identified 5 principal components with eigen values >1. The first two principal components explained the majority of the variance in the data and were therefore retained for analysis. The first principal component (PC1) accounted for 37.96% of the variance and displayed the most positive correlating adjectives of "content/relaxed", with the most negative correlating adjectives of "fearful/bored". The second component (PC2) explained 15.67% of the variance and comprised of the most positive correlating adjectives of "lively/inquisitive" and most negative correlating adjectives of "apathetic/bored". Table 5 displays the full list of adjectives for both components with associated correlation value. Figure 8 displays the relationship between all variables in PC1 and PC2.

Results of the mixed effect linear model for the combined results for Group 1 and Group 2 cows are presented in Table 6. Treatment period had a significant effect on PC1, with cows scoring higher values on this component during the choice period (when cows had access to both the outdoor yard and the novel object, both at the beginning and at the end of this phase) compared to the baseline weeks. Cows also scored significantly higher on this component when they solely had access to the outdoor yard, compared to the baseline week. Higher scores on PC1 reflected cows being assessed as more content, relaxed and positively occupied compared to fearful and bored. Treatment period also had a significant effect on PC2, with cows scoring higher values across all treatment periods compared to baseline weeks. Higher scores on PC2 were indicative of cows being assessed as more lively and inquisitive compared to apathetic and bored. The difference in PC1 and PC2 between treatment period are presented graphically in Figure 9.

An overview of the study results are presented in Table 7.

Weather

The mean (±SD) air temperature (°C) throughout treatment periods were: 3.62 + 4.10 (baseline), 6.46 + 3.74 (Group 1 outdoor yard, Group 2 indoor novel object), 2.73 + 4.27 (Group 1 indoor novel object, Group 2 outdoor yard), 7.05 + 3.26 (early choice period) and 6.36 + 6.67 (late choice period).

Discussion

Summary

The current study is the first to utilise QBA to assess dairy cows' affective states in response to a potentially positive welfare intervention. The intervention consisted of manipulating the standard housing conditions of commercially housed dairy cows. Diversification of the environment through environmental enrichment, which offers opportunities for exploration, control and choice, has been suggested as one way to offer confined

TABLE 3 Group 2: Principal components 1 and 2, displaying associated correlations between variables and principal components for each behavioural descriptor.

Descriptor	PC1	PC2
Active	0.73	0.57
Relaxed	**−0.85**	0.22
Fearful	**0.77**	0.10
Agitated	0.60	0.40
Calm	−0.84	−0.02
Content	**−0.87**	0.25
Indifferent	0.65	−0.27
Frustrated	0.66	0.51
Friendly	−0.01	0.53
Bored	**0.79**	**−0.43**
Playful	0.51	0.49
Positively occupied	−0.82	0.31
Lively	0.47	**0.70**
Inquisitive	0.11	**0.69**
Irritable	0.23	0.32
Uneasy	0.74	0.03
Sociable	−0.01	0.55
Apathetic	0.71	**−0.32**
Happy	−0.69	0.46
Distressed	0.33	−0.25

The bold values indicate the two most positive and two most negative correlating adjectives.

animals positive experiences (12). In line with this theory, the hypothesis of the study was that offering additional environmental resources would have a positive impact on cows' affective states. The results support this hypothesis, with more positive affective states being indicated during intervention periods, when cows had access to additional environmental resources.

PC1 (affective mood) and treatment period

Group 1 cows scored significantly higher on PC1 during both stages of the choice period, when cows had access to both resources and also when they just had access to the indoor novel object compared to standard housing conditions. Similar results were shown for Group 2 cows, however only during the late choice phase compared to baseline conditions. Combined results from both groups showed that cows scored higher on PC1 during both stages of the choice phase and when cows had access to the outdoor yard compared to standard housing conditions. The most positively correlated adjectives on PC1 were relaxed, content and positively occupied, all with positive emotional connotations. The most negatively correlating terms were fearful and bored, therefore this principal component could be representative of general affective mood, on a scale from negative (lower scores) to positive (higher scores).

These results suggest that when cows had access to additional environmental resources, they were more relaxed, content and positively occupied than when in standard housing conditions. Although the behavioural descriptors were not analysed in isolation, reference should be made to why cows may have scored higher on these terms during treatment periods. Positively occupied is arguably the least complicated term to interpret. It seems plausible that increasing the behavioural activities available within the cows' environment would increase the amount of time they spent positively occupied. This has previously been observed in calves, where the provision of four different types of enrichment simultaneously resulted in calves spending more time interacting with enrichment compared to when only one single item was provided (58). Similarly in pigs, the simple provision of four instead of two wooden beams, increases both the frequency and duration of manipulation bouts (59) and increasing the amount of straw available increased both the time spent manipulating the straw and pigs' simultaneous straw use (60).

Cows appearing more relaxed and content would be in line with an overall shift to a more positive affective mood. It is possible that as animals spend more time positively occupied with the environment, they use more positively motivated energy, which could be linked to being more relaxed/tired out. Dairy cows provided with overnight pasture access have been shown to have longer overnight lying durations compared to continually housed cows (61). Longer durations of sleep behaviour have been observed in rats provided with environmental enrichment, compared to rats housed in standard cages (62). Furthermore, rats housed in more complex environments with a choice of simultaneously available enrichment resources compared to having only one type of enrichment, have been shown to sleep more, spend more time in enrichment-directed behaviour and less time inactive while awake (63, 64).

The results from PC1 also suggest that cows were less bored and fearful when they had access to additional environmental resources. Research on boredom in animals suggests that it may be reduced by providing additional behavioural opportunities through environmental enrichment (65–67). It therefore seems biologically plausible for cows to have appeared less bored when they were provided with two additional environmental resources. Understanding why a simple change in environmental conditions may reduce wider negative affective states, such as apathy and fear however, is more challenging. In human psychology, the experience of boredom is described as unpleasant and distressing (68). Given that this is an under-researched area in animals (69), it is possible it is an equally aversive experience and research has started to suggest this, for example animals will choose aversive experiences over monotony (65, 70). In animals, depression-like symptoms appear to be induced by barren housing, which may develop from unavoidable chronic stressors of the environment (71). Proxies of low mood, one symptom of depression, such as negative information processing, have shown to be changed in pigs, but not dairy cows, through environmental enrichment (19, 72). When pigs were moved from barren to enriched housing, they showed decreased negative information

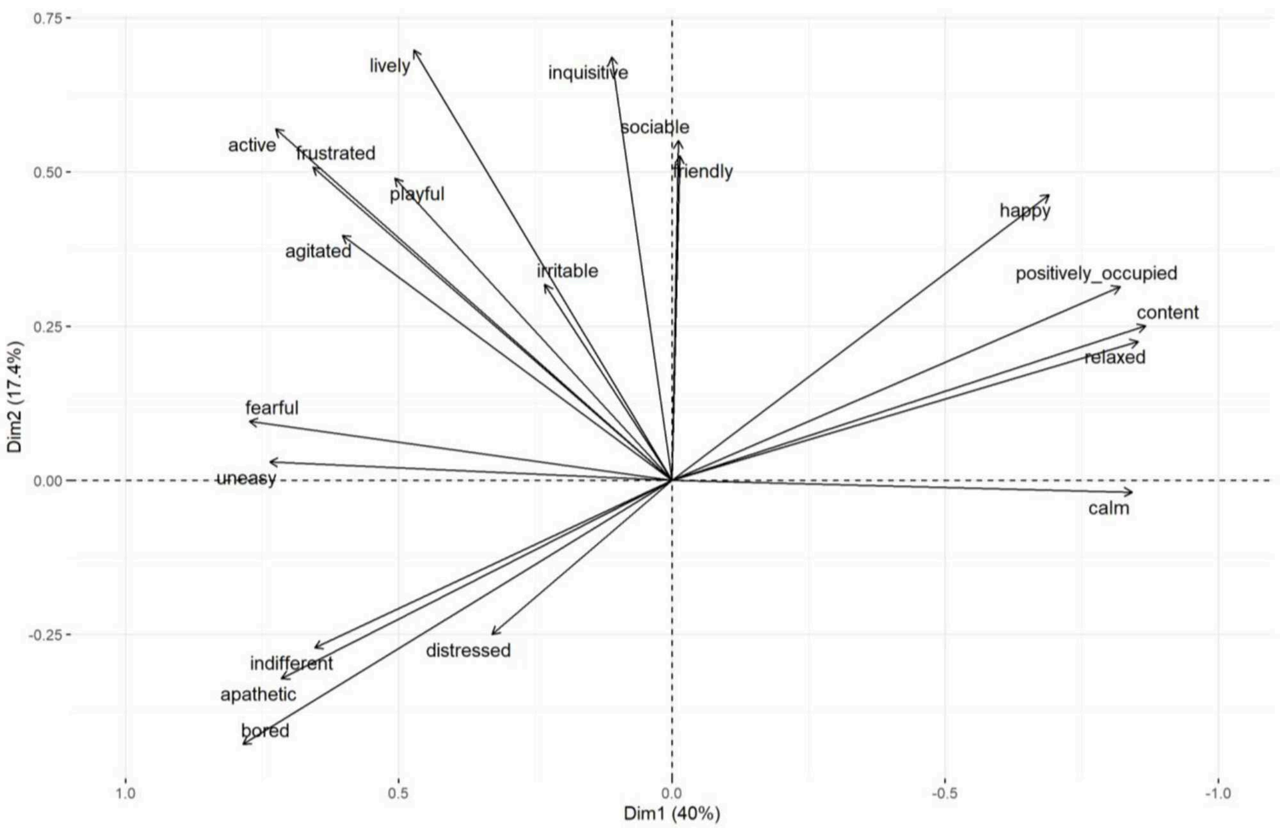

FIGURE 6
Group 2: Variable correlation plot displaying the relationship between all variables in PC1 (Dim1) and PC2 (Dim2) and each terms value figure in contribution to the principal component.

TABLE 4 Group 2: Results of the linear model assessing PC1 and PC2 scores attained during the different treatment periods.

Coefficients of the model	Estimate	Confidence interval (95%)	P-value
QBA PC1			
Reference: Baseline housing conditions Intercept	1.93		
Choice phase (early)	−2.27	−4.91–0.38	0.09
Choice phase (late)	−5.37	−8.01 to −2.72	<0.01
Indoor novel object	−0.41	−3.05–2.24	0.76
Outside	−1.62	−4.26–1.03	0.22
QBA PC2			
Intercept	−2.80		
Choice phase (early)	3.83	2.40–5.25	<0.01
Choice phase (late)	2.80	1.38–4.22	<0.01
Indoor novel object	3.24	1.81–4.66	<0.01
Outside	4.13	2.71–5.55	<0.01

Choice phase (both resources simultaneously available), indoor novel object only and outside (access to the outdoor yard only).

processing in cognitive bias tests. This result was exemplified in pigs that were transferred from enriched to barren housing, showing higher levels of negative information processing in cognitive bias tests when compared to that of pigs that had always been managed in barren housing (19). Dairy cows were housed in conditions aimed to elicit a contrast in positive and negative affective states (72). The 'positive' housing provided additional space, enrichment and social stability, with a 'negative' condition featuring overcrowding, removal of enrichment and social instability. The contrasting housing conditions however, failed to influence responses to a judgement bias test. Further research is needed to understand the impact that housing conditions have on both positive and negative affective states in animals.

Boredom is correlated with anxiety and fear in people (73, 74), and therefore it is possible that these negative affective states are also linked in animals. Given the association between these states, reducing boredom could simply be paired with overall reductions in negative affective states such as fear, or providing more time filling environmental activities could act as some form of distraction from triggers of fear and anxiety. Increasing animals' time in positively engaging behaviours would likely decrease time spent in empty or boredom-like situations, where cows may be more aware of surroundings and

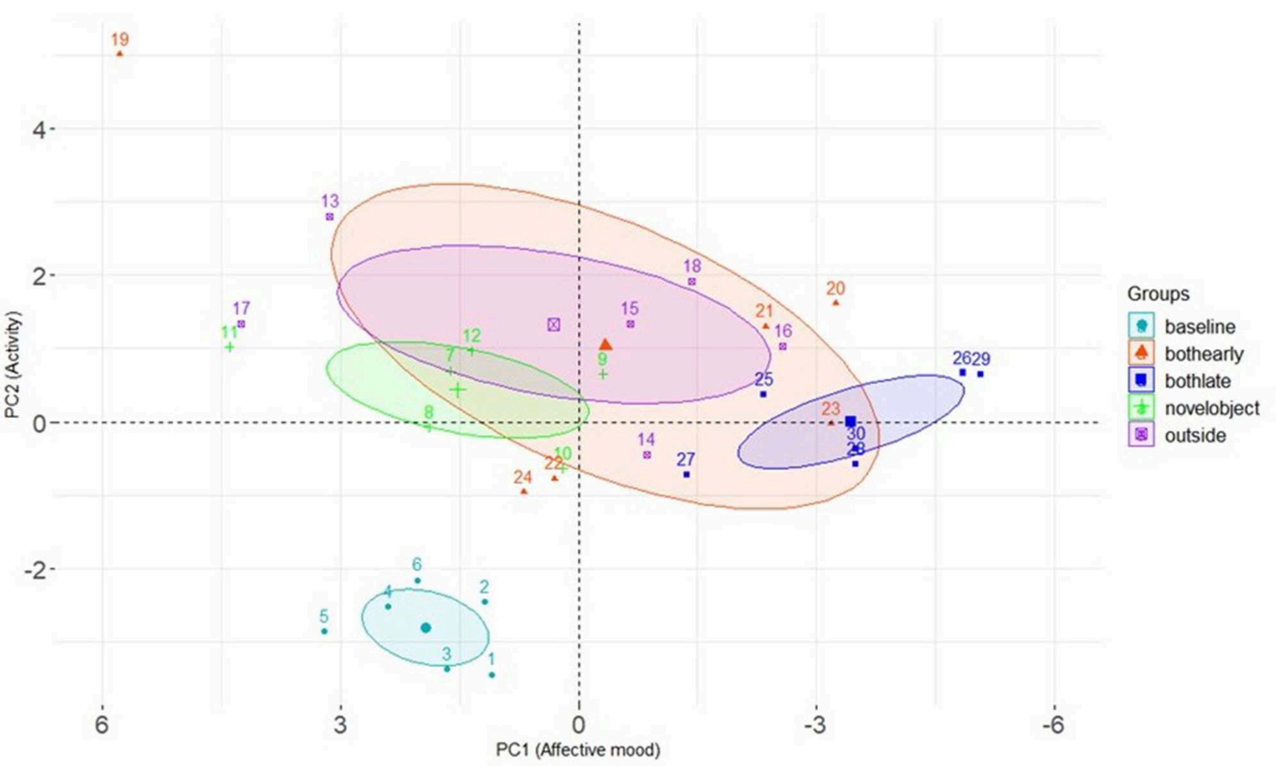

FIGURE 7
Group 2: Biplot displaying all QBA assessment scores in terms of PC1 and PC2. Each separate point displays one assessment date. Points are coded per treatment period [baseline, novel object, outside, both resources (early) and both (late)] as indicated on the plot. Group means are in bold and ellipses indicate the 95% confidence intervals of the group mean.

potential threats. Interestingly, anxiety behaviours in rats and mice have shown to be reduced through the use of environmental enrichment (75–77) but this link has yet to be explored in dairy cows.

The potential benefits in behaviour and welfare of dairy cows facilitated by different forms of environmental enrichment has started to be explored (29). However, the impact of enrichment on cows' affective wellbeing has received little research. Results indicating enhanced affective states have been shown in calves housed in pairs compared to individually, and in calves housed in enriched compared to unenriched environments (15, 16). A small number of studies have explored the association between the level of housing confinement and affective states in dairy cows. Reduced reward anticipation has been displayed in dairy cows with access to pasture (25), however mixed results have been shown when evaluating eye temperature between these conditions, a physiological indicator of stress (78). QBA has indicated better affective states in dairy cows in loose housing systems compared to tethered systems and during the early stage of a housing period compared to during the late stage of housing (13, 14). The results of the current study, indicating more positive affective states in cows with outdoor access, appear consistent with these findings. The significant results from PC1 which are suggestive of an overall shift to a more positive mood, including cows appearing less bored, persisted during the late stage of the choice period, when cows appeared to have started to show some level of habituation to the indoor novel object. However, despite some decline in time spent using one of the enrichments, the majority of both groups were still interacting with it. Although further replication of the current study would be beneficial, the similarity between results for the two groups of cows shows the repeatability of the study findings.

PC2 (activity) and treatment period

Results from Group 2 and both groups combined, showed that cows scored significantly higher on PC2, during all treatment periods compared to standard housing conditions. The most positive correlating adjectives on this component were lively and inquisitive, with the most negative correlating terms being apathetic and bored. This component therefore, appears to represent a combination of activity and valence. Enrichment is known to increase exploration and associated activity (79, 80). Research in calves has shown that simple housing modifications, such as social housing and additional space are associated with higher levels of play behaviour (81, 82). Dairy cows and heifers have also been shown to display increased activity and play behaviour with decreased access to exercise (83, 84) suggesting a motivational need for locomotory behaviour which is limited during confinement. Therefore, it would be understandable for cows to appear more active, lively or inquisitive when

TABLE 5 Group 1 and Group 2: Principal components 1 and 2, displaying associated correlations between variables and principal components for each behavioural descriptor.

Descriptor	PC1	PC2
Active	−0.64	0.59
Relaxed	**0.86**	0.17
Fearful	**−0.81**	0.01
Agitated	−0.57	0.39
Calm	0.66	−0.10
Content	**0.87**	0.26
Indifferent	−0.70	−0.32
Frustrated	−0.65	0.43
Friendly	0.10	0.29
Bored	**−0.80**	**−0.40**
Playful	−0.36	0.57
Positively occupied	0.82	0.17
Lively	−0.40	**0.66**
Inquisitive	−0.26	**0.60**
Irritable	−0.27	0.40
Uneasy	−0.69	−0.004
Sociable	−0.12	0.53
Apathetic	−0.67	**−0.41**
Happy	0.73	0.44
Distressed	−0.38	−0.18

The bold values indicate the two most positive and two most negative correlating adjectives.

provided with enrichment resources. Furthermore, the provision of access to outside space was likely facilitative to increased exercise in the current study. Again, when compared to the field of human psychology, exercise is a known and widely used treatment for anxiety and depression (85, 86) and it could be possible for a similar relationship to exist in animals. A review on the literature on the benefits of exercise for dairy cows has confirmed that increasing the movement opportunity provided by housing has a positive effect on activity level and can benefit cow health, behaviour and welfare (87). The provision of diverse environments offering wider behavioural activities is already suggested to be one of the first strategies for mitigating boredom in confined animals (67). Inactivity, one suggested behavioural expression of boredom in animals (88) has been shown to decrease in multiple species when provided with more complex environmental opportunities (65, 89, 90). Concurrent with the knowledge of the relationship between environmental enrichment and boredom, it is tenable that the cows in the current study displayed a decreased behavioural expression of boredom when provided with access to two additional environmental resources.

The temperature throughout the study remained well within the thermal comfort zone for dairy cows (91, 92). In addition to this, the temperature between treatment periods varied within a small range of 4.32°C, therefore was unlikely to have impacted cow behaviour or affective states.

QBA and study limitations

QBA is utilised for its on-farm practicality, requiring little time to complete when compared to other farm assurance assessments (45), and requiring no resources or technical equipment. Its practicality as an on-farm measure of welfare assessment, including aspects of positive welfare, was observed within the present study however it should be mentioned that some terms are more challenging to assess than others. Although QBA does not assess the physical behaviours performed by the animal, the particular behaviour an animal is engaged in can affect the ease with which the expressive qualities of that behaviour are assessed. Behaviour may therefore carry more weight in our interpretation of animals' quality of experience for some terms compared to others. For example, a cow that is positively occupied could be observed and its style of behaviour could be assessed for varying terms, such as how tense or relaxed it may appear. However, a cow standing or lying completely motion less, is more challenging to assess for its level of happiness or frustration for example. The expressive qualities of animals therefore appear to be more difficult to assess when less active, with fewer visual cues. Overall terms were assessable, yet a small number were much more challenging to assess, the term happy being one such example. Although the concept of QBA is to use interpretation of animals' expressive demeanour to make the assessments, a certain level of knowledge as to how these affective descriptions may be behaviourally expressed within a certain species is needed for guidance. Very little is known about how animals express happiness, therefore making a visual judgement of an animal's level of happiness is a challenging task, which ties in to the current complexities of trying to evaluate animals' affective experiences (10). QBA has proven itself as a reliable measure of making inferences about animals' differing affective states (10), yet the potential for it to be considered as anthropomorphic is frequently mentioned (30, 31, 93). This criticism could potentially be controlled, by using careful consideration of the terms used for assessments or by also using the free choice profiling approach, where assessors generate their own terms.

A potential limitation of the QBA assessments conducted within this study was the inability to blind the assessor to study treatments. Therefore, the assessor was aware of when cows were housed in standard and enriched conditions. It is possible that this could introduce an element of unconscious assessor bias, due to interventions having the potential to be linked to moral connotations, for example one treatment being perceived as better for welfare than another. Evidence of this contextual bias has previously been observed whilst using QBA (94, 95). Tuyttens et al. (94) recruited veterinary students to assess the welfare of laying hens, using QBA from video recordings. The same video clip from one group of hens was split into two separate clips and students were informed that one showed hens from an organic farm, with the other showing hens from a conventional farm. Students gave lower scores for negative descriptors and higher

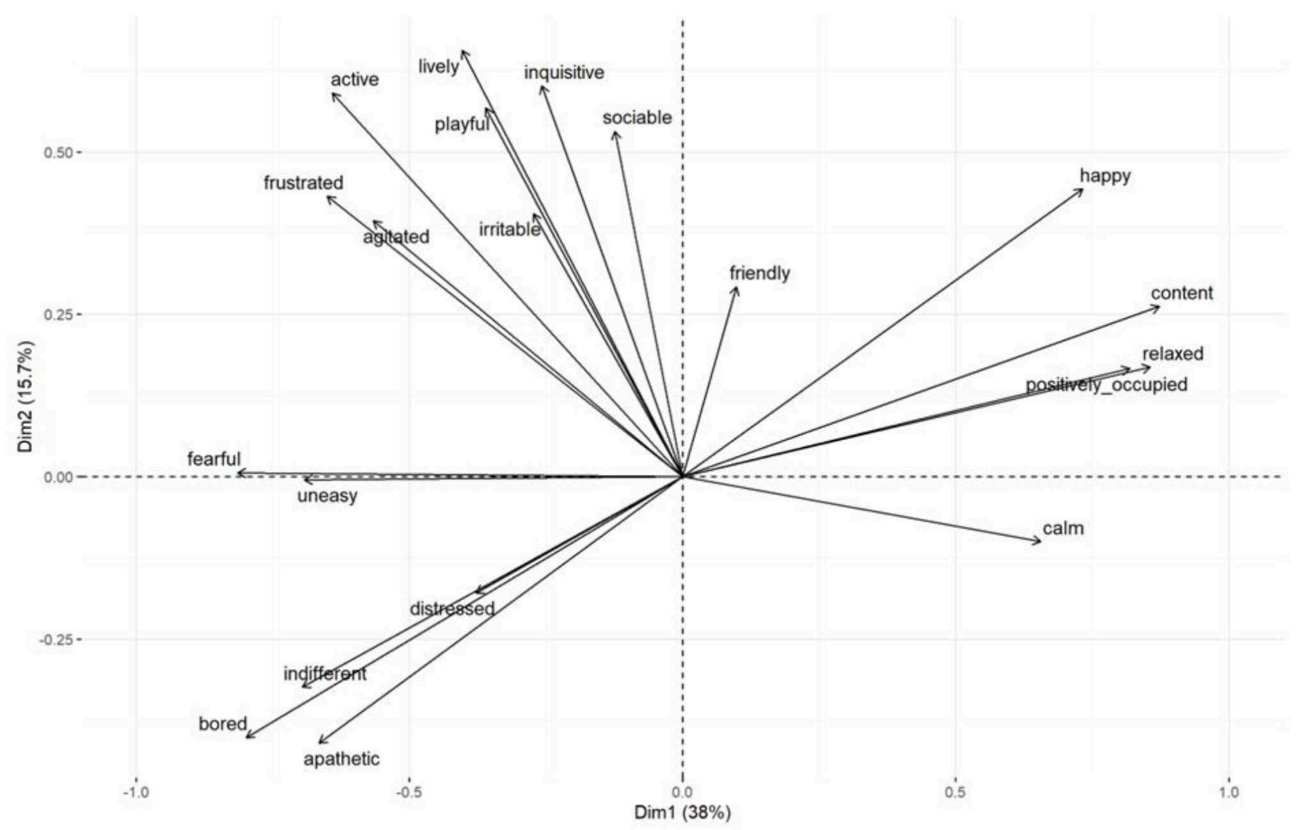

FIGURE 8
Group 1 and 2 combined: Variable correlation plot displaying the relationship between all variables in PC1 (Dim1) and PC2 (Dim2) and each terms value figure in contribution to the principal component.

TABLE 6 Group 1 and 2 combined: Results of the linear model assessing PC1 and PC2 scores attained during the different treatment periods.

Coefficients of the model	Estimate	Confidence interval (95%)	P-value
QBA PC1			
Reference: Baseline housing conditions Intercept	−2.49		
Choice phase (early)	3.62	1.99–5.25	<0.01
Choice phase (late)	5.65	4.02–7.27	<0.01
Indoor novel object	1.34	−0.29–2.96	0.11
Outside	1.85	0.22–3.48	0.03
QBA PC2			
Intercept	−1.72		
Choice phase (early)	2.74	1.48–4.01	<0.01
Choice phase (late)	1.59	0.33–2.86	<0.01
Indoor novel object	2.31	1.04–3.58	<0.01
Outside	1.97	0.70–3.23	<0.01

Choice phase (both resources simultaneously available), indoor novel object only and outside (access to the outdoor yard only).

scores for positive descriptors when under the impression that the hens they were scoring were from an organic rather than a non-organic farm. The magnitude of this relationship was positively correlated with students opinion regarding hen welfare in these different systems. Wemelsfelder et al. (96) investigated the impact of being contextually aware of the animals' environment on QBA results. Video recordings of 15 pigs interacting with a novel object were digitally extracted and applied to both an indoor and outdoor setting and the resultant video clips were analysed by blind observers. There was a strong correlation between the indoor and outdoor variants of video clips across both QBA components. Environmental background did however have an effect on one of the QBA components (confident/content–cautious/nervous) but not the other (playful/active–bored/lethargic), implying that pigs observed in an outdoor setting were perceived to be more confident and content and less cautious and nervous than when these same pigs were observed against an indoor background. Thus, although different contexts led to slight shifts in assessors' scorings in this study this did not lead to significant misinterpretations of the pigs body language. One of the underpinning concepts of QBA is to evaluate not just how animals are behaving, but how they are interacting with their environment (30, 97), which evidently also requires knowledge of the environmental situation. Therefore, the assessor's use of knowledge regarding

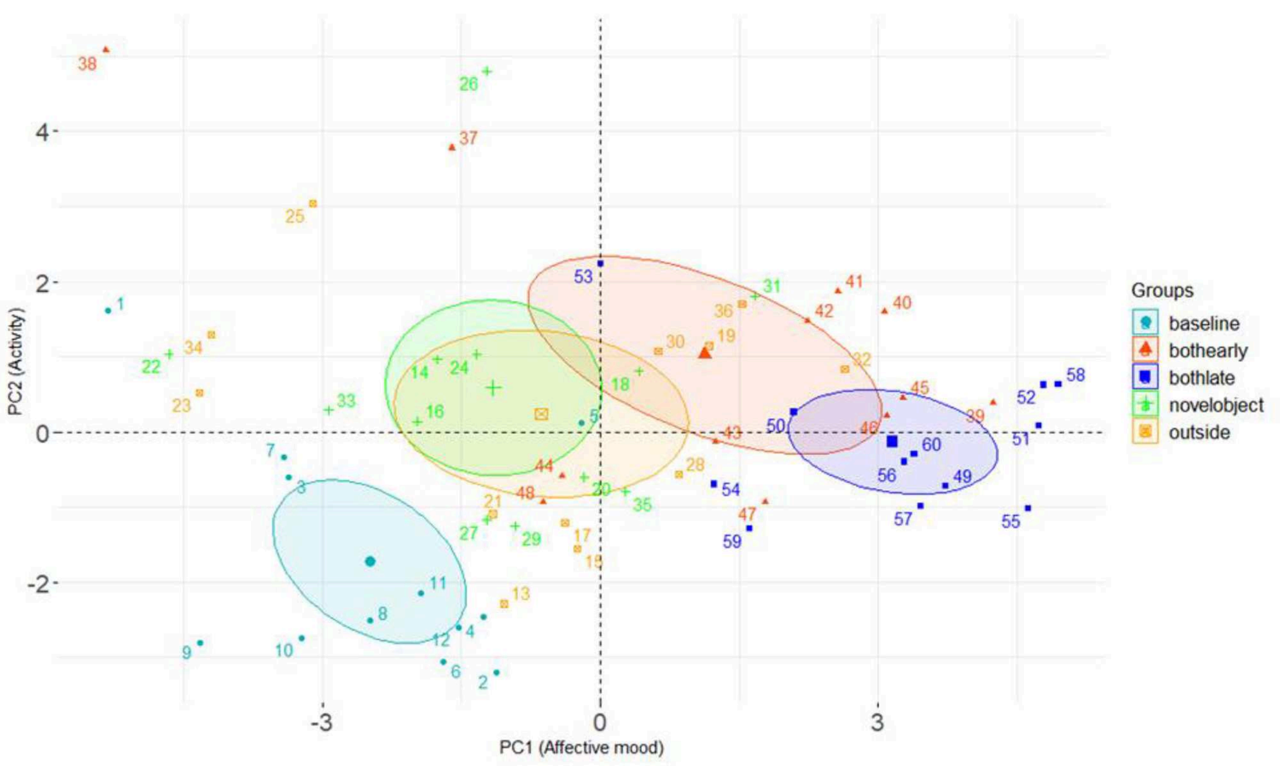

FIGURE 9
Group 1 and 2 combined: Biplot displaying all QBA assessment scores in terms of PC1 and PC2. Each separate point displays one assessment date. Points are coded per treatment period [baseline, novel object, outside, both resources (early) and both (late)] as indicated on the plot. Group means are in bold and ellipses indicate the 95% confidence intervals of the group mean.

the animal's environment does not seem unreasonable. This contextual bias is a potential weakness of the use of QBA, when used in certain situations, where different farming systems, conditions or study interventions may be perceived to provide different levels of welfare. Ideally observers should be blind to such background conditions, and in many QBA studies they are (41), however in live assessments of changing on-farm housing conditions that is not possible to achieve. QBA has been practically implemented in industry as an on-farm welfare assessment (36, 37) despite this potential bias risk, due to contextual awareness of surroundings.

Results of the current study could have been strengthened through use of a combination of positive welfare indicators. For example, correlates of enhanced affective states have been demonstrated between QBA results and ear position in calves and lambs (98, 99) and QBA and positive social behaviour in dairy cows (46). Divergences may also highlight where studies making reference to changes in affective states require further replication or validation. For example, Carreras et al. (20) evaluated the affective states of pigs housed in an enriched (solid floor, straw and increased space allowance) or a barren (decreased space allowance, no straw, slatted floors) environment and found QBA results, cortisol concentrations and carcass wounds to be indicative of better welfare states in the enriched conditions. However, no differences were detected in the cognitive bias testing. Similarly, Vitali et al. (100) evaluated the welfare status of pigs housed in mechanically compared to naturally ventilated housing and found QBA to identify pigs in mechanically ventilated buildings to be associated with more positive affective states. Interestingly, pigs in mechanically ventilated buildings also performed higher levels of stereotypical and negative social behaviours and showed a higher general level of inactivity, all behaviours associated with negative affective states (88, 101, 102).

Conclusions

Qualitative behavioural assessment was used to identify differences in cow behavioural expression and, we therefore hypothesise, in associated affective state, between periods when the cows were housed in standard commercial conditions and periods when they were housed in enriched conditions. The enriched conditions provided additional environmental resources. Our results indicate that the simple housing modifications, access to a novel object and to outdoor space, are likely to positively impact the affective lives of commercially housed dairy cows. The results are biologically plausible and suggest

TABLE 7 Summary of results displaying PC1 and PC2 for each group and their associated most positive and negative correlating adjectives.

	Principal component	Treatment period
Group 1	PC1 **content/relaxed** – bored/fearful	**Indoor novel object** only Outside only **Choice phase (early)** **Choice phase (late)**
	PC2 **lively/playful** – bored/apathetic	Indoor novel object only Outside only Choice phase (early) Choice phase (late)
Group 2	PC1 **content/relaxed** – fearful/bored	Indoor novel object only Outside only Choice phase (early) **Choice phase (late)**
	PC2 **lively/inquisitive** – bored/apathetic	**Indoor novel object only** **Outside only** **Choice phase (early)** **Choice phase (late)**
Combined	PC1 **content/relaxed** – bored/fearful	Indoor novel object only **Outside only** **Choice phase (early)** **Choice phase (late)**
	PC2 **lively/inquisitive** – apathetic/bored	**Indoor novel object only** **Outside only** **Choice phase (early)** **Choice phase (late)**

Treatment periods which had a significant effect on principal components are indicated for each group. Treatment periods in bold indicate where significantly higher scores were being attained on a principal component. Adjectives in bold are the most positively correlating terms for that principal component.

that some level of positive experience may be facilitated through simple modification to the housed environment 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 reviewed and approved by the University of Nottingham, School of Veterinary Medicine, and Science Ethical Review Committee.

Author contributions

AR contributed to study conception and design, on farm management of the study including data collection, data analysis, interpretation of data, and writing of the manuscript. MG contributed to study conception and design, data analysis, interpretation of data, and reviewing the manuscript. LR, JK, and NE contributed to study conception, design, and reviewing the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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Stayability in Simmental cattle as affected by muscularity and body condition score between calvings

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The present study aimed to investigate the association between stayability (STAY) traits, muscularity, and body condition score (BCS) in the Italian Simmental dual-purpose cows. Data were collected from 2,656 cows linearly scored in their first lactation from 2002 to 2020 and reared in 324 herds. The binary trait STAY, which is the ability of a cow to stay in the herd, was obtained for each cow-lactation available up to parity 5 (from STAY1-2 to STAY4-5). Analysis of STAY was carried out using logistic regression, considering the fixed effect of energy corrected milk, conception rate, somatic cell score, and muscularity or BCS predicted at different time points. The herd of linear classification and residual error were the random effects. Primiparous cows with a medium BCS and muscularity in early lactation presented a more favorable STAY across life compared to thinner ones ($P < 0.05$). In fact, cows with an intermediate BCS/muscularity were more likely to stay in the herd after the third lactation (STAY3-4), compared to those presenting a lower BCS/muscularity ($P < 0.01$). However, cows whose muscularity was high were generally less likely to start the third lactation compared to the others. A potential explanation for this could be the willing to market cows with good conformation for meat purpose. Simmental is in fact a dual-purpose breed known for the good carcass yield and meat quality. This study demonstrates how muscularity and BCS available early in life can be associated with the ability of Simmental cows to stay in the herd.

KEYWORDS

culling, dual-purpose cows, functional longevity, resilience, survival rate

Introduction

Herd-testing for daily milk yield and composition is one of the major sources of information for the genetic evaluation of dairy cows and quantification of herd productivity and profitability (1). For several decades, in European and Northern American countries, dairy breeding objectives included mainly traits related to milk production (2). Although being one the key drivers of profitable dairy farming, the genetic improvement for such characteristics has led to deterioration of functional traits, such as longevity and fertility, due to the antagonistic genetic correlations existing among these traits (3–5). For these reasons, nowadays breeding objectives of dairy cattle include a plethora of economically relevant traits not necessarily directly related to milk productivity (2, 6). In dairy farming, high culling rates indicate poor animal welfare, suboptimal farming conditions, and inefficient use of animal resources, impairing the sustainability of the dairy sector (7). According to Allendorf and Wettemann (8), high replacement rates cause a decrease in herd productivity followed by augmented replacement costs (9). Indeed, in Holstein-Friesian cows, milk production per lactation is maximized at the third lactation (10) and cows usually finish paying back their initial rearing cost at the end of their second lactation (11). Thus, culling cows before that moment has a detrimental impact on farmers' profitability (12, 13). Moreover, low culling rates may also improve the environmental footprint of dairy farms because of the lower number of heifers required in the herd (14).

In dairy cows, productive lifetime is defined as the period during which the animal is in production in the herd (15). Instead, longevity can be described in different manner in dairy cattle: e.g., by means of age at last calving, number of lactations started or completed, number of days from first calving to culling, age at culling, and survival at various ages or parities. Longevity combines all the characteristics that are directly associated with a cow's ability to successfully stay and perform in the herd (16). For this reason, some authors (17–19) have opted for the term "stayability" (STAY). This trait can be considered somehow equivalent to longevity, but it is usually expressed in a binary trait where 1 and 0 indicate if the animal remains in the herd and produces up to a specific moment or not, respectively (20). Cow's STAY is a key component of profitability in dairy production, as long-living cows allow to achieve the same herd production with a lower replacement rate. This implies that replacement costs can be reduced and that surplus newborn calves, preferably crossbred, can enter the beef market (21).

Conformation traits, or type traits, have been used for indirect selection to improve productive life due to their correlation with survival (22). Although collection of such phenotypes is consuming in terms of time and labor, the main advantage of type traits is that they are available early in life (2), and, indeed, several authors reported correlation of some type traits with longevity in different dairy cow breeds. Jovanovac and RaguŽ (23) reported that udder and body conformation traits, as well as muscularity and size traits, could be used as predictors of STAY and longevity in Croatian Simmental dairy cows. Schneider et al. (24) reported that udder and feet and legs traits had a strong relationship with functional herd life in Quebec Holsteins. In addition, Imbayarwo-Chikosi et al. (25) reported that chest width and rump angle were strongly associated with the risk of culling in South African Holstein dairy cows. To the best of our knowledge, no studies have attempted to identify factors associated with STAY in Italian Simmental cows, whose breeding objectives include both dairy and beef attitudes. This would be important to inform farmers in optimizing management and culling decisions based on some conformation traits, recorded within the national recording scheme.

Therefore, the aim of the present study was to retrospectively explore the variability of STAY in Italian Simmental cows and quantify its association with muscularity and BCS.

Materials and methods

Database

The present study was conducted using data retrieved from the National Association of Italian Simmental Cattle Breeders (ANAPRI, Udine, Italy) database that were collected between January 2002 and December 2020. Data was recorded on 2656 Italian Simmental dual-purpose cows reared in 324 dairy herds located in Emilia Romagna region, in North-eastern Italy. This region has a large number of dairy farms [3,519; BDN-Anagrafe Zootecnica Nazionale, (26)] that greatly contribute to the regional economy [60% of the regional gross saleable production; ISTAT, (27)]. The majority of the farms involved in the present research were multi-breed, i.e., the 97% of farms reared both Simmental and Holstein cows. Out of these, in 15 farms the number of Simmental cows was equal or above 50; only 2 of them had more than 130 heads. Only cows which were linearly classified in their first lactation were considered in the present study.

Data provided by ANAPRI included information regarding the cows' lactations estimated by the Italian Breeders Association (AIA, Rome, Italy), namely whole lactation milk and solids yield, and test-day milk records with the daily milk yield, gross composition, and somatic cell count (SCC, cells/mL). Linear type traits scores, measured once in life (in primiparous cows) by trained personnel were also present.

Phenotypes

Stayability

This trait was calculated in the lactation set, and it was defined for each cow-lactation up to the fifth, based on the presence or absence of the subsequent calving date (Table 1). Briefly, a STAY equal to 1 was assigned if a calving date was present after the previous lactation, otherwise STAY was considered equal to 0. The value was recursively set at 0 for all parities after the one incurring the culling date. This resulted in five different variables for each cow: STAY1-2, STAY2-3, STAY3-4, and STAY4-5.

Milk traits

The energy corrected milk (ECM) was obtained from the actual lactation data according to the formula proposed by Dairy Records Management Systems (28):

$$\boldsymbol{ECM} = (\boldsymbol{MY} \times \mathbf{0.327}) + (\boldsymbol{FY} \times \mathbf{12.86}) + (\boldsymbol{PY} \times \mathbf{7.65})$$

where *MY, FY, and PY* indicate the kg of milk, fat, and protein produced within the lactation.

Milk SCC was converted to somatic cell score (SCS) according to the formula proposed by Ali and Shook (29) to achieve normal distribution:

$$\boldsymbol{SCS} = \mathbf{3} + \log_2(\boldsymbol{SCC}/\mathbf{100{,}000}).$$

Test-day SCS values were then averaged within each lactation, in order to be merged with STAY phenotypes and be used as an indicator of the cow's udder health.

TABLE 1 Definition and descriptive statistics of the stayability traits.

Trait	Definition	Cows[a]	Rate[b]
STAY1-2	Stayability as a primiparous cow: survived to 1st lactation = 1; departed during 1st lactation = 0.	2,656	0.98
STAY2-3	Stayability as a second-parity cow: survived to 2nd lactation = 1; Departed during 2nd lactation = 0.	2,603	0.70
STAY3-4	Stayability as a third-parity cow: Survived to 3rd lactation = 1; Departed during 3rd lactation = 0.	1,819	0.62
STAY4-5	Stayability as a fourth-parity cow: survived to 4th lactation = 1; Departed during 4th lactation = 0.	1,127	0.57

[a]Number of cows survived. [b]Proportion of cows survived (STAY = 1).

Morphological characteristics

Linear-type traits can be scored on any days in milk (DIM) in primiparous cows. However, the morphological traits considered in the present study (muscularity and BCS) are known to vary within lactation, suggesting that observed differences among cows can be due also to the moment in which they were scored, i.e., stage of lactation. For this reason, following Buonaiuto et al. (30), individual muscularity and BCS lactation curves were adjusted through random regression analysis, allowing daily individual prediction of both traits to be present. In such a way, the differences in the expected muscularity and BCS among cows at the same DIM becomes independent from the number of days post-calving at linear type scoring. Subsequently, average lactation profiles were estimated for cows belonging to different classes of age at first calving (30), in order to evaluate the absolute growth rate (AGR) of both muscularity and BCS. The AGR was calculated according to the formula used by Handcock et al. (31):

$$AGR = \frac{(BT_x - BT_y)}{(t_x - t_y)}$$

where BT_x and BT_y are the predicted muscularity or BCS at xth and yth DIM, t_x is the initial age of the cow (in days), and t_y is the final age (in days) (30).

For further statistical analysis, only muscularity and BCS data predicted at four specific moments during the lactation were considered (30):

i. at the onset of lactation (5 DIM for both traits; Figure 1);
ii. at the nadir of muscle and/or fat reserves losses (first null AGR), i.e., the moment where the uptake from body reserves stops in Simmental (85 and 45 DIM for muscularity and BCS, respectively);
iii. at the maximum AGR, i.e., when the greatest recovery of muscle/fat reserves is observed in Simmental (180 and 160 DIM for muscularity and BCS, respectively);
iv. at the second null AGR, representing the moment from which cows start to lose again muscle/fat reserves (280 DIM in both traits);

Subsequently, cow-specific predictions of muscularity and BCS were merged to the lactation data.

Statistical analysis

Muscularity, BCS, ECM, and SCS (lactation average) were grouped into 5 classes based on quintile distribution for each individual variable, as: low, medium-low, medium, medium-high, and high. Records belonging to different lactations were analyzed separately, meaning that the effect of parity was not accounted for in the statistical models. Muscularity and BCS predicted at each given time point during the first lactation were included. Therefore, STAY1-2, STAY2-3, STAY3-4, and STAY4-5 were analyzed 4 times, by considering at each run classes of muscularity and BCS predicted at one out of the four different time points considered.

A logistic regression model was fitted with the GLIMMIX procedure using SAS software, version 9.4 (SAS Institute INC., Cary, NC):

$$y_{lmnopqr} = \mu + MU_l + BCS_m + ECM_n + CR_o + SCS_p + Herd_q + e_{lmnopqr},$$

where y is STAY1-2, STAY2-3, STAY3-4, or STAY4-5; μ is the overall intercept of the model; MU_l is the fixed effect of the lth class ($n = 5$) of muscularity predicted at each specific aforementioned time point; BCS_m is the fixed effect of the mth class ($n = 5$; defined according to quintiles) of BCS predicted at each specific aforementioned time point; ECM_n is the fixed effect of the nth class ($n = 5$) of ECM; CR_n is the fixed effect of the nth class (0 vs. 1) of conception rate at first insemination, where 1 indicates that only a single insemination is needed to achieve pregnancy and 0 otherwise; SCS_o is the fixed effect of the 0th class ($n = 5$; defined according to quintiles) of milk SCS; $Herd_p$ is the random effect of the pth herd ($n = 324$) of linear classification, assumed to be distributed as $\sim N\left(0, \sigma_H^2\right)$, where σ_H^2 is the herd variance; and e is the random residual term, assumed to be distributed as $\sim N\left(0, \sigma_e^2\right)$ where σ_e^2 is the residual variance. For the fixed effects of muscularity and BCS the first class (low) was kept as the reference, and each odds ratio (OR) was considered significant when the 95% CI did not include 1.

Results and discussions

Overview of the studied population

The Italian Simmental cows included in the present research presented relatively high production levels compared to those reported by Cziszter et al. (32) for Fleckvieh (Austrian Simmental) cattle and by Erdem et al. (33) for Simmental cows reared in Turkey. The evolution of yield traits from parity 1 onwards in Italian Simmental cows are shown in Table 2. Descriptive statistics (mean ± standard deviation) indicated that the average MY in this study increased gradually from parity 1 to 3 (Table 2) and then decreased (parity 4: 6,660.04 ± 2,688.70 kg). The MY trend across parities was

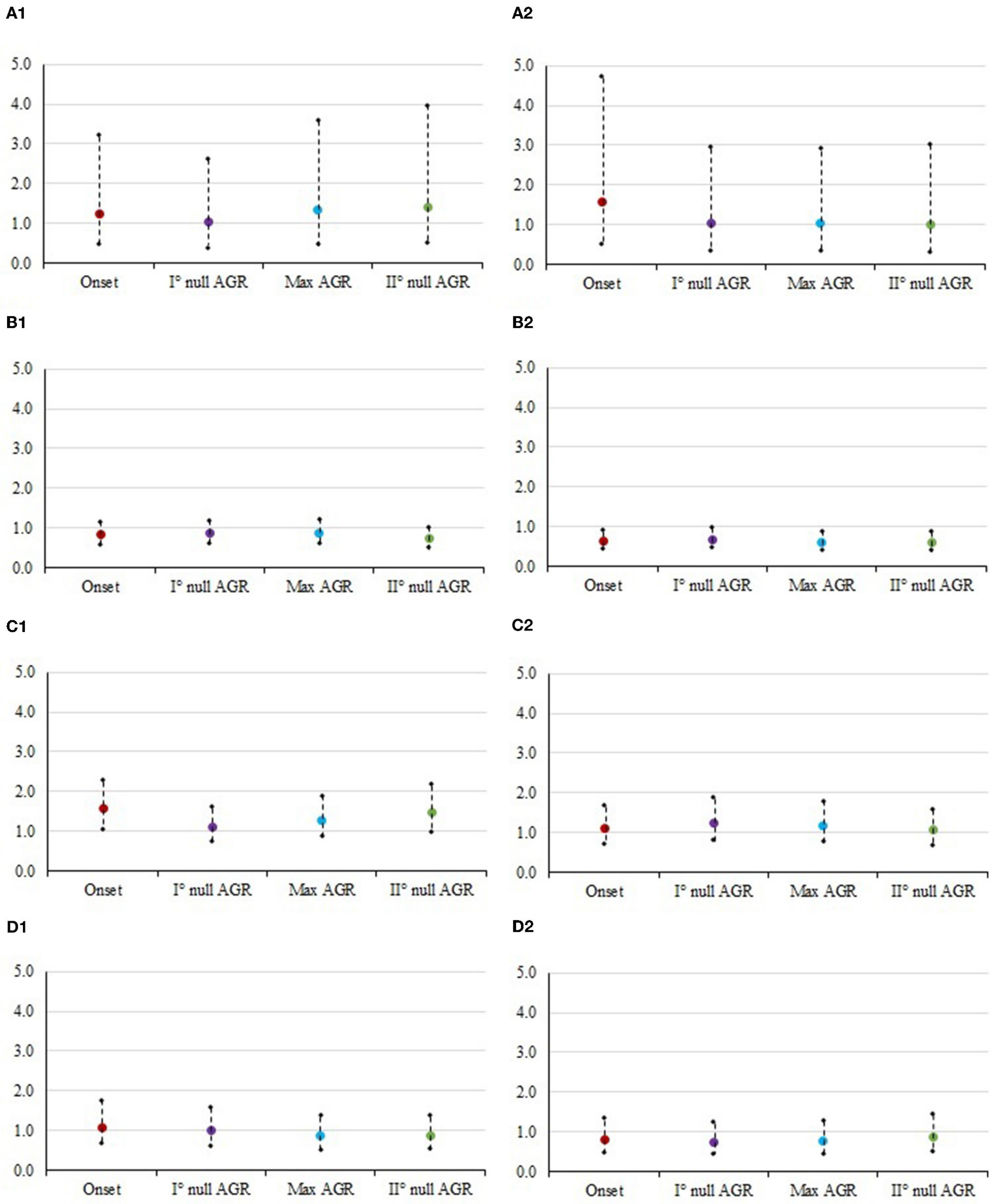

FIGURE 1
Odds ratios and 95% confidence interval for the risk of culling at each class of muscularity in different timepoints. The panels contain: (1) Mid vs. Low. **(A1)** STAY1-2, stayability as a primiparous cow, **(B1)** STAY2-3, stayability as a second-parity cow, **(C1)** STAY3-4, stayability as a third-parity cow, **(D1)** STAY4-5, stayability as a fourth-parity cow. (2) High vs. Low. **(A2)** STAY1-2, stayability as a primiparous cow, **(B2)** STAY2-3, stayability as a second-parity cow, **(C2)** STAY3-4, stayability as a third-parity cow, **(D2)** STAY4-5, stayability as a fourth-parity cow. For muscularity, timepoints were selected according to the absolute growth rates (AGR) trends reported by Buonaiuto et al. (30): onset of lactation = 5 DIM; I° null AGR = 85 DIM; Max AGR = 180 DIM; II° null AGR = 280 DIM.

TABLE 2 Overview of Simmental cows' productivity[a] and fertility[b] performance in different parities.

Parity	Trait	Mean	Median	SD	Coefficient of variation, %	Min.	Max.
1	Milk yield (kg)	6,847.58	6,702	2,152.30	31.43	149	14,754
	Fat yield (kg)	264.39	255	85.03	32.16	22	589
	Protein yield (kg)	242.59	236	76.83	31.67	6	517
	Calving age (month)	29.54	29	4.19	14.17	20	41
	IFS (n)	83.31	75	39.78	47.75	2	218
	Days open (n)	120.79	100	86.36	71.49	0	532
	Calving interval (d)	410.73	391	76.70	18.67	283	666
2	Milk yield (kg)	6,861.69	6,932	2,541.66	37.04	92	14,750
	Fat yield (kg)	265.71	262	94.81	35.68	18	589
	Protein yield (kg)	245.23	245	86.60	35.31	19	512
	Calving age (month)	43.28	42	5.37	12.41	32	65
	IFS (n)	81.17	74	38.08	46.91	6	218
	Days open (n)	109.96	92	87.54	79.61	0	858
	Calving interval (d)	401.99	382	70.17	17.46	276	663
3	Milk yield (kg)	6,947.05	7,015	2,698.66	38.85	354	14,739
	Fat yield (kg)	270.51	269	99.87	36.92	9	583
	Protein yield (kg)	247.56	247	91.72	37.05	13	517
	Calving age (month)	56.51	56	6.34	11.22	43	82
	IFS (n)	80.78	73	36.28	44.91	6	217
	Days open (n)	107.11	88	89.73	83.78	0	476
	Calving interval (d)	400.17	378	70.87	17.71	272	665
4	Milk yield (kg)	6,660.04	6,726	2,688.70	40.37	127	14,652
	Fat yield (kg)	264.56	261	97.00	36.66	3	588
	Protein yield (kg)	240.95	237	86.30	35.82	5	514
	Calving age (month)	69.23	68	7.00	10.11	53	99
	IFS (n)	80.51	72	38.25	47.51	11	218
	Days open (n)	97.62	85	83.09	85.12	0	430
	Calving interval (d)	399.19	379	68.07	17.05	301	658
5	Milk yield (kg)	6,745.17	6,827	2,920.80	43.30	355	14,489
	Fat yield (kg)	265.82	261	103.18	38.82	14	585
	Protein yield (kg)	242.58	237	94.23	38.85	12	509
	Calving age (month)	82.03	81	7.75	9.45	65	113
	IFS (n)	81.91	73	37.83	46.18	1	212
	Days open (n)	98.61	79	89.37	90.63	0	494
	Calving interval (d)	402.74	382	67.60	16.79	282	645

[a]Based on all test-day records. [b]IFS, Interval form calving to first insemination.

similar to that reported by different authors (34, 35) for different European populations of Simmental cows. Milk composition plays an important role in countries like Italy where approximately the 75% of the total national milk annually produced is used for cheese manufacturing (36–38). As a matter of fact, fat and protein content together with pH and acidity are essential factors during milk processing into finished dairy products (39).

Along with the high productivity, the population studied was characterized by a composition comparable to that of specialized dairy breeds, with an average fat and protein content of 3.79 and 3.48%, respectively (data not shown). Parity-specific descriptive statistics of fat and protein yield, both used to calculate ECM, are reported in Table 2. Data observed are similar to that reported by other authors (40, 41) for Simmental dairy cows. By using test-day records of the first 150 DIM, Costa et al. (42) reported an average milk, fat, and protein yield of 4,157, 167.5, and 136.4 kg, respectively in Fleckvieh cows. In the same DIM window, these authors reported fat and protein content to average 4.03 and 3.28% (42). Numerous studies have investigated the effect of parity on yield traits using both test-day records or lactation data. According to the literature (43, 44) the positive correlation between MY and parity observed until a certain parity order could be attributed to the udder development/size, i.e., to the increasing number of functional secretory cells, and to the different requirements of primiparous and multiparous.

The cows' productive level and milk quality can be evaluated simultaneously by the means of ECM. The ECM can be considered as a key parameter for STAY in dairy cows as it directly affects the farm profit and, consequently, may have an effect on culling decisions (45). The culling of unproductive cows is necessary to keep the herd profitable and is thereby done on a voluntary basis. In the field, the real objective is to reduce the involuntary culling, e.g., elimination of cows—perhaps with a good MY—due to scarce fertility, severe disease, or acute inflammation (46). Well managed herds show high survival rates, thus a great proportion of mature cows and, consequently, a lower replacement rate (47).

In the present research the highest and the lowest CI value were observed in 1st (410.73 ± 76.70 d) and 4th lactation (399.19 ± 68.07 d) and, overall, the mean CI is similar to that reported in previous research in Italian Simmental cows (30). Dry period length averaged between 75.28 ± 29.22 (parity 1) to 81.29 ± 30.65 (parity 4). Across lactation the median of dry period ranged from 72 (for parity 1) to 77 (parity 2 and 4).

An overview of the investigated STAY traits and their definition is reported in Table 1. In particular, the survival rates were 98, 70, 62, and 57% (Table 1) and of the initial 2,656 cows present in parity 1 only 642 survived until parity 5 (24%; data not shown). Results are in general difficult to be compared with the literature due to scarce information available on such traits, especially for Simmental. In Holstein, Hardie et al. (19) reported that 84% (at parity 1) and 80% (at parity 2) of Holstein cows in US organic herds were able to survive and continue their productive career. Moreover, Garcia-Peniche et al. (47), report that from 38 to 43% of Holsteins stayed until 5 years of age, completing an average of 2.12 to 2.22 lactations. In the official annual report of Zuchtdata (48) the average number of calvings is equal to 3.83 for Austrian Fleckvieh while the average productive life is estimated at 3.66 years. The 24.17% of the Italian Simmental dual-purpose cows involved in this study achieved parity 5 (Table 1) which greatly differs from what has been reported for other breeds. As an example, Hare et al. (49) reported that US Holsteins dairy cows' population experienced a serious drop in survival to parity 5: from 24.2% recorded in 1980 to 14.3% in 1998. In Ireland, Williams et al. (50) reported that only 13% of the Holsteins cows involved in their study survived to the fourth lactation. Similar results are reported by Hardie et al. (19) that observe only 14% of US organic Holstein dairy cows reach parity 5. It is worth to highlight that, for the purpose of this study, data provided by ANAPRI belong exclusively to cows that were linearly classified during the first lactation, thus with a BCS and a muscularity score available. It derives that non-linear classified scored cow (e.g., for early culling in parity 1 before scoring) are not accounted for in this study. For this reason, results of this study may be interpreted in the light of absence of data from early culled cows.

According to Padilla et al. (51), a gradual age-related body deterioration is common to most animals, including dairy cows (52) and in livestock species this can affect both health and fitness of producing animals. In the past, the selection of high-producing dairy cows has favored larger more angular females, which resulted in skinny with poor carcass yield characteristics. Differently, dual-purpose cows as Simmental are characterized by a long and muscular body, that makes back and buttocks convex in most of the cases. This different body conformation was also observed by Knob et al. (53), who reported BCS of Simmental cows (and their crosses) to be approximately 1 point higher that of Holstein cows in all stages of lactation. Differences in body conformation can partly justify the survival rate observed in dairy vs. dual-purpose breeds (18). It is important to consider that, in the case of dual purpose breeds, culling could be influenced by external and economic factors, e.g., the market demand and price of milk and meat and the feed cost. Generally, when heifers of dual-purpose breeds are abundant and meat low-priced, farmers tend to cull more than usual, increasing the herd replacement rate.

Sources of variation of stayability

Results from the analysis of variance for STAY traits are summarized in Table 3. In the case of muscularity and BCS effect, the odds ratio estimates are depicted in Figures 1, 2, whereas estimates obtained for levels of SCS and CR are presented in Table 4. Overall, the effect of ECM, CR, and SCS were always significant indicating that some odds ratios differed ($P < 0.001$; Table 4), with the only exception of STAY1-2. Apart from severe reasons, in fact, Italian Simmental cows, whose average productive life is 3.3 lactations, are generally kept in the herd at least until second calving, i.e., regardless of the performance (26). This may partly explain why STAY1-2 was not affected by the above-mentioned fixed effects. Inclusion of ECM, CR and SCS allowed to account for that variability related to productivity level, udder health, and fertility. Odds ratio of these effects (Table 4) generally indicate that STAY is associated with SCS levels. In fact, as SCS increases, the risk of culling also increased; similar trends were observed for ECM. Regarding CR, we observed a higher risk of culling in cows with low CR (Table 4). However, although the odds ratio showed an association for all these effects, the significances was always >0.05.

Muscularity was significant for STAY1-2 at the onset of lactation and for STAY3-4 in second null AGR and in late lactation. On the other hand, BCS was significant during all the phases considered for STAY2-3 ($P < 0.05$). STAY3-4 was significantly ($P < 0.05$) affected by cow's BCS during the max (around 160 DIM), and the second null AGR (around 280 DIM).

Figures 1, 2 show the odds ratio of the risk of culling from STAY1-2 to STAY 4-5 in cows showing different muscularity and BCS level. Although not significant in most of the cases (Table 3),

TABLE 3 F-values and significance of fixed effects[a] included in the analysis of stayability traits.

Trait	Moment[b]	MU	BCS	ECM	CR	SCS	Herd variance	RSD
STAY1-2	Onset	3.00*	0.73	1.39	2.03	1.26	0.08	0.30
	I° null AGR	1.28	0.61	1.36	1.99	1.16	0.09	0.33
	Max AGR	1.59	0.58	1.32	2.13	1.21	0.08	0.31
	II° null AGR	0.77	1.44	1.32	1.88	0.97	3.39	3.16
STAY2-3	Onset	2.12	3.69*	47.98***	97.59***	5.05**	0.28	0.10
	I° null AGR	1.45	3.86**	47.92***	98.36***	5.04**	0.28	0.10
	Max AGR	2.04	2.69*	47.75***	98.24***	5.06**	0.28	0.10
	II° null AGR	1.87	2.95*	47.51***	95.98***	4.77**	0.29	0.10
STAY3-4	Onset	0.27	1.90	34.31***	74.07***	5.22**	0.45	0.14
	I° null AGR	0.40	1.19	34.15***	75.05***	5.07**	0.44	0.14
	Max AGR	1.00	2.84*	34.77***	75.08***	5.22**	0.45	0.14
	II° null AGR	2.72*	2.50*	34.74***	76.29***	5.22**	0.43	0.14
STAY4-5	Onset	0.61	0.52	24.47***	56.35***	3.25*	0.23	0.15
	I° null AGR	0.60	0.37	24.42***	56.48***	3.29*	0.22	0.14
	Max AGR	0.39	0.45	24.47***	55.55***	3.47**	0.21	0.14
	II° null AGR	0.43	0.79	24.72***	55.77***	3.67**	0.23	0.14

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. [a]MU, muscularity; BCS, body condition score; ECM, energy corrected milk; CR, conception rate; SCS, somatic cell score; RSD, residual standard deviation. [b]For either the MU or BCS effect. Based on absolute growth rates (AGR) trends reported by Buonaiuto et al. (30): onset of lactation = 5 days in milk; I° null AGR = 85 days in milk for MU and 45 for BCS; Max AGR = 180 days in milk for MU and 160 for BCS; II° null AGR = 280 days in milk.

the odds ratio generally indicates that animals with an average condition in terms of both muscularity and BCS are exposed to a lower risk of culling compared to cows with lower (sub-optimal) scores.

In particular, Italian Simmental dual-purpose cows with medium BCS at the beginning of lactation are more likely (1.3 times greater in the case of STAY1-2) to complete the lactation compared to those with lower condition (Figure 2A). From the moment of greatest recover of muscle and fat reserves (approximately at 180 DIM) to the moment after which animals lose again muscle and fat tissue (approximately at 280 DIM), dairy cows with medium conditions are more likely to stay in the herd, compared to cows with low condition (Figures 1, 2). An example is given by the odds ratios of STAY2-3 which are depicted in Figure 1B; in fact, the cows' muscularity had a strong impact on productive life, and therefore in the ability to stay in the herd. Indeed, at STAY2-3, cows whose muscularity was classified as high are less likely to stay in the herd compared to cows with a low muscularity, with odds ratio at DIM 5, 85, 180, and 280 being lower than unity and equal to 0.639, 0.690, 0.619, and 0.612. Conversely, at STAY2-3, cows with high BCS (Figure 2A) are more likely to stay in herd compared to cows with a low BCS, especially at 45 (odds ratio = 1.404) and 180 DIM (odds ratio = 1.310). The odds ratios (Figure 2C) show how, in parity 3, the BCS has a strong and significant impact on the cows' STAY (STAY3-4), with cows in the medium class being more likely to stay in the herd compared to those in the low class; the odds ratio at 45, 160, and 280 DIM was 1.470, 1.639, and 1.724, respectively. The same can be valid for cows with high BCS compared to those with low BCS (5 DIM: 1.129, 45 DIM: 1.102, 160 DIM: 1.005, 280 DIM: 1.100). During STAY3-4 cows with a medium muscularity condition are more likely to stay in the herd compared to cows with low muscularity, presenting an odds ratio of 1.569 at 5 DIM. At 280 DIM, the moment after which animals lose again muscle tissue, cows with medium BCS are significantly more likely (odds ratio = 1.724) to continue their career compared to those whose condition was classified as low. Similar result could be observed at STAY4-5 (Figure 1D1), in particular, cows with medium muscularity at onset of lactation (5 DIM) are more likely to stay in herd compared to cows with low conditions (odds ratio: 1.091). In general, the result depicts a fall in the probability to stay in the herd for cows with high muscularity and BCS conditions (Figures 1D2, 2D2). For example, cows with high muscularity conditions are less likely to stay in herd compared to cows with low conditions (odds ratio: 5 DIM: 0.806, 45 DIM: 0.784, 160 DIM: 0.775, 280 DIM: 0.872, Figure 1D2).

Potential reasons that can explain some of the results observed may be related to the status of negative energy balance (NEB) that commonly occurs in the periparturient period (54). Grummer et al. (55) estimated energy balance to be around−5.8 Mcal/d when cows are close to parturition, with peaks up to−20 Mcal/d during the 1st month of lactation. Plaizier et al. (56) reported that, in addition to NEB, cows can also experience a negative nitrogen balance in the 1st days after calving. During this phase, dairy cows, especially high-producing ones, cannot fulfill the energy deficit by increasing their feed intake (57). According to what has been reported by Straczek et al. (58), lactating dairy cows are characterized by high plasma levels of leptin, an anorectic hormone, directly related to a high loss of body condition caused by intensive lactogenesis. Therefore, cows are forced to mobilize body reserves, like fat and muscle tissue (33, 58–60). Indeed, even if body fat tissue is identified as the major body source of energy reserves, the catabolism of protein may also contribute to nutrient requirements especially in primiparous and/or early lactation (61). According to Komaragiri

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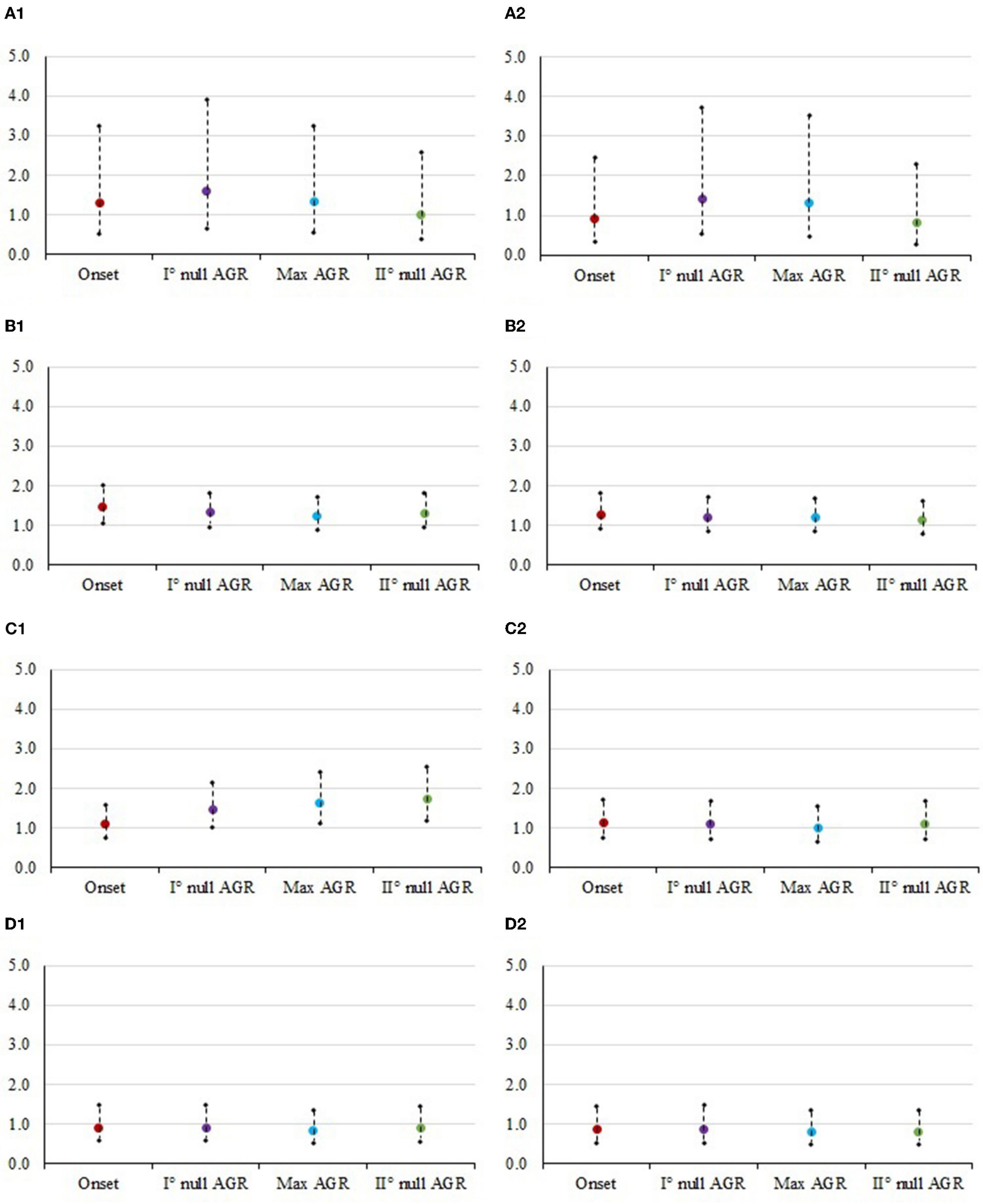

FIGURE 2
Odds ratios and 95% confidence interval for the risk of culling at each class of body condition score in different timepoints. The panels contain: (1) Mid vs. Low. **(A1)** STAY1-2, stayability as a primiparous cow, **(B1)** STAY2-3, stayability as a second-parity cow, **(C1)** STAY3-4, stayability as a third-parity cow, **(D1)** STAY4-5, stayability as a fourth-parity cow. (2) High vs. Low. **(A2)** STAY1-2, stayability as a primiparous cow, **(B2)** STAY2-3, stayability as a second-parity cow, **(C2)** STAY3-4, stayability as a third-parity cow, **(D2)** STAY4-5, stayability as a fourth-parity cow. For body condition score, timepoints were selected according to the absolute growth rates (AGR) trends reported by Buonaiuto et al. (30): onset of lactation = 5 DIM; I° null AGR = 45 DIM; Max AGR = 160 DIM; II° null AGR = 280 DIM.

TABLE 4 Odds ratios (95% confidence interval) for the risk of culling at each class of energy corrected milk, somatic cell score, or conception rate estimated from the different time points[a].

Trait	Moment[a]	Energy corrected milk[b]		Conception rate[c]	Somatic cell score[b]	
		Medium	High	0	Medium	High
STAY1-2	Onset	0.55 (0.21–1.42)	0.36 (0.14–0.93)	1.54 (0.85–2.78)	1.61 (0.68–3.83)	1.11 (0.52–2.40)
	I° null AGR	0.55 (0.21–1.42)	0.37 (0.15–0.95)	1.53 (0.85–2.77)	1.61 (0.68–3.84)	1.13 (0.53–2.44)
	Max AGR	0.55 (0.21–1.43)	0.37 (0.15–0.96)	1.55 (0.86–2.80)	1.66 (0.70–3.96)	1.13 (0.53–2.44)
	II° null AGR	0.45 (0.16–1.29)	0.33 (0.11–0.94)	1.56 (0.83–2.92)	1.57 (0.62–3.99)	1.02 (0.44–2.37)
STAY2-3	Onset	5.34 (3.91–7.29)	4.60 (3.40–6.23)	0.35 (0.28–0.43)	1.29 (0.95–1.74)	1.01 (0.76–1.36)
	I° null AGR	5.33 (3.90–7.27)	4.62 (3.41–6.26)	0.35 (0.28–0.43)	1.28 (0.95–1.72)	1.01 (0.75–1.35)
	Max AGR	5.27 (3.86–7.19)	4.61 (3.41–6.24)	0.35 (0.29–0.43)	1.30 (0.96–1.75)	1.00 (0.75–1.35)
	II° null AGR	5.28 (3.87–7.21)	4.60 (3.40–6.23)	0.36 (0.29–0.44)	1.28 (0.95–1.72)	1.00 (0.75–1.34)
STAY3-4	Onset	5.65 (3.90–8.16)	6.26 (4.29–9.14)	0.35 (0.28–0.45)	1.64 (1.16–2.32)	1.21 (0.86–1.71)
	I° null AGR	5.64 (3.90–8.15)	6.18 (4.24–9.02)	0.35 (0.28–0.45)	1.62 (1.15–2.29)	1.22 (0.86–1.72)
	Max AGR	5.79 (4.00–8.39)	6.39 (4.37–9.34)	0.35 (0.28–0.45)	1.64 (1.16–2.32)	1.24 (0.88–1.75)
	II° null AGR	5.71 (3.94–8.25)	6.30 (4.31–9.20)	0.35 (0.27–0.44)	1.63 (1.15–2.31)	1.24 (0.88–1.75)
STAY4-5	Onset	6.11 (3.86–9.66)	7.32 (4.53–11.83)	0.32 (0.24–0.43)	1.80 (1.17–2.78)	1.23 (0.80–1.88)
	I° null AGR	6.03 (3.82–9.52)	7.23 (4.74–11.67)	0.32 (0.24–0.43)	1.83 (1.19–2.82)	1.23 (0.80–1.87)
	Max AGR	5.96 (3.78–9.39)	7.22 (4.48–11.64)	0.32 (0.24–0.44)	1.86 (1.21–2.86)	1.23 (0.80–1.87)
	II° null AGR	6.03 (3.82–9.51)	7.21 (4.47–11.61)	0.32 (0.24–0.43)	1.90 (1.23–2.94)	1.25 (0.82–1.91)

[a]For either the muscularity (MU) or body condition score (BCS) effect. Based on absolute growth rates (AGR) trends reported by Buonaiuto et al. (30): onset of lactation = 5 days in milk; I° null AGR = 85 days in milk for MU and 45 for BCS; Max AGR = 180 days in milk for MU and 160 for BCS; II° null AGR = 280 days in milk. [b]"Low" is the reference class. [c]CR = 1 (reference class) indicates that only a single insemination was needed to achieve pregnancy, otherwise CR = 0.

et al. (62), during early lactation a cow can lose around 20 kg of muscular tissue and between 8 to 57 kg of body fat. In particular, van der Drift et al. (54) found out that fat mobilization that starts immediately after calving continues up to the 8th week after parturition. Also Schäff et al. (63) observed that skeletal muscle mobilization takes place, starting immediately after calving, but the duration was shorter. In fact, it stopped at about 5 weeks postpartum, with a peak mobilization rate during the first 2 weeks of lactation (63). Findings by Megahed et al. (60) are in accordance with our results, particularly with the fact that body condition of cows facing up to the second calving is crucial to deciding their survival in the herd. Indeed, Megahed et al. (60) reported a greater periparturient mobilization of backfat and skeletal muscle in primiparous than in multiparous cows. The reasons for such greater mobilization in younger animals could be related to the reason they have not finished their growth yet (64) and that they have to cope also with growing requirements, in addition to production and maintenance. Straczek et al. (58) reported that Simmental cows have a greater capacity to adjust the NEB state compared to Holsteins, restoring earlier the BCS loss after the lactation peak. Consequently, cows with good conditions at the onset of second lactation are more prone to perform better along the lactation and to be more resilient to the different metabolic disorders and reduced fertility (65). It is worth considering that farmers rearing Simmentals may decide to cull cows with higher muscularity at a certain point for beef purposes in order to increase the herd profit. Although cows with high BCS are more likely to stay in herd compared to those with low BCS (Figure 2B), the odds ratios for cows with medium BCS were always the highest. Similar results were observed by Erdem et al. (33), who suggested that rearing cows with moderate BCS conditions can be considered an important approach for the herd management. This implies that farmers prefer to cull fat cows to leave space for animals with a medium condition. Probably, dairy farmers are interested to rear cows with appropriate BCS (around 3.0 on a 5-point scale) because these parameter plays an important role in maintaining the health status of lactating cows. As reported by Yasothai (66), dairy cows presenting a severe BCS loss during lactation are exposed to several reproductive problems resulting in longer intervals between first ovulation and estrus, more days open, and lower first-service conception rates. Moreover, literature demonstrates that dairy cows with BCS greater than 3.5 tend to exhibit several metabolic disorders, such as hypocalcaemia, fatty liver, oxidative stress and ketosis (67–70). In addition, fat or over-conditioned dairy cows are at higher risk of developing a combination of metabolic, digestive, infectious and reproductive conditions known as the "fat cow syndrome" (71, 72). Bahrami-Yekdangi et al. (73) reported that in over-conditioned cows (BCS > 3.75; odds ratio = 1.27) the incidence of dystocia was larger than in other cows. An excessive accumulation of body fat predisposes to more insulin resistance, especially during the prepartum, a metabolic disorder with characteristics similar to human type 2 diabetes (74, 75). A transitory phase state of insulin resistance is generally considered a homeorhetic adaptation during early lactation, which provides glucose supply to the mammary gland limiting glucose utilization by insulin-responsive peripheral tissues, such as skeletal muscle or adipose tissue (76). Furthermore, insulin resistance can increase lipolysis of adipose tissue, and the accumulation of non-esterified fatty acids leads in turn to increased insulin resistance. In addition, the high culling risk generally observed for fat cows could be related to the negative relationship between high BCS and milk production (77).

Conclusions

In the present study data of Italian Simmental cows were used to investigated the relationship between STAY and type traits, namely muscularity and BCS. The results indicate that cows characterized by a medium BCS/muscularity are more likely to stay in the herd compared to those with extreme body conditions, i.e., they are more likely to close the lactation and then start the subsequent one. Results of this study provide new insights into the survival and culling of Italian Simmental cattle population. Apart from productivity, in dual-purpose cows type traits and STAY are connected, being indicators of direct voluntary culling with a direct effect on farm's profitability. Further studies should disclose genetic architecture of STAY taking into account muscularity, BCS, and productive performance.

Data availability statement

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

Ethics statement

This study did not require manipulation or modification of the usual handling of the animals, since we have worked directly with the routine records provided by the breeders' associations.

Author contributions

Conceptualization: AF, LD, and GV. Methodology: GV and LD. Software: AC and GV. Formal analysis and writing—original draft preparation: GB, GV, and AC. Data curation: LD, AC, GB, and GV. Writing—review and editing: GV, GN, AC, and NL-V. Visualization: AF, DC, AP, and LM. Supervision: GV, AF, and NL-V. Project administration: GV and AF. All authors have read and agreed to the published version of the manuscript.

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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.

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Welfare implications on management strategies for rearing dairy calves: A systematic review. Part 1–feeding management

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Introduction: Calves are very susceptible to stress in the early stages of life, and it is necessary to ensure maximum welfare. Feeding management has been identified as a major risk factor for calf health and welfare at this stage. However, the management protocol for calf rearing and its impact on animal welfare is unclear. A systematic review of different management strategies for rearing dairy calves according to the three spheres of animal welfare was conducted using an electronic search strategy. In this review, management strategies were studied to identify scientific gaps, to know the welfare problems of these animals in order to prioritize actions and future research and to study the interpretive approach of this management from the three welfare spheres.

Methods: A protocol was used to analyze and extract information from the studies. Of the 1,783 publications screened, only 351 met the inclusion criteria for the management or welfare of calves' items.

Results: The publications identified in the search can be divided into two main groups feeding and socialization, based on the main topic of the publication. The main topics that emerged from the search in the feeding management group were milk replacer, colostrum, and weaning, divided into the three main areas of biological functioning and health, natural life and affective states or cognitive judgement.

Discussion: The main issues to be addressed were the different types of feed consumed by animals from birth to weaning and the weaning management. It has been found that the most researched issues are colostrum and solid starter feed management. Unresolved issues were highlighted, such as the lack of a clear protocol for the administration of milk replacers to reduce hunger and the best management of weaning to reduce stress.

KEYWORDS

rearing calves, Holstein calves, welfare, feeding management, animal production, dairy sector

1. Introduction

One of the major challenges in livestock production's is to ensure animal welfare at all stages of rearing. In dairy cattle, calf rearing is one of the most challenging aspects of animal welfare and the second-highest variable cost after feeding (1). Furthermore, optimizing calf rearing has a massive impact on the future production of the cow, thus making it a key issue for welfare, production, and economic sustainability.

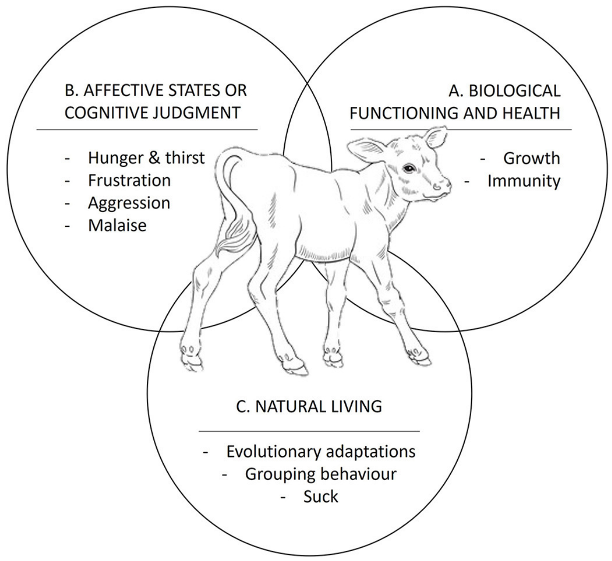

FIGURE 1
The three spheres of animal welfare and corresponding keywords: **(A)** biological functioning and animal health, **(B)** affective states or cognitive judgement, and **(C)** natural living (Adapted from 9).

To ensure animal welfare, it is necessary to know how to assess it. There have been significant changes in the assessment of animal welfare in recent decades. The current scientific approach to animal welfare by science is not yet standardized. Although there is a scientific process and an increasing consumer demand for animal welfare, regulations are only focus on the basics (2, 3). One of the reasons for this lack of specific regulation may be the lack of consensus on the concept of animal welfare. In recent years, there has been an evolution from avoiding negative experiences to exploring positive experiences for animals, recognizing that good welfare, a "*good life*," is not only about preventing negative states, but also about promoting positive experiences and emotional states (4–6). Positive animal welfare and its evaluation emphasizes resources valued by animals, positive emotions, and the natural behaviors that animals are motivated to perform (5).

It is therefore essential to define animal welfare before evaluating any management strategy. Animal welfare indicators can be grouped into three basic concepts (represented by spheres) first defined by Fraser et al. (7) and later adapted for dairy cattle by von Keyserlingk et al. (8). The three key spheres are (i) biological functioning and health, where good health indicates the correct physiological functioning of the animal; (ii) affective states or cognitive judgement, which considers how the animal feels when experiencing and perceiving its environment (8); and (iii) natural life, which refers to the evolutionary adaptation suffered of the animal to its environment, such as gregarious behavior (9) (Figure 1).

Ensuring optimal welfare in all three spheres during the rearing period has a direct impact on calf development. This is important because it has been shown that optimal development during the early stages of an animal's life influences its future (10), for example, neonatal diarrhea and other neonatal parameters have an economic cost and are associated with adverse effects on future cow production and reproductive performance (11, 12). This concept implies that rearing a healthy calf up to puberty under the highest welfare conditions will result in optimal production in future lactations (13). Growth rate during the first 6 months of life has been shown to be a direct determinant of age at first calving (14). In addition, body weight at first calving is associated with higher milk yield in the first lactation (15). Therefore, the efficiency of the dairy system can be improved through optimal calf rearing, a lower age at first calving, optimized future performance (16, 17), reduced rearing costs and shorter non-productive periods.

Dairy calves are highly susceptible to stress throughout their rearing period, but the most critical period is before weaning. There are many stressors during the pre-weaning period. The first stressor is the separation from the mother (18) and the potentially negative effects of human-animal interaction (19). Later in the animal's life, transport to a new location (20) and other management practices, such as pain during the disbudding (21), discomfort due to suboptimal housing conditions, and the limited opportunities for social interaction with their conspecifics (22), can also affect animal welfare. In addition, dietary management is key to the proper physiological and immune development of the animal (23). However, it is necessary to examine the interpretation of this management from an animal welfare perspective.

Significant changes in calf management have occurred over the last few decades, and many different realities have coexisted (24, 25) due to the diversity of production systems around the world. Despite the existence of some calf rearing guidelines such as FAO (26) and NASEM (27), there is little research on how management or the lack of an appropriate management affects welfare. Farm management strategies need to accurately identify, target, and intervene when different calf stressors occur. Focusing on feeding programs (16, 28) and social management (20) are high potential strategies that farmers can implement to avoid welfare problems. It is also important to address the lack of standardized and universal good management practices related to the welfare of dairy calves.

However, there is an unclear protocol available in the literature to ensure the highest welfare from feeding and social management strategies for preweaned dairy calves to have a base on which all realities can be established. In addition to studying how each of the management strategies affect the three spheres of animal welfare. Furthermore, no literature review has been undertaken to examine all these issues.

For these reasons, the first part of this systematic review was undertaken to systematically map the research in feeding management strategies and identify any existing scientific gaps in knowledge. This work is also intended to prioritize actions and future research, as well as exploring the interpretive approach to this management. However, such a comprehensive review is lacking in the current state of knowledge. The following research question was formulated: What is known from the literature about the feeding management of preweaned calves and how does it affect welfare? What needs to be investigated?

TABLE 1 Approach and structured steps used to search the literature for this review.

	Three spheres of animal welfare		
	Biological functioning and health	Affective states or cognitive judgment	Natural living
Population	Dairy OR calf OR calve*		
Intervention	farm* OR wean* OR rear* OR "milk feed*" OR starter Or colostrum OR additi* OR "solid feed*"	"individual hous*" OR "pair hous*" OR "milk bucket" OR bottle* OR deprivat* OR enrich*	"early separat*" OR "pair hous*" OR mother OR separat* OR "milk bucket" OR bottle* OR "social group*" OR "social environment*" OR nipple
Comparison	health OR disease* OR infecti* OR disorder* OR mortality OR longevity OR liveability OR pathogen* OR phatologic* OR cull* OR metabolic* OR perform* OR "body condition*" OR develop* OR immun* OR environment OR ruminat* OR rumen*	behavio* OR stereotyp* OR environment* OR "fear test" OR "open field" OR "novel object test" OR "restrain test" OR "behavio test*"	behavio* OR stereotyp* OR environment* OD "maternal bond*"
Outcome	perform* OR feed OR milk OR consumption OR intak* OR starter OR "body weight" OR weaning* OR OR growth OR "early digest*" OR APPs OR cortisol	fear OR hunger OR learning OR stress OR cortisol OR aggressi* OR optimist* OR possitiv* OR react* OR upset* OR cognit* OR judg* OR pain* OR mal* OR discomfort* OR thirst* OR anxiet* OR affect*	behavio* OR "social interact*" OR activ* OR "social buffer*" OR explorat* OR aggressi* OR upset* OR playful* OR suckling* OR adapt* OR group* OR greg* OR play* OR rest* OR voc*

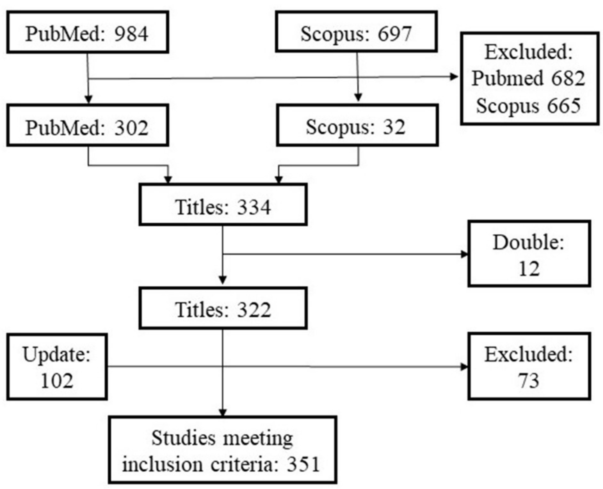

FIGURE 2
Search process for identifying publications on feeding and social management strategies in calf rearing.

2. Materials and methods

A systematic review was used to address our research objectives. The literature search was conducted according to the PRISMA guidelines (29). PRISMA stands for Preferred Reporting Items for Systematic Reviews and Meta-Analyses. These guidelines provide an evidence-based minimum set of items for the methodology and identification of publications and reporting in this review.

2.1. Search terms and search strategies

As a first step, the authors discussed the objectives of the search and the inclusion/exclusion criteria. It was decided to identify preweaned calves' feeding and social rearing strategies and to analyze their impact on the three welfare domains. Other management issues, such as disbudding, transport, or veterinary treatment, as well as more specific issues, such as milk composition or osmolarity, were not investigated. The search included literature published between the years 1975 and 2022. Only studies published in English and with a full scientific text available were included.

The search terms were defined using the PICO approach (population, intervention, comparison, and outcome) (30), modified for the study objectives (Table 1).

2.2. Data extraction and search process

The searches were performed on May 27th, 2021. The defined search terms resulted in two databases in Pubmed and Scopus, which yielded 984 publications (Pubmed) and 697 (Scopus). This means that the search identified 1,681 publications as potentially relevant. An update of the search was performed on July 26th, 2022, just before the manuscript was finalized, using the same search terms but restricting the search to the period after the original searches were performed, thus including literature between May 27th, 2021, and July 26th, 2022, and yielding 102 new results.

After the initial search, the publications were scanned in several steps (see Figure 2). The papers were transferred to Abstrackr (31), a web application that facilitates the screening of systematic reviews by title and abstract. The publications considered relevant in terms of management or welfare issues in each of the Abstrackr filters were combined, resulting in a single dataset of 334 publications. These studies were included in a database with title, authors, journal, year of publication and DOI.

The same person (first author) filtered all the papers, and each author double-checked for each 25% of the papers. In this study, the level of agreement between the authors was 86.3%, with 80% or more being strong agreement, as reported elsewhere (32).

The updated search identified an additional 102 publications. Only 29 of the new articles were considered as relevant. The 80.2% were excluded throughout the review as they did not meet the

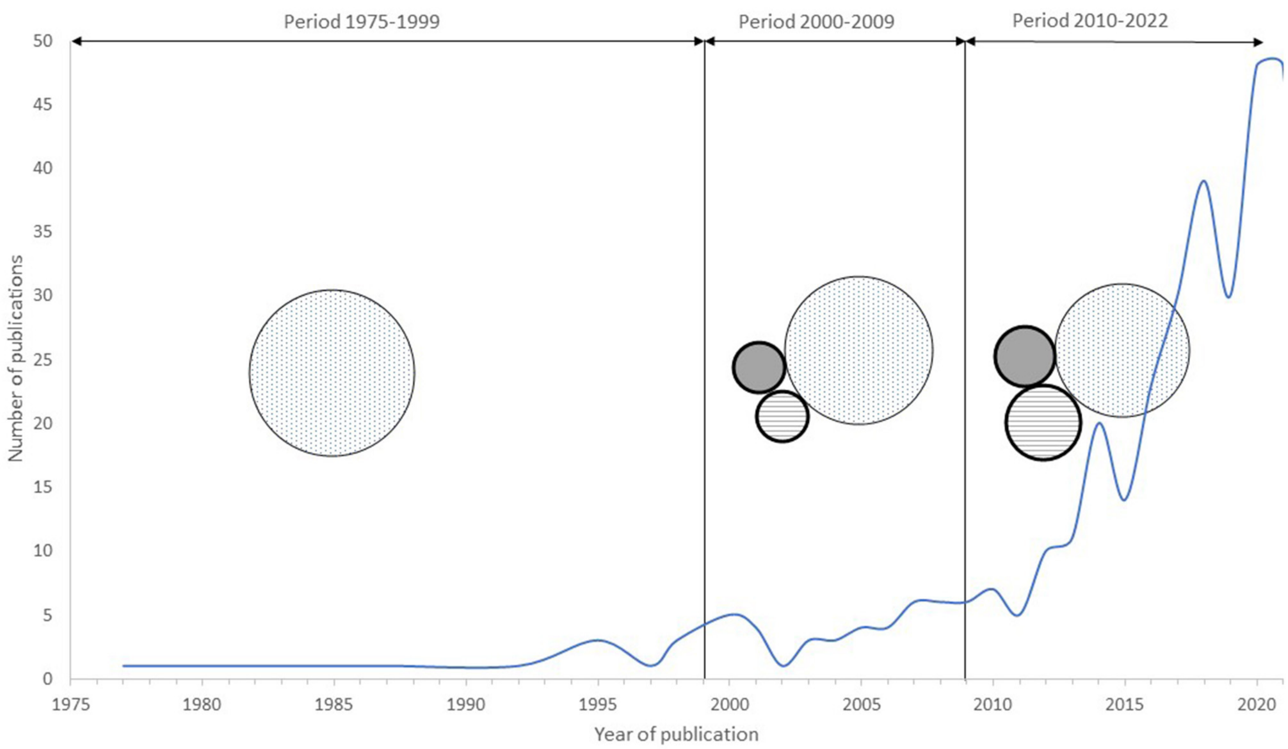

FIGURE 3
Distribution of publications according to the year of publication and three spheres of animal welfare. The blue line represents the number of publications per year related to animal welfare during the rearing of Holstein's calves. The size of spheres is weighted by the percentage of publications related to each animal welfare sphere. Dotted spheres represent biological functioning and health, spheres with horizontal lines refer to natural living, and gray spheres refer to affective states or cognitive judgements.

inclusion criteria. Abstracts were removed if they did not relate to the welfare, feeding, or social management strategies of dairy calves.

After screening the titles and abstracts, the search results were refined using the screening tool "Rayyan" (33), where duplicates were removed and 322 publications were relevant to be included as results of the systematic review search. Each of the remaining publications was examined by reading the abstract and categorized according to animal welfare sphere and management resources. For animal welfare, publications were grouped into three spheres of biological functioning and health, affective states or cognitive judgement and natural living. As there is an interrelationship between the spheres, when publications addressed welfare from more than one area, they were included in the corresponding groups. Clustering was done according to: colostrum, milk replacer, started feed, weaning, mother bonding, social interaction, and human interaction. After a full reading of the abstract, a complete reading was performed to sort into the correct category if this information was unclear. After updating and screening, 351 studies met the inclusion criteria, as shown in Figure 2.

3. Results

3.1. Study characteristics

Based on the available scientific publications, there has been a noticeable upward trend since 1975 which continues up to the present day, with 82% of the publications having been published in the last 10 years (62% of which have been published in the last 5 years). The scientific research can be broadly grouped roughly under three broad, interrelated headings of welfare: 68.1% relates to biological functioning and health, 18.9% to natural living, and 13% to affective states or cognitive judgement. However, publications with the last two major components were published very recently, in the last decade. From 1975 to 2000, all the publications were related to biological functioning and health. In 2001–2010, 80.5% corresponded to biological functioning and health, 9.7% to natural living, and 9.8% to affective states or cognitive judgment. In particular, in the last interval from 2011 to 2022, 65.2% of the publications covered biological functioning and health, 20.9% natural living, and 13.9% affective states or cognitive judgment (Figure 3).

The 351 studies were published in 50 journals representing 49% of the Journal of Dairy Science articles. Preventive Veterinary Medicine represents 5.9%, Animals 5.4%, Journal of Dairy Research and PLoS One 3.7% each, and Frontiers in Veterinary Science 1.7%. The remaining 30.6% is spread over 44 other journals.

In this first part, we analyze all the feeding management techniques and their impact on welfare. According to the specific topic addressed, the publications can be classified, from most to least number, into general management (22.5%), milk replacers (20.5%), colostrum (19.7%), social interactions (16.9%), weaning (8.8%), mother bond (5.4%), started feed (3.4%) and human-animal relationship (2.8%) (Figure 4). Although many topics were addressed, even when dealing only with management practice were considered, the studies could be divided into two main groups according to the nature of the practices: (i) feeding and (ii) social management. As these groups are so large, they are considered separately.

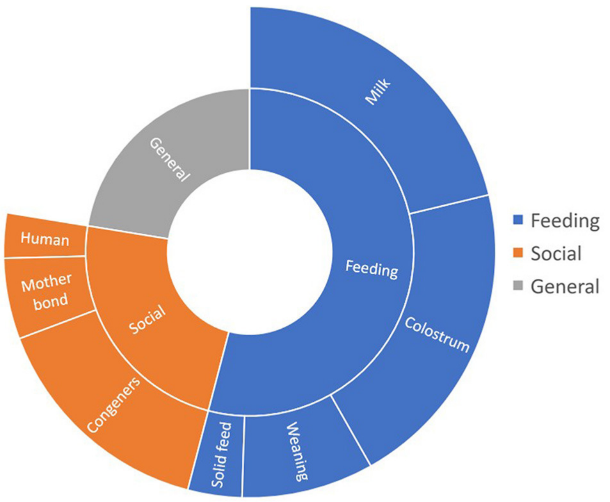

FIGURE 4
Distribution of papers by topic of publications when searching for feeding and social management strategies. The size of each is proportional to the number of publications found.

3.2. Synthesis of results of feeding management

Feeding in the early stages of calf life is critical for good development. Several studies have investigated the effect of feeding management techniques during the early stages of calf development, particularly in preweaned calves. Compared to the framework of the three spheres, all the different steps of feeding management have been studied in a compartmentalized manner. Under the umbrella of feeding management each component of colostrum, liquid feed, solid feed starter, and weaning strategies are evaluated and analyzed in Figure 5.

4. Discussion

Despite the importance of the neonatal and infant period for appropriate physical, behavioral, and cognitive development into adulthood (34), the literature review over the last two decades has produced many publications on different management strategies, but few studies from the perspective of the three welfare domains.

However, there has been a shift in the approach to animal welfare assessment, incorporating animal-based indicators related to affective states and natural living. The application of this new welfare knowledge will improve the daily lives of animals.

4.1. Feeding management for welfare

Feeding management during the first period of calf life is crucial to ensure their development, welfare, and productivity

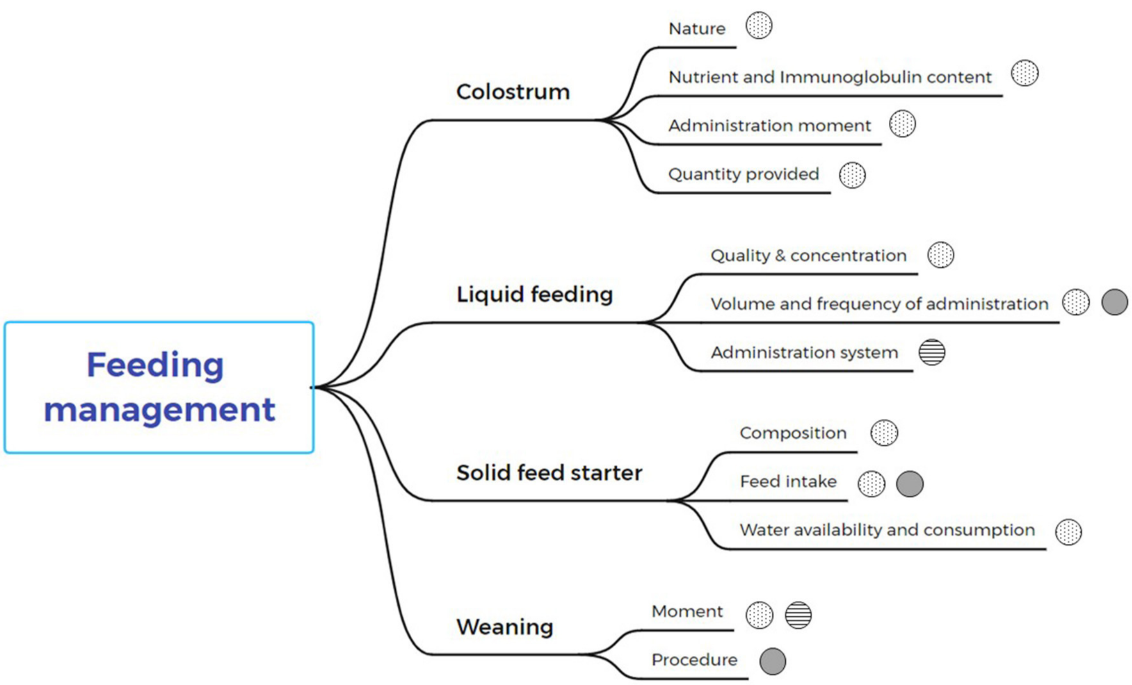

FIGURE 5
Critical points of each phase of feeding management are identified and their relationship to animal welfare spheres. Dotted spheres represent biological function and health, spheres with horizontal lines relate to natural living, and gray spheres relate to affective states or cognitive judgement.

TABLE 2 Cut-off points for passive immunity transfer in calf serum according to total serum protein and IgG concentration from different review studies and the equivalence measured with a Brix refractometer.

	Failure	Fair	Good	Excellent	Authors
Total serum protein (g/l)	<52		>52		(42–44)
	<58–63		>58–63		(45)
IgG concentration (g/l)	<10				(46–48)
	<10	10–18	18–25	>25	(35, 49)
	<20–25	>20–25			(45)
Brix (%Brix)	<8.1%	8.1–8.8%	8.9–9.3%	>9.4%	(35)

(23). The effect of feeding strategies on the development of preweaned calves has been reported in several of the papers reviewed in this analysis. In addition, the effect of each feeding management practice on animal welfare has been investigated in several publications. Thus, as shown above, in Figure 5, the key aspects of each feeding management are explained from the (a) correct colostrum administration (35), (b) liquid feeding until weaning (36, 37) (c) feeding with solid starter feeding (38, 39) and (d) weaning management (40).

4.1.1. Colostrum management

According to the studies reviewed, colostrum intake affects welfare from a biological function and health perspective, as it is essential for the immunity of the calf. Publications have shown that it is crucial to provide sufficient quantities of high-quality colostrum with nutritional and immunoglobulin content and to achieve this immunity in the 1st h of life. High-quality colostrum has an IgG concentration >50 g/L (41). In the studies reviewed, two approaches were used to assess the impact of colostrum on calf immunity, colostrum characteristics and passive immunity assimilation in the calf (see Table 2) (50).

In terms of colostrum characteristics, maternal and commercial substitutes have been studied as two types of colostrum (according to their nature). Commercial substitutes have adequate IgG absorption and are less likely to be microbiologically contaminated (51). However, when maternal colostrum is offered, calves show increased growth at weaning, improved immune and metabolic development, and higher of blood IgG concentrations (51–53). The quality of maternal colostrum varies depending on the individual cow and environmental management factors. For example, several studies have shown that multiparous cows produce better colostrum than younger cows, as it has a higher concentration of IgG and better nutritional properties (46, 54). However, a proper vaccination protocol and adequate dry cow feeding are essential to reduce passive transfer failure (54).

The time of collection and the time between collection and administration are also important. If the quality of the colostrum is poor or if it is administered at an inappropriate time, the transfer of passive immunity will fail. This leads to a decline in the wellbeing of the biological function and health. Therefore, the longer it takes to collect the colostrum after calving, the lower the IgG concentration will be (46), and its administration to the calf must be carried out in the shortest possible time (55).

If colostrum cannot be administered immediately, hygiene and storage practices are considered key factors. Under poor hygienic conditions, colostrum may be bacterially contaminated (50, 55). If it is not possible to maintain optimal hygiene, heat treatments such as pasteurization at 60° for 60 min (41, 50, 51) or high-pressure treatment at 400 MPa for 15 min (56) can be used. These treatments reduce the concentration of pathogenic bacteria and maintain IgG quality (56, 57). In addition, colostrum can be stored frozen as freezing and thawing do not affect IgG concentrations as long as thawing is performed au bain-marie and the temperature does not exceed 40°C (58).

It is also the key to assessing colostrum quality. The Brix refractometer is an accurate, acceptable, and rapid tool for assessing colostrum quality evaluation tool with excellent repeatability (59, 60). Accordingly, colostrum can be classified as good if >22% Brix and poor if <18% Brix (61). It is important to note that mixing poor quality colostrum with good quality colostrum is not recommended (62, 63). Although the quality of colostrum has been extensively studied, its relationship to the quantity to be administered has not been established. Therefore, increasing the amount of colostrum, reducing the time between birth and colostrum administration, or increasing the amount of whole milk after colostrum have been recognized as good practices (42, 62, 64) and improve welfare from a biological function and health perspective.

As mentioned above, the characteristics of the colostrum are as important as the immunity assimilation of the calf. The success or failure of passive immunity transfer has been extensively studied (35, 43, 46). For example, a relationship has been found between successful passive transfer and a lower likehood of developing enteric or respiratory disease has been found (65). In addition, lower concentrations of IgG and total serum protein in the first 3 days of life are associated with reduced growth rates (43, 46). Based on the literature reviewed, the cut-off values for transfer failure and the calf serum IgG concentration measured with a Brix refractometer are shown in the table below (Table 2).

All of the above mentioned assumes that good quality and quantity of colostrum is essential for calf rearing and to ensure welfare from a biological function and health perspective at this stage (42, 49, 66). In short, the best colostrum management protocol, with less passive transfer failure, is administer a volume of high-quality colostrum that is equivalent to 10–12% of their body weight in the first 2h and an additional meal corresponding to 5% of body weight 6–8 h later to reduce morbidity and mortality (67).

4.1.2. Liquid feeding management

According to the studies reviewed, the management of liquid feeding affects animal welfare in all three spheres (23). From the point of view of biological function and health, it is essential to provide liquid feeding of good quality, concentration and volume so that the animal is well nourished. In addition, the amount of liquid feeding and the frequency of feeding will affect affective states or cognitive judgement, as calves properly fed should not suffer from hunger. The delivery system also affects the natural living sphere, as nipple-feeding is more similar to natural sucking behavior.

Calves must be adequately fed to meet their nutritional requirements and to support the development and maturation of the gastrointestinal tract's, allowing the calf to digest and absorb nutrients (23, 38, 68). Insufficient milk intake slows postnatal growth and can affect the development of organs such as the intestines and the mammary glands (23). Liquid feed intake also influence solid feed intake (69) and calf growth. According to Soberon et al. (70), the higher the average daily gain during preweaning, the more milk will be produced in the first lactation. Epigenetic programming, which is still under investigation, suggests that diet is one of the most important environmental factors influencing the genetic expression of milk production (70). However, the optimal feeding strategies (38) are highly uncertain in the studies reviewed. In addition, adjustments to the management in calf feeding practices will inevitably be required. At this stage, different alternatives have been studied, taking into account the type of liquid diet and supplement, the amount and concentration, the frequency of administration or the method of administration. Regardless of the strategy adopted, correct implementation of hygiene is essential to prevent health problems in calves, reduce the burden of pathogenic bacteria and break the chains of infection. For example, there are several studies that focus primarily on the cleaning of artificial nipples and buckets, as these are presented as the central critical point (71).

On the other hand, no significant improvement in calf development was found in relation to milk type. The reviewed publications have focused on the use of a milk replacer, transition milk (72), or discarded milk (73). However, when using milk replacer, the most critical factor is to maintain a protein content above 28% (74, 75), as milk protein content is directly related to daily gain (76). Fat content must be maintained in the range of 17–25% (72, 74, 77). It is important that the milk replacer is of high quality, as poor-quality milk replacers can affect welfare through morbidity (diarrhea) and also hunger through starvation (78).

There is a wide variety of feeding protocols in the reviewed bibliography, and there is no consensus on the best practice. Traditionally, restricted feeding has been used to promote solid feed intake, but these restrictions have resulted in malnutrition and immunosuppression (38), contributing to a negative welfare status. In contrast, other authors have investigated *ad libitum* milking administration protocols, with growth benefits but delays in rumen development as animals consume less solid feed (79, 80). Therefore, a balance needs to be found between encouraging the calves to start eating solids and avoiding starvation if they are fed with milk only. Other protocols involving the amount, frequency or concentration of milk have also been reviewed. For example, feeding 20% of the calf's bodyweight in milk has been shown to reduce feed intake and rumen development before weaning (23, 81). Alternatively, rumen development is better at 10% of the bodyweight (81). In contrast, some protocols provide an amount of milk regardless of body weight, with varying amounts and concentrations, as shown in Table 3. The optimal number of dosed meals per day is not known (86).

TABLE 3 Quantities and concentrations of milk fed to calves, according to different studies.

Quantity	Concentration (powdered milk)	Author
6 L/d	750 g/d	(80)
5–9 L/d	–	(82)
4.4 L/d	660g/d	(77)
6–8 L/d	–	(83)
3.8 L/d 3.8, 5.6, 7.2 L/d	454 g/d 454, 681, 908 g/d	(84)
4.7 L/d	660 g/d until 39 d 330 g/d since 42 d	(52)
9L/d during 3–28 d 5l/d during 29–42 d	941 g/d 778 g/d	(85)

Several authors have pointed out that the feeding protocol has a significant impact on welfare. Depending on the protocol, the calves may suffer from hunger or frustration, which would worsen animal welfare at affective states or cognitive judgment and natural living spheres. In order to know whether the animals are hungry, non-nutritive oral behaviors (87, 88), cross-sucking (89) and vocalizations (90, 91) could be studied. In addition, when animals do not feel hungry, they engage in more locomotor play, which is a positive indicator of welfare (82, 85). Despite the lack of a clear protocol on the amount, concentration, and frequency of administration in the review results, several authors have reported better results in terms of health and growth outcomes with fixed amounts of liquid feed at higher nutrient densities throughout the lactation period compared to a gradual increase (74, 83, 84).

Regardless of the protocol used, there are several ways to offer milk. Bucket feeding is far removed from the natural sucking behavior of the animal, and teaching animals to drink from a bucket requires training and effort. Up to 60% of calves know how to drink milk from a bucket at 3 days of age t (92). Another option is to use bottles with nipples, which are more compatible with the natural living sphere. With this method, animals show less non-nutritive sucking (88). In addition, throughout the literature reviewed, the method of feeding has been modernized with the introduction of automatic milk feeders, which are introduced to animals at around 5 days of age and can be housed in groups of 10–15 calves (93, 94). These feeders accurately control animal milk intake (95), but their effect on calf welfare is still being investigated.

For all of the above, the authors emphasize the need to provide good quality milk and choose an appropriate feeding protocol, with a fixed amount of milk offered at the beginning and gradually reduced as weaning approaches, to meet the calves' nutritional needs of the while avoiding hunger (38, 96). It is also important to monitor animal behavior to know if they are hungry if there is an increase in vocalizations or non-nutritive oral behaviors. Further research is needed to know the best amount, concentration, and frequency that ensure the best animal welfare in the three spheres.

4.1.3. Solid feed starter management

The literature reviewed shows that solid feed management has a significant impact on growth and welfare. At the level of affective states or cognitive judgement, correct feed management helps to reduce hunger or digestive discomfort. Diet composition, intake and water availability are essential for ruminal development, and therefore affect animal welfare through the biological function and health sphere.

Proper rumen development during the preweaning is critical. Solid feed intake plays a fundamental role in rumen development and maturation. The milk feeding protocol has a major influence on solid feed intake, and high liquid diet feeding programmes may compromise solid feed intake in the first few weeks of life (38, 68, 69). The most important factor in promoting solid feed intake is the decrease in milk available after 40 days of age, as this can lead to malnutrition before this time (96). In addition, social contact, which will be discussed in more detail in Part Two, also appears to influence intake, with social animals consuming more solid starter feeds (97).

A solid diet should provide the protein and energy necessary for calf growth (an average of 23.4% protein and 32.3% starch on a dry matter basis) (39, 98). In addition, the method of feeding, the palatability of the solid food, and the amount consumed are also important for the calf growth and the avoidance of digestive distress (98) which would reduce the welfare at the level of affective states or cognitive judgement.

In addition to starter feed, calf feeding practices should include the provision of water *ad libitum* to maximize starter intake and weight gain. Weight gain is reduced when animals are deprived of water (99), and animal welfare deteriorates (21).

On the other hand, it is currently debated whether the inclusion of forage in the starter diet can benefit calves (39). Forage feeding has been promoted from a welfare perspective. Some authors report benefits such as alleviation of ruminal acidosis, promotion of ruminal microbial diversity and abundance (100) as well as higher average daily gain. The importance of feeding hay not only for rumen development, but also for reducing stress during the weaning process (101). Others have found negative effects of including hay, such as a reduction in solid starter consumption (39), which is crucial because when calves have consumed enough starter, it is time to wean them (102).

4.1.4. Weaning management

Weaning has also been the subject of much research, as it is a turning point in the intensive calf feeding management and can cause a great deal of stress. Weaning is a very stressful event for the animals and a challenge for the farmer (103, 104). It also affects animal welfare at the level of biological functioning and health as it radically changes the diet and the calves need to have a proper rumen development. From the point of view of affective states or cognitive judgement, the procedure used to carry out weaning can cause anxiety and frustration. Finally, at the level of natural living, this event causes behavioral changes in the calves.

TABLE 4 Weaning strategies and their effects that each of them has on the calf, according to the different authors.

Weaning strategy	Effect	Authors
Wean for age	Easier farm management	(96, 107)
Wean for solid feed intake	Ensured ruminal development	(102, 105)
Abruptly weaning	High stress Not accustomed to eating solid feed	(103)
Gradual weaning	Higher feed consumption Less abnormal behavior	(96) (109)
	Greatest underlying frustration	(103)
Dilute weaning	Less frustration	(110)

Weaning is the most important nutritional transition for young calves. On intensive dairy farms, calves are weaned earlier than in the wild, where weaning occurs at around 6 months (104). In the studies reviewed, it was found that the timing of weaning can be decided the basis of two main parameters in order to minimize adverse effects. Either it can be programmed according to the age of the animal or the amount of solid starter food consumed (105). In addition, weaning can be managed gradually (removal of feed), by diluting the milk, or abruptly by removing access to liquid feed (106).

As explained above, milk restriction is commonly used to encourage solid food intake to facilitate early weaning, but it can compromise calf growth if done too early (107). The earliest age at which this procedure can be done is 40 days, as it can cause malnutrition if done earlier (96). In all the studies reviewed (103, 108, 109), this weaning is carried out up to 62 days.

In addition, solid feed intake is considered the key parameter in deciding when to wean calves, and it has been suggested that calves are ready for weaning when they have consumed a minimum quantity of 0.9–1 kg of solid feed for three consecutive days (102) or 15 kg of cumulative non-fiber carbohydrates (52). The problem with deciding when to wean an animal using this method is that many calves are weaned at an older age than if age had been the deciding factor, and very individualized management is required (105).

The weaning protocol has also been widely discussed, and each strategy has a different effect (Table 4). Gradual weaning is carried out by removing meals. This encourages a greater consumption of solid feed and helps to develop the rumen better (96, 104, 109). It is the most similar to natural weaning (111), although it has been shown to cause a more prolonged frustration in the animal. In contrast, abrupt weaning removes meals all at once and causes less frustration (103). However, some animals may be unwilling to consume the minimum amount of solid feed, especially if they are on *ad libitum* milk allowances (109). Finally, the last option is to dilute the milk replacer until only water remains, and then remove the nipple, which causes minor frustration (110).

However, regardless of how weaning is performed, it is a stressful process for calves (i.e., the daily gain decreases the day after weaning, and calves have high cortisol concentrations (112). It is known that calves increase the frequency of vocalizations during this period, a measure of stress and distress (90, 104), but there is still a lack of knowledge on how to minimize the stress suffered during this period. However, the effects of this process on affective states or cognitive judgement have not been investigated.

Weaning management is therefore important as it must be carried out to avoid decreasing nutrient intake and weight loss. Best management practices show a gradual reduction in milk offered from 40 days of age and complete weaning when they consume more than 1 kg of feed for three consecutive days.

5. Conclusions

Calf welfare is not sufficiently considered when making management adjustments. There are still many common calf feeding management practices applied, paricularly in the dairy farm sector, that are detrimental to the health and welfare of calves. Understanding the welfare problems caused by management and the consequences of not doing so, will help to prevent future problems. A standardized protocol helps to have a basis on which to build on according to different production systems. The most studied issues are colostrum and solid feed starter management. However, with all the information reviewed, the most important gaps in knowledge are the lack of a clear protocol for administering milk replacers to reduce hunger and the best management of weaning to reduce stress. Collaboration between the scientific research community and the dairy sector is essential to establish management standards and ensure the success of farm systems adaptated to support proper growth, ensure health and welfare, and facilitate weaning.

6. Implications

This paper provides an overview of the feeding management strategies used in the rearing of Holstein calves and how this management affects the three spheres of animal welfare. Understanding the influence of management on welfare helps to prevent future problems. From the information reviewed, the best protocol, according to the authors, is detailed below. In addition, the authors have produced a table (Supplementary Table 1), suggesting different management practices and their impact on each of the spheres of animal welfare and the missing gaps that need to be investigated in the future.

Based on the information reviewed, some advice could be summarized to optimize calf management protocols in terms of feeding management.

The most important aspect of colostrum management is to collect and administer it as soon as possible after birth, in the first 2h. If possible, pasteurize it to minimize the microbial load. Calves should drink a high-quality colostrum with a minimum of 22° Brix, and a good volume corresponding to 10–12% of their body weight. With regard to liquid feeding, it is essential to provide a high-quality milk substitute (>28% protein, 17–25% fat in powdered milk) and optimal hygiene. A fixed amount of 6–7 liters with a minimum of 660 g of milk powder in two or three daily feeds is recommended. The solid feed starter should provide the protein and energy needed for calf growth, and the animals must have continuous access to water. It is important to facilitate an increase in the rate of feed intake during the first few weeks of age to promote the correct rumen development of the calf. Finally, the best protocol for weaning is to gradually reduce the amount of milk offered from 40 days of age and to wean completely when calves consume more than 1 kg of feed for three days.

Data availability statement

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

Author contributions

PC, AV, FE, and IB-P contributed to the conception and design of the study. PC and IB-P organized the database. PC drafted the first manuscript. All authors contributed to the revision of the manuscript, read, and approved the submitted version.

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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.

The reviewer MC declared a shared committee Red CIBA and ISAE South West Europe Region with the authors to the handling editor.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1148823/full#supplementary-material

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Welfare implications on management strategies for rearing dairy calves: A systematic review. Part 2 – Social management

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Introduction: Raising a healthy calf up to puberty is essential for optimal farm performance. It is therefore, it is necessary to promote animal welfare from the three spheres during this short period. Social management has been postulated as essential in lowering stress and consequently improving calf welfare during this period. Only the health sphere has been studied for a long time, but more recent studies have recently promoted positive experiences and emotional states from affective states or cognitive judgment and natural living spheres. A systematic review of different management strategies in rearing dairy calves according to the three spheres of animal welfare has been conducted using an electronic search strategy.

Methods: The analysis and extraction of information from the studies were performed according to a protocol. From 1,783 publications screened, only 351 met the inclusion criteria.

Results: The publications identified in the search can be divided into two main groups, feeding and social management, based on the main topic of the publication. This review provides an overview of social management, understood as the calf's interaction with others around it.

Discussion: Primary social management issues that emerged were social housing with congeners, separation from the mother and human-animal interaction, distributed in the three broad spheres of animal welfare. The review highlights unresolved questions about how social management practices affect the three spheres of animal welfare at this life stage and the need to standardize good socialization practices for this stage. In conclusion, all the information shows that social housing has improved animal welfare from affective states, cognitive judgment, and natural living spheres. However, gaps in research were identified in relation to the optimal time to separate the calf from the mother, the optimal time to group with conspecifics after birth and group size. Further research on positive welfare through socialization are needed.

KEYWORDS

rearing calves, Holstein calves, welfare, social management, animal production, dairy sector

1. Introduction

Infancy is one of the most important periods of development for mammals, with the environment playing a crucial role (1). In the case of calves, welfare in the early stages of life is one of the most challenging tasks on a dairy farm. Ensuring the best welfare from all three spheres (biological functioning and animal health, affective states or cognitive judgement, and natural living) during the rearing period has a direct influence on calf development and maybe a possible preventive solution to future problems (2). Furthermore, there are also regulations that set minimum standards for the protection of calves, which can be used as a guideline for rearing animals (3).

The recent development of not only avoiding negative experiences but also seeking positive ones (4–6), coupled with consumer demand, has led to increased socialization studies (7). Social interactions have been studied, from maternal bonding to interaction with humans and conspecifics.

Bonding with the mother and the best time for separation still need further study (8). Traditionally, dairy calves are separated from their dam within hours after birth and reared artificially, but in recent years cow-calf contact rearing has received more attention as a more natural system (9, 10).

Furthermore, a good relationship between cattle and humans is important as it allows for reduced stress responses to routine management practices, thus improving welfare (11). The quality of the human-animal relationship plays an essential role in defining the welfare of the animals (9) and, unquestionably, in dairy animals the human-animal interactions are more frequent and more intensive than in the other farm. From birth to adulthood calf are in contact with humans as some procedures are performed daily such as milking (11).

Finally, social interaction between calves is very important, as cattle are a predatory and gregarious species and being together is an important safety factor for them (12). Current research shows the benefits of rearing calves in pairs or small groups. Housing calves with at least one other calf can improve consumer perception, animal welfare, calf growth (7) and cognitive development (1). Although many welfare improvements have been seen, the effect on health is less clear (7), but the future trend is towards social housing.

Therefore, the second part of this systematic review (2) aims to provide a detailed overview of the different social management practices and their impact on the welfare of preweaned calves. In addition, this review aimed to identify gaps in knowledge for further research.

2. Materials and methods

The systematic review protocol is described in detail in the first part of this systematic review (2).

2.1. Search terms and search strategies

The aim of this search was to identify social management strategies and analyze their effect on the welfare of preweaned calves. The search terms and strategy are available elsewhere (2). In addition, relevant references found during the update and review process have been included in the manuscript.

2.2. Data extraction

A data extraction form and screening process was developed for this systematic review, which is available elsewhere (2). As mentioned in the first part, the publications were clustered according to: colostrum, milk replacer, started feed, weaning, separation from mother, animal-human interaction and interaction with congeners. The last three groups will be developed in this review.

3. Results

3.1. Synthesis of results of social management

Several studies have analyzed the effects of social management techniques on early calf development, particularly in preweaned calves. All the different social strategies are examined in a disaggregated way compared to the framework of the three welfare spheres.

Social management practices were broadly described in the search as practices that affect animal welfare. Calves are gregarious animals, so social management greatly impacts their welfare. Under the umbrella of social management, shown in Figure 1, separation from mother, human-animal interaction and conspecific interaction are assessed and analyzed.

4. Discussion

Although the neonatal and infant periods are important for adequate physical, behavioural, and cognitive development into adulthood, literature reviews over the past two decades have resulted in many publications on different management strategies, but few studies addressing the three spheres of animal welfare. However, there has been a shift in approaches to animal welfare assessment to include animal-based indicators related to emotional state and natural life. The application of this new welfare knowledge will improve the daily lives of animals.

Management has been shown to significantly influence the welfare of preweaned calves. The first part, looked at feeding management (2), so in this part, we will look at social management.

4.1. Social management for welfare

Social management was found to be critical in the early stages of calf development. As shown above, in Figure 1, social management practices such as (a) separation from the mother (13), (b) human-animal interaction (14), and (c) conspecific interaction with their congeners (15) have been widely described in the reviewed literature as practices that affect animal welfare. In the case of conspecific interaction, individual and group housing of different sizes have been studied. The level of socialization affects the sphere of affective states or cognitive judgement and natural life and, to a lesser extent, the sphere of biological functioning and health.

4.1.1. Separation from the mother

One of the most common practices in dairy farming is to separate calves from their mothers immediately after calving (16). However, consumers question the ethics of this practice (17), and calf rearing with cow contact has increased in recent years (8).

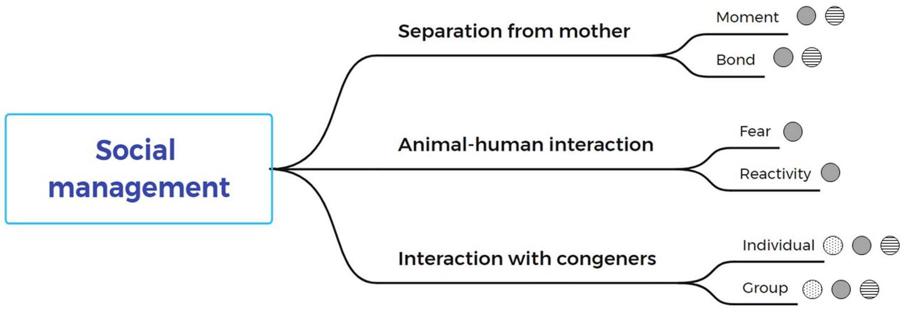

FIGURE 1
Critical points of each phase of social management and their relationship to animal welfare spheres. Dotted spheres represent biological functioning and health, spheres with horizontal lines relate to natural living, and gray spheres relate to affective states or cognitive judgments.

Leaving calves with their mothers can have health and psychological benefits. Calves gain more weight and have fewer diseases (13), have more favorable emotional states (17), and show more playful behaviour (18). However, the longer the calf separated from its mother, the stronger the bond is formed and therefore the more negative the behavioural response after separation (13, 19).

In addition to separation time, other separation strategies have also been investigated (8, 20, 21). There are four main rearing systems described in the literature. Firstly, full contact systems where cow and calf have unrestricted access to each other. Second, partial contact systems which can be implemented in a variety of ways: with restricted suckling systems, where there is brief contact only for suckling; with half day contact systems, where the cow and calf are housed together during the day or night, and finally with cow systems, where a cow suckles 2 to 4 calves, usually without milking (10).

With regard to full contact, some "anti-suckling devices" have been used; such as nose rings or nose flaps, although this device allows the calf to be with the mother, it seems to cause frustration. Wenker et al. (21) reported that animals with full contact but nose-flap were more stressed than those with partial contact. In addition, the combination between full contact and abrupt weaning stresses cows and calves (20). Partial contact, on the other hand, has been shown to reduce abnormal behaviour such as cross-sucking and no differences in health have been found (8). Half-day contact seems particularly promising as animals become accustomed to separation, experience positive humane treatment and calves can learn to use a milk feeder to prevent stunting after weaning (10). All of these strategies have a direct effect on affective states or cognitive judgment (17).

Although Nicolao et al. (20) have shown that the best compromise between cow milk yield and calf welfare is a long period of cow-calf contact between the morning and evening milking, more research is needed to investigate strategies to improve the process of debonding and weaning.

4.1.2. Animal-human interaction

Establishing good human interaction improves welfare (9) and reduces animal fear and distress from affective states or cognitive judgement. Workers without training or low job satisfaction elicit higher responses in the avoidance test, which is why calves are more fearful of them (14). Poor management affects the approach distance of calves to humans as the reactivity is due to the constant stress of poor management. The animal has also been shown to maintain this response. Human contact on farms is a very important factor to be consider and has been little studied, probably because of the complexity of the assessment (22). More research is needed in this area.

4.1.3. Interaction with congeners

Finally, housing and social interaction with other calves significantly impact animal welfare in terms of social management. Animals can be housed individually, in pairs, or groups, although individual housing is the most common practice. It has been shown that calves housed individually have consistent behavioural and developmental deficits. In contrast, social housing, whether in pair-housed or group-housed, has been shown to improve production rates through grain consumption and grow as well as or better than individual housing (7), in addition, to improving cognitive, and behavioural parameters related to affective states or cognitive judgement and natural living (1).

Although individually housed calves have complete control over their health and food intake, the literature shows that they have deficits at the level of affective states or cognitive judgement and natural living. Calves deprived of contact with other animals show greater anxiety responses to the environmental novelty test (15, 23, 24), greater anxiety when encountering other calves (25), cognitive and learning deficits (26, 27), and play deprivation (28).

In the case of pair-housing, the optimal time to put the animals together after birth is still under investigation. However, in the available studies, have found no difference between pair-housing immediately after birth or at 3 weeks of age (29, 30). Animals have better productive parameters than when housed individually: they consume more solid feed (24, 31, 32), they have a higher average daily gain both before (1, 29, 33) and after weaning (34) and they achieve a higher weight at weaning (35). When calves are housed in pairs, their welfare is improved in terms of biological functioning and health.

Studies on the effects of social housing on health, studies are controversial. On the one hand, some studies show an increased risk of disease (36, 37), while other studies have shown no risk to the health of socialized calves (25, 38, 39).

The most significant difference in welfare between calves' pair-housed and individual housed calves in behavioural responses, affecting spheres of affective states or cognitive judgement and natural life. In the studies reviewed, it is clear that social housing provides a greater opportunity for natural social behaviours, and animals are more exercised (33). Calves spend more time resting with their partner (35, 40), improve their affective appearance (41), show less stress, and increase their motivation to play (42). It is well known that when the animals are healthy, they are more motivated to play (43) whereas when they are sick they spend more time lying down and eat less (44). These behaviours may therefore be useful in detecting health problems. In terms of responses to tests of novel social and environmental situations, paired calves have been shown to be less reactive and more curious (23, 25). In addition, this type of housing may alleviate some of the negative aspects of weaning, as this stress is cushioned by social support (38, 45, 46), and less non-nutritive oral behaviours are observed (34). Finally, calves housed in pairs are better prepared to live in groups after weaning (47).

However, studies have shown that pair-housing also has its limitations. In social housing, competition for feed and cross-sucking problems have been reported (48). Cross-sucking is a welfare problem defined as sucking on any part of the body of the calves in the same pen and it can lead to abscesses in the ears and belly button (33, 49, 50). An excellent way to reduce this behaviour is to offer the milk with slow-flow nipples (51) or with anti-sucking devices (52), while competition can be avoided by using long barriers that occupy the front half of the calf during feeding (48).

Calves have been shown to change their behaviour to accommodate mates (40) and to display more natural behaviour (53, 54) when they are with their peers. There is currently, a lot of interest in taking social housing for calves a step further by housing them in group. In this case, there must be an equal or greater number of teats than animals in the group because otherwise, competitiveness increases and feeding time decreases (55, 56). Introducing new precision livestock farming technologies can facilitate this type of housing, as automatic feeders or remote monitoring systems improve individual attention and save labor, even for grouped calves (15, 57).

5. Conclusion

There is currently no clear agreement on all issues relating to calf social management strategies and their impact on welfare. An understanding of welfare issues by management can help prevent future problems. From all the information reviewed, the most important gaps in knowledge are the optimal time to separate the calf from its mother, and further research into the positive welfare benefits of socialization with humans and congeners. Collaboration between scientific research and the dairy sector is essential to establish management standards that support proper growth, ensure health and welfare, and facilitate weaning.

6. Implications

This paper provides an overview of the social management strategies used in the rearing of Holstein calves and how this management affects the three spheres of animal welfare. Understanding the influence of management on welfare helps to prevent future problems.

Based on the information reviewed, some recommendations can be summarized to optimize the social management of calves. Separation from the dam should occur immediately after birth. In addition, good human-animal interaction is essential to implement. In terms of socialization with conspecifics, housing in pairs or groups immediately after birth improves animal welfare.

In addition, the authors have produced a table (Supplementary Table S1), suggesting different management practices and their impact on each of the spheres of animal welfare and the missing gaps that need to be investigated in the future.

Data availability statement

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

Author contributions

PC, AV, FE, and IB-P contributed to the conception and design of the study. PC and IB-P organized the database. PC wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1154555/full#supplementary-material

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