GRAZING BEHAVIOUR AND WELFARE OF RUMINANTS

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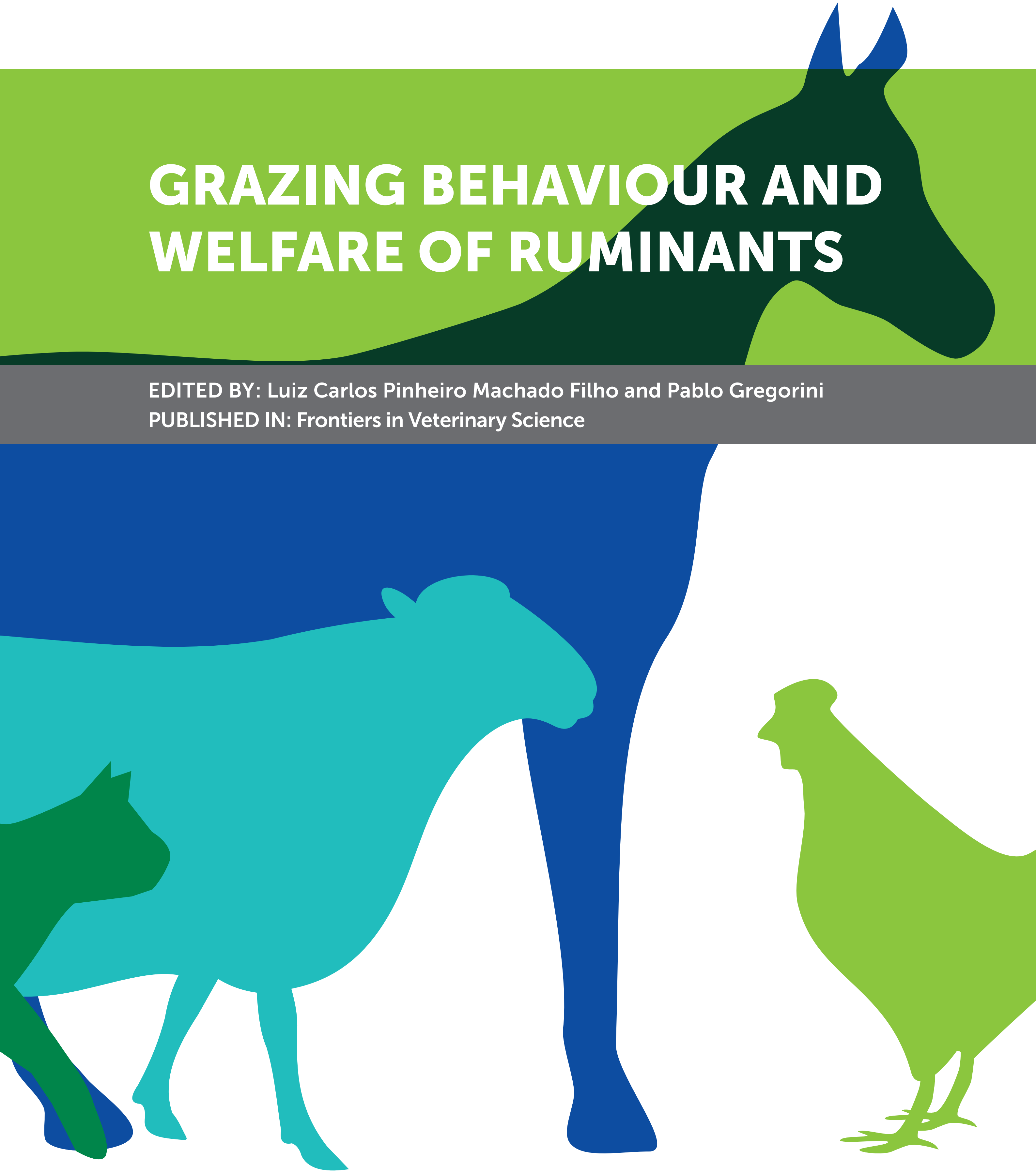

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GRAZING BEHAVIOUR AND WELFARE OF RUMINANTS

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EDITORIAL
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Editorial: Grazing Behavior and Welfare of Ruminants

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Keywords: diet diversity, sustainable farming, social behavior, grazing patterns, lameness

Editorial on the Research Topic

Grazing Behavior and Welfare of Ruminants

The domestication and use of animals for our benefit entails responsibility for their quality of life (1). Animal welfare is a prerequisite for any ethical and sustainable animal production system to be socially defensible and acceptable (2). In nature, animals evolved in a changing environment and developed adaptive mechanisms to increase fitness (3). Cattle evolved in extensive grasslands and rangelands, in herds and families with complex social hierarchy and adapt to challenges posed by their environment through natural selection. Grazing animals face a number of challenges, including tick-borne diseases and lack of access to water and shade. Under human control, it is our responsibility to help animals to cope with such stressors and provide them a good life. This Research Topic aims to identify stressors present in pastoral husbandry systems; assess to the extent they affect health, welfare and production, and propose solutions to mitigate or overcome stressors.

To evaluate the quality of life of animals, assessing welfare conditions is necessary. A number of parameters to evaluate the welfare of grazing ruminants are proposed and are summarized by Barrell. Those parameters should include not only poor states of welfare or physiological measures, such as cortisol, endorphins, plasma serotonin, heart rate variation, etc., but also positive states. As sentient individuals, welfare assessment should include their emotional status. It is likely that to assess welfare condition in grazing ruminants, a variety of tools should be used in the methodology, rather than being reliant on a single measure (Barrell). Welfare is a more complex issue than solely the animal's condition. Welfare became a strong demand from urban societies, but they are raised on farms. Therefore, farmers have to be involved in the debate on animal welfare as part of a broader debate on environmental management, markets and social expectations. Welfare issues have to be resolved in the context of the farming system, and not considering only the experiences of the animal. More sustainable and equitable farming practices are to be built, through dialogue with all involved actors (Fisher). On this debate, pastoral systems have much to contribute. For example, overall costs of production can be reduced if heifers are raised on pasture, compared to confinement housing options (Hawkins et al.).

Grazing is a natural behavior of ruminants (4), and to offer them this opportunity is of paramount importance regarding their welfare. Providing access to alternative forms of outdoor space for ruminants appears to be less attractive for cows than pasture (Smid et al.). In natural conditions, ruminants explore and graze a diverse range of habitats. While exploring diverse habitats and swards ruminants are able to express individual grazing pattern and feed preferences, as well as personalities, with the later being regulated by social and biophysical environments, as well as the emotional state of the animal (Moreno Garcia et al.). Natural pastures offer a diverse range of forage, allowing individuals within herds to have consistent differences in grazing patterns. Such differences are best recorded by the visual observation of the foraging behavior. Ruminants have circadian rhythms and observations from dawn to midnight can represent grazing activities for the entire day (Jochims et al.).

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Diet diversity is important for the animal nutrition, having an impact on animal and human health. Grazing a diverse arrangement of plants provide to ruminants different sources of primary nutrients such as protein and carbohydrates, and secondary compounds, e.g., phenolics and terpenes (Beck). A diverse pasture provides the animal a wider range and greater amount of phytonutrients, such as terpenoids, phenols, carotenoids, and anti-oxidants, than a monotonous swards, and this more than fed grain-based animals. Further, phytochemical rich diverse swards and its management improve animal health and these nutrients also benefit human health (5–7).

Besides swards' botanical composition, pasture and grazing management affect defoliation strategies by grazing ruminants, from bite features to meal behavior to daily grazing patterns. Sheep on low-intensity/high-frequency grazing strategy bitten on the "top stratum" of the plants' canopy, whereas on high-intensity/low-frequency strategy sheep mostly bitten on "grazed plants." Selecting a diet of better nutritive value, sheep on low-intensity/high-frequency grazing had greater nutrient intake. Consequently, blood parameters of these sheep were positively associated with nutritional status and immune response to stress (Zubieta et al.), with possible positive consequences on their welfare.

Because of seasonality in pasture production and / or quality ruminants can face periods of unfulfilled nutrient demands and sometimes hunger. Among other solutions, silage, hay or other supplements offered on pasture are widely used to compensate the shortage of pasture during low season (8). As a social species, cattle and other ruminants have an internal hierarchy, where dominant animals have priority in accessing resources over subordinate ones, especially when resources are limited (9). Competition among animals then occurs, and low ranking animals have to develop strategies to access the resources. In a daily rotational pasture management system, when grain supplement was offered at the time of paddock entry, subordinate heifers could choose to graze fresh pasture, instead of competing for grain with dominant ones. On the other hand, offering supplements after 12 h of entering the paddock, resulted in a higher number of agonistic interactions and less time of grazing during grain offer (Bica). Therefore, supplementing at the time of entering the paddock reduce fights and offers subordinate heifers an opportunity to graze high-quality pasture, improving their welfare.

When proper sources of water are not offer, thirst becomes another critical challenge to grazing ruminants. Comparing the behavior and performance of grazing steers in the context of water availability in troughs or in ponds, troughs were superior with steers gaining 29% more weight ($P \leq 0.007$) than their counterparts drinking from ponds (Bica et al.).

Identified as one of the three most common health issue affecting dairy cows (Sadiq et al.), lameness also occurs in pasture based dairy systems (10). In grazing cows, lameness can be a further problem as they have to walk more (11). Regardless of being on pasture or confined, a study showed that preventive hoof trimming was effective in reducing the prevalence of lesions (Sadiq et al.). Notwithstanding, trimmed cows that spent more time on pasture, have a lower incidence of hoof lesions (12).

Except for extensive management systems, the formation of new groups of animals and the consequent movement of animals from one group to another is a common routine in animal husbandry, including grazing ruminants. Group changing may lead to social instability and stress, implying potentially negative effects on animal welfare (Sosa et al.). As to mitigate the stressful consequences, whenever it is possible, familiar individuals should be transfered as a group and juveniles with a familiar adult. The presence of familiar adults among juveniles in a new group is likely to bring more stability and reduce aggression (13).

Grazing ruminant production systems have the potential to allow the animals to express their natural behavior, maintain health, and experience positive emotional states. The challenges ruminants face on pasture (differences in vegetation, topography, weather changes, social interactions, etc.) may also be viewed as sources leading to positive emotional states, since they present complex problems, that can be successfully solved. The diversity occurring in natural systems may improve animal welfare and prepare the animal for an efficient adaptation to environmental challenges (Villalba and Manteca).

AUTHOR CONTRIBUTIONS

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

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ORIGINAL RESEARCH
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Impact of Group Management and Transfer on Individual Sociality in Highland Cattle (*Bos taurus*)

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The sociality of cattle facilitates the maintenance of herd cohesion and synchronization, making these species the ideal choice for domestication as livestock for humans. However, livestock populations are not self-regulated, and farmers transfer individuals across different groups. Individuals consequently have to adapt to different group compositions during their lives rather than choose their own herd mates, as they would do in the wild. These changes may lead to social instability and stress, entailing potentially negative effects on animal welfare. In this study, we assess how the transfer of Highland cattle *(Bos taurus)* impacts individual and group social network measures. Four groups with nine different compositions and 18 individual transfers were studied to evaluate 1) the effect of group composition on individual social centralities and 2) the effect of group composition changes on these centralities. This study reveals that the relative stability of dyadic spatial relationships between changes in group composition or enclosure is due to the identities of transferred individuals more than the quantity of individuals that are transferred. Older cattle had higher network centralities than other individuals. The centrality of individuals was also affected by their sex and the number of familiar individuals in the group. This study reveals the necessity of understanding the social structure of a group to predict social instability following the transfer of individuals between groups. The developing of guidelines for the modification of group composition could improve livestock management and reduce stress for the animals concerned.

Keywords: livestock, social network, animal welfare, pastureland, applied ecology, bovines

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INTRODUCTION

Animal farming began in the Holocene [about 7,500 years BC; (1–3)], when humans domesticated aurochs (*Bos primigenius*), the ancestor of *Bos taurus*. Humans mainly chose cattle for their social nature, which facilitates the maintenance of herd cohesion and synchronization and simplifies the locating of groups in pastureland and the coordination of movements such as transhumance (4, 5). Social groups can regulate their own composition in the wild, with individuals migrating or groups splitting when competition for food becomes too high, for instance (6, 7).

This self-regulation is not possible for livestock. Farmers transfer individuals to different groups throughout their lives to facilitate genetic mixing and reproduction, or to manage pastureland activities (8–10). Such changes may result in periods of social instability and stress (11, 12). These frequent changes in group composition modify the social organization and stability of groups, with possible implications for animal welfare (13) and health (14).

Like their wild counterparts, domestic bovines show strong social behaviors with stable and long-term dyadic relationships when possible, i.e., when the group composition is also stable (12). Boyland et al. (15) showed that cattle form strong relationships with specific partners. These preferential associations are dependent on different socio-demographic factors such as sex and age, as well as dominance, kinship or familiarity with other group members. Two individuals that are the same age or arrive in an enclosure at the same time will have a higher probability of developing a strong relationship than other individuals (16, 17). Many behavioral experiments have shown that cattle are able to discriminate between *familiar* and *unfamiliar individuals*, hereafter defined as individuals a bovine has spent time with, or unknown/new individuals, respectively (18, 19). Adding new individuals to the group disrupts the contact between familiars and aggressive behavior increases (10). This suggests that prioritizing good and stable relationships in a group of animals enhances the wellbeing of individuals by decreasing their stress and reinforcing their social status. The use of this principle for livestock management is encouraged (8, 13, 15).

In physiological terms, social stress may lead to decreased food ingestion, lower milk production and even ceased reproduction for cows (8), and can also have a strong impact on the behavior, cognition and health of calves (14). This stress can be reduced by the presence of familiar individuals during transfer (20, 21). The impact of such transfers is also dependent on the sex of individuals: the removal of males from an enclosure leads to stronger cohesion between females, whilst the removal of females does not influence associations between males. These remain basic due to the sexual segregation observed in cattle (6, 22). Females are more involved in group social cohesion than males; this is probably because they are the phylopatric sex, like in some primates species (23).

It appears necessary to understand the social structure of a group to predict any social instability that could occur through the transfer of an animal. Taking this factor into consideration would make livestock management more efficient and less stressful for animals (8). This study uses social network analysis (24) to assess how group composition affects social centralities of Highland cattle (*Bos taurus)* and how the transfers of these individuals impact their social relationships.

Highland cattle are originally from the Scottish Highlands in the United Kingdom. Like most domestic ungulates, this is a social species with sexual segregation (6). This breed is particularly suitable for eco-grazing, as it is adapted to a wide temperature range and has a non-selective diet. Many French natural reserves and national parks have imported Highland cattle in order to maintain ecosystem biodiversity (25–27). These Highland cattle populations with different group compositions can be observed in a wide study permitting a more detailed understanding of how the age ratio, sex ratio and size of group compositions affect the social centrality of cattle and how the transfer of individuals between groups impacts sociality and its dynamic in this species. We studied different compositions (nine in total) of four groups over a 6-month period. We first assessed which sociodemographic factors (sex, age, dominance rank, and group size) influence the social centrality of Highland cattle, which was measured using eigenvector centrality (or popularity, i.e., how well an individual is connected to its neighbors, but also how well its neighbors are connected) and the strength of associations (or social activity, i.e., how often an individual is seen in the proximity of other specific group members) (24). In a second step, changes in group compositions in terms of group size, age or sex composition were examined to determine how they affected the associations and social centrality of individuals. This enabled us to measure the changes in dyadic relationships and in individual centrality according to the changes in group composition. We worked both on transferred and resident individuals.

Following the previous results on sociality in cattle (18, 28, 29), we made the following hypotheses:

1. *Effects of socio-demographic factors.* Social centrality is expected to be influenced by the age, sex and dominance rank of group members and the number of familiar individuals they have in the group (17, 28–31). Older individuals were expected to have higher dominance rank and higher social centrality (29). Familiar individuals or those of the same sex and age should also show stronger dyadic associations (6, 32).
2. *Effects of group composition changes*. After a transfer, fewer changes in eigenvector centrality and strength of associations were expected in older, dominant individuals, whilst the opposite was expected in younger, subordinate individuals in the new group composition. Indeed, older or dominant cattle have stronger relationships that are more easily maintained (29, 30). Concerning familiarity, we expected that individuals with a higher number of familiar individuals (for instance three or four) to show a lower impact on their social centrality than the individuals with no or few familiar individuals (i.e., one or two). We further predicted that resident individuals, i.e., those who experienced the arrival of a newly transferred individual in their group, would be less impacted than those being transferred (10). We suggest that the number of transferred individuals is not the only factor affecting social relationships and believe that the social role of removed or newly added individuals can have strong consequences on the social structure. We expected the removal or addition of specific individuals such as a bull or an older individual, specifically an older female, to strongly impact the social relationships of all other individuals because they no longer play their specific social role within the group (29–31).

MATERIALS AND METHODS

Ethical Note

This study was based on the observation of animals, and no handling or invasive experiments were involved. Our study was

TABLE 1 | Characteristics of the four Highland cattle group sites.

Observation site	GPS coordinates	Area (m^2)	Observation time	Number of changes in group composition
Robertsau (Rob)	48.611237, 7.806514	5 enclosure changes: 66,438; 32,801; 44,028; 80,501; 33,637; 44,028	Period 1: 14/04/15–28/08/15, Period 2: 22/01/16–29/04/16	2
Niedersteinbach (Nie)	49.029522, 7.720504	86,787	Period 1: 14/04/15–28/08/15	1
Sturzelbronn (Stu)	49.057404, 7.580153	112,273	Period 1: 14/04/15–28/08/15	2
Rolbing (Rol)	49.10545, 7.26120	71,454	Period 2: 22/01/16–29/04/16	None

approved by our research institution (IPHC, agreement n°H-67-482-18). It was carried out in full accordance with our national ethical guidelines and complied with European animal welfare legislation. CS is habilitated to perform such behavioral studies on animals (level 1, R-45GRETA-F1-04). Every effort was made to ensure the welfare of the animals and minimize disturbance by researchers present in the field. Animal transfers were a result of agricultural management/farmer choices, not related to the study.

Observation Sites and Study Subjects

We studied the effect of group composition and the effect of change in group composition in four groups of Highland cattle (**Table 1** and **Figure 1**). Group composition change is defined as changing a minority of the individuals at the study location (Robertsau, Niedersteinbach, and Sturzelbronn by either adding some new individuals or removing some individuals from the group; Rolbing is not listed because no transfers were made involving that location). The four groups were located in the Grand Est region of France (see **Figure S1** for a map of the different locations). Enclosure size did not have an effect on aggression in the group or the cohesion of group members (correlation test with permutations between the enclosure size and the mean number of aggressions per day per individual: $N = 11$, rho $= -0.30$, pperm $= 0.317$; correlation test with permutations between the enclosure size and the mean number of 3 m proximity per scan per individual: $N = 11$, rho $= -0.37$, pperm $= 0.214$).

Group composition changes were made by the farmer, either for the needs of farmland management or for breeding reasons. In particular, the non-castrated bull was transferred between the groups in order to copulate with females. Castrated bulls, which are known to be less aggressive than bulls (33, 34) were also transferred into groups with juveniles to decrease the stress of the latter. Juveniles were transferred away from their mothers to facilitate new gestation. Females were generally transferred for pastureland management (25–27). The authors did not contribute to the management decision concerning the time of transfer or the choice of individuals transferred. These four groups were chosen for their group size and their contrasting group compositions (i.e., only females with juveniles, females with a bull, juveniles and bullocks; females with different vs. similar ages). The groups were large enough to permit social network analysis (24, 35, 36). The group compositions were selected to study the impact of group composition on individual social centrality and how the changes of group composition affect these centralities.

Water was supplied via a water pump for the Robertsau group, whilst the three other groups had access to a river. Enclosures were all composed of similar vegetation: mainly grass (more than 90% of groundcover, surface area estimated with GIMP 2.9), wetland, some bushes and some small areas of forest/trees, as indicated in **Figure S1**. Animals were supplied with hay during winter. Twice a week, hay was placed at different locations across a surface area of about seven acres to avoid resource competition. Observations were carried out over two periods: one in 2015, from April 14th to August 28th, and the second in 2016, from January 22nd to April 29th. During the two periods, composition was changed in all groups except the Rolbing group (**Table 1** and **Figure 1**). Each group member was identified according to physical traits such as coat color and horn shape. These physical traits had been clearly identified for each individual prior to the study.

Changes in Group Composition

Group composition changes are summarized in **Figure 1**. A total of nine group compositions were observed for these four groups (**Table 2**) and concerned 18 individual transfers.

Data Scoring

Data were scored by two observers located 2–10 m from the animals. While both observing, one communicated what they saw, and the other wrote it down as confirmation of what they observed. This allowed behaviors to be confirmed by two observers. Cattle were already habituated to human presence and were not disturbed by the observations, which were made once a week over a 6-h period between 9 a.m. and 5 p.m. The groups were not observed during rainy or snowy days or during the weekends. Sampling frequency for each group composition is given in **Table 2**.

The group social network was defined and scored using dyadic spatial associations (13, 15). Spatial associations were defined according to the nearest neighbor (closest individual

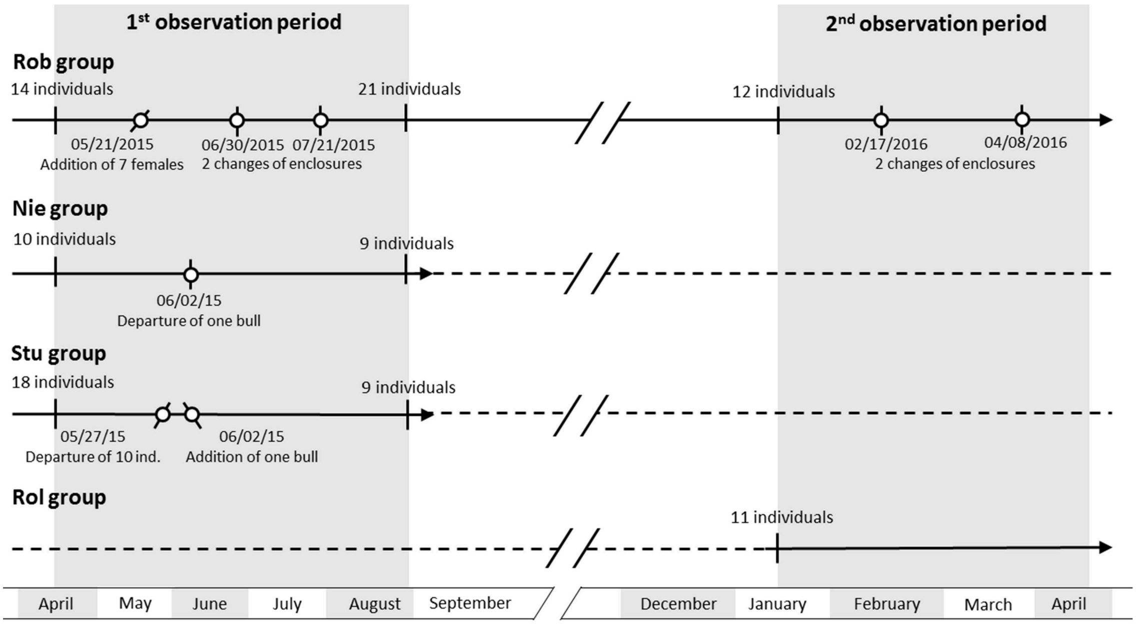

FIGURE 1 | Chronological scheme of the composition changes in all four groups. Solid lines indicate the period of observation, whilst dashed lines indicate an absence of observation. Dots indicate changes in group composition or enclosure. Forward and backward strokes indicate the addition and departure of individuals, respectively. A vertical stroke indicates a change of enclosure.

TABLE 2 | Group size, number of scans and observation days, number of agonistic interactions, sex ratio, and age ratio for each group composition (including changes of enclosure).

Group composition	Number of scans (and days)	Number of agonistic interactions	Group size	Sex ratio	Age ratio
Niedersteinbach 1	429 (7)	150	10	1 M–9 F	9 A–1 J
Niedersteinbach 2	922 (13)	74	9	9 F	8 A–1 J
Robertsau 1	207 (6)	214	14	14 F	9 A–5 J
Robertsau 2	211 (7)	369	21	21 F	11 A–10 J
Robertsau 3	118 (4)	278	21	21 F	11 A–10 J
Robertsau 4	221 (7)	557	21	21 F	11 A–10 J
Robertsau 5	174 (4)	104	12	12 F	4 A–8 J
Robertsau 6	321 (6)	233	12	12 F	4 A–8 J
Robertsau 7	272 (4)	99	12	12 F	4 A–8 J
Rolbing	416 (9)	74	11	7 M–2 CM–2 F	2 A–9 J
Sturzelbronn 1	172 (4)	83	18	3 M–15 F	8 A–10 J
Sturzelbronn 2	133 (2)	48	8	8 F	8 A
Sturzelbronn 3	899 (13)	266	9	1 M–8 F	1 A–8 A

For sex ratio, M, Male; F, Female. CM indicates castrated males. For age ratio, A, Adult; J, Juvenile ($\leq$2yo).

whatever the distance) and were scored every 5 min with the instantaneous sampling method (37). This means that every 5 min (one scan), the value "1" was recorded in a matrix if individual A was the nearest neighbor of individual B and "0" in all other cases. We summed all scans in one matrix for each group composition, thus obtaining the absolute frequencies of nearest neighbors. Dyadic spatial association was defined as the absolute nearest neighbor frequency between each dyad of group members. The total number of scans is indicated in **Table 2**. We obtained 72 scans during usual days. However, we could obtain less than 72 scans during some days due to different perturbations mostly very bad weather or farmers' intervention. The "nearest neighbor" approach is more appropriate for this kind of study (i.e., evaluating the effects of group composition on social network) than the "five meter proximity" concept (38, 39). Spatial proximity matrices and nearest neighbor matrices are highly correlated (Mantel test with 1,000 permutations: $r \geq 0.78$, $p \leq 0.0001$). Given these two points, we chose the "nearest neighbor" approach to measure associations.

Observers also scored spontaneous agonistic interactions using the behavioral sampling method (37) in order to assess the dominance hierarchy of each group composition. We scored supplanting, avoidance and aggression as agonistic interactions. We measured each agonistic interaction as an event, however long it lasted. We scored this interaction between individual A and individual B as "1" in a matrix of agonistic interactions. We then summed all dyadic agonistic interactions for each group composition period. Agonistic interactions, considered to be the best choice of dominance index (40, 41), were used to calculate the Modified David's Score (MDS). David's score is based on an unweighted and a weighted sum of the individual's dyadic proportions of wins combined with an unweighted and a weighted sum of its dyadic proportions of losses (41). Animals that usually dominate have high positive scores, and those that are usually dominated have largely negative scores. Individuals were ranked from the highest to the lowest MDS, with the individual with the highest value ranked first in the dominance hierarchy and the individual with the lowest value ranked last. SocProg 2.6 (42) was used to calculate MDS values for each group composition, and scoring began on the eighth day following transfer. We did not take the first days of observations into account in our calculation because of the instability of social and hierarchical relationships during this period. Whilst the number of aggressions were higher during these first days compared to stable periods, many agonistic behaviors were bidirectional, meaning that the hierarchy was still not established. These agonistic behaviors did not fit with the dominance ranking we observed in the stable periods.

Basing our analysis on the time intervals between group composition changes, we defined familiarity as the number of familiar individuals in the group, meaning the number of individuals a group member is with / has been with for more than 3 months (35). The examination of the pedigree of each individual revealed that kinship association matrices would be difficult to obtain for each group composition due to missing data or very close genetic proximity between familiar individuals. We therefore preferred to analyse familiarity and did not assess the effect of kinship. Moreover, kinship is very difficult to study in ungulate groups, where the composition changes frequently (10, 12, 18, 19, 21).

Social Network Analysis

Social network analysis (SNA) is an increasingly widespread tool for the study of sociality and its dynamic (24, 38, 43, 44). Indeed, social relationships can evolve over time because of changes in the social strategies of group members, and the arrival or departure of individuals through births, deaths, migrations or transfers. Specific tools were developed in SNA to analyse these changes and their causes (45–48). SNA has also been recognized as a reliable tool for animal welfare and conservation (13, 49, 50).

During data analysis, the matrices of spatial associations obtained per observation day were added together for each group composition. Each dyad of individuals thus obtains a spatial association weight that indicates whether or not these two individuals were frequently observed together. The spatial associations for each group composition were used to calculate the eigenvector centrality coefficient and the strength of associations of each individual (24). These measures were calculated using SocProg 2.6 (42).

Eigenvector centrality is a commonly used measure of individual centrality, and indicates the popularity of an individual (51). This coefficient is defined as a measure of how well an individual is connected to its conspecifics, and also reveals the connections of the group members to which it is connected (52).

The strength of associations is the sum of each node's edge values, and indicates the social activity of an individual (51). The individual with the strongest and most numerous associations has the highest strength value (24). In this study, strength indicates the number of times an individual was observed as the nearest neighbor of another individual. Indeed, in a given scan sampling, one individual might be observed several times as the nearest neighbor of the other group members (maximum $= N -$ 1, where N is the group size).

These two variables are correlated but are by no means collinear (Pearson correlation test, $r = 0.16$, $p = 0.03$).

Statistical Analyses

Do Dyadic Spatial Associations Depend on Shared Characteristics Among Dyads?

In a first step, we assessed how the weight of dyadic spatial associations was influenced by socio-demographic factors such as sex, age and dominance. Matrix correlations were made with a Mantel test with 1,000 permutations to check whether individuals sharing similar characteristics (similar age, dominance rank or sex) have stronger dyadic associations than individuals that do not share similar characteristics. This is called homophily, i.e., the tendency of individuals to associate and bond with similar congeners (53, 54). Using Socprog 2.6, we then created matrices for age differences (0: dyad individuals have the same age, 1: an age difference of approximately 1 year, and so on), dominance rank differences (0: dyad individuals have the same rank, 1: a difference of one dominance rank, and so on) and sex difference (0: same sex, 1: different sex). These three matrices were calculated for each group composition and correlated to the dyadic spatial association matrices for each group composition. The "CombinePValue" package in R 3.24 was used to combine the p-value of all group compositions and obtain global statistics. The goal here was to test whether vectors of p-values are significant when combined and to confirm or negate the possible effect of a given socio-demographic factor at the population level.

How Does a Change of Group Composition or Enclosure Affect Dyadic Spatial Associations?

A Mantel test with 1,000 permutations in SocProg 2.6 was used to correlate the dyadic association matrices after a change (transfers or enclosure change). Only individuals that were present in the two adjacent matrices for each matrix (ex: Rob1-Rob2, Rob2-Rob3, Stu1-Stu2, etc.) were retained. The correlation coefficient was then correlated with the number of individuals transferred between two group compositions using a Spearman correlation test with permutations (library R "Coin," R 3.24).

How Do Sociodemographic Factors Influence Individual Centralities?

GLMMs [R package "lme4"; (55)] were used to test whether the eigenvector centrality and the strength of associations were affected by the following independent sociodemographic variables: the age of individuals, their sex, their dominance rank and the number of familiar individuals they were associated with in the group. The experimental units we used were the eigenvector centrality for a first GLMM and the strength of associations for a second GLMM, per individual and per group composition. Prior to GLMMs, the eigenvector centrality and the strength of associations were corrected using the group size for each composition in order to control for the mathematical effect of the number of nodes on network metrics. For the regression $y=ax+b$, y (the eigenvector centrality or the strength of associations) was multiplied by b. The identity of individuals was included as a random factor.

How Do Changes in Group Composition Affect Individual Centralities?

Two further GLMMs were carried out using the differences in eigenvector centrality and in strength of associations between two compositions as positive or negative values. The experimental units we used were the eigenvector centrality difference for a first GLMM and the association strength difference for a second GLMM, per individual and between two group compositions. Effect variables were the age of individuals, the number of familiar individuals in the new group, the difference in dominance rank between the two compositions (negative or positive values) and the total number of added or removed individuals. Changes of enclosures without adding or removing individuals were considered as "0" changes in the analyses. This makes it possible to compare networks where the transfer of individuals occurs to those without transfers. The identity of individuals was included as a random factor. The sex variable was not included in the model testing the differences between two group compositions because only four males (one adult and three juveniles) were transferred to another group, meaning that the sample size was too low, and the sex variable was correlated

TABLE 3 | Correlations of dyadic associations (DyaAsso) matrices with matrices of characteristic differences (age, dominance, and sex).

Group composition	DyaAsso-dominance	DyaAsso-sex	DyaAsso-age	Dominance-age
Niedersteinbach 1	$p = 0.332$ ($r = 0.06$)	$p = 1$ ($r = -0.15$)	$p = 0.039$ ($r = 0.16$)	$p = 0.007$ ($r = 0.43$)
Niedersteinbach 2	$p = 0.302$ ($r = 0.10$)	NA (just one sex)	$p = 0.431$ ($r = -0.01$)	$p = 0.448$ ($r = 0.08$)
Robertsau 1	$p = 0.036$ ($r = -0.29$)	NA (just one sex)	$p = 0.004$ ($r = -0.15$)	$p = 0.002$ ($r = 0.52$)
Robertsau 2	$p < 0.001$ ($r = -0.40$)	NA (just one sex)	$p < 0.001$ ($r = -0.32$)	$p < 0.001$ ($r = 0.63$)
Robertsau 3	$p < 0.001$ ($r = -0.21$)	NA (just one sex)	$p = 0.001$ ($r = -0.19$)	$p < 0.001$ ($r = 0.55$)
Robertsau 4	$p < 0.001$ ($r = -0.40$)	NA (just one sex)	$p < 0.001$ ($r = -0.32$)	$p < 0.001$ ($r = 0.70$)
Robertsau 5	$p =0.001$ ($r = -0.35$)	NA (just one sex)	$p = 0.008$ ($r = -0.25$)	$p = 0.042$ ($r = 0.28$)
Robertsau 6	$p < 0.001$ ($r = -0.43$)	NA (just one sex)	$p = 0.009$ ($r = -0.23$)	$p = 0.036$ ($r = 0.28$)
Robertsau 7	$p =0.004$ ($r = -0.30$)	NA (just one sex)	$p = 0.006$ ($r = -0.24$)	$p = 0.013$ ($r = 0.39$)
Rolbing	$p =0.015$ ($r = -0.30$)	$p = 0.006$ ($r = 0.31$)	$p < 0.001$ ($r = -0.36$)	$p = 0.035$ ($r = 0.43$)
Sturzelbronn 1	$p =0.028$ ($r = -0.13$)	$p = 0.168$ ($r = 0.05$)	$p = 0.948$ ($r = -0.10$)	$p < 0.001$ ($r = 0.63$)
Sturzelbronn 2	$p =0.592$ ($r = -0.03$)	NA (just one sex)	$p = 0.262$ ($r = 0.10$)	$p = 0.046$ ($r = 0.34$)
Sturzelbronn 3	$p =0.006$ ($r = -0.42$)	$p = 1$ ($r = -0.16$)	$p = 0.708$ ($r = -0.03$)	$p = 0.349$ ($r = 0.05$)
Global	**$p = 1.019e-13$** **NEG ($r = \|0.26\|$)**	**$p = 0.087$** **POS($r = \|0.17\|$)**	**$p = 7.728e-12$** **NEG($r = \|0.19\|$)**	**$p =2.584e-23$** **POS($r = \|0.41\|$)**

The last column also indicates the tests between matrices for age difference and dominance difference. NA, Non-Applicable. For the global value, POS indicates that most of significant correlations were positive; NEG indicates that most of significant correlations were negative. Bold values indicate the global statistical analyses calculated by combining P-values.

with the age of individuals in the model (male individuals were the only representatives of their age group (i.e., adult or juvenile) on transfer in all cases).

The time period was not included as random factor in our GLMMs because the variation of temperatures between the two periods (Period 1 and Period 2) was less than the difference in temperatures over a day (independent sample test with permutations: $z = 4.76$, $p < 0.0001$) and because the social behavior of cattle did not change during the daytime (the changes in dyadic associations between Period 1 and Period 2 are not more numerous than the changes within each period: $r = 0.6$ vs. $r = 0.58$). Although activity changes according to the temperature, social behavior does not (35). In addition, the period is not dissociated from the group composition, which has already been taken into account in our model. Taking both factors into account could lead to false interactions, influencing the statistical significance of our results [false positive or false negative (56)].

For each GLMM, multi-model inferences and Node label permutations were run. These are detailed in the **Supplementary Material**. GLMM diagnostics (i.e., residual normality distribution plot and multicollinearity between dependent factors) were carried out to evaluate the validity of the final models. We checked for multicollinearity of the predictor variables by calculating the variance inflation factor [VIF, R package "car," (57)]. In all cases, the predictor variables had a VIF value of between 1.02 and 1.9, indicating that the predictor variables were not correlated. The significance level was set at 0.05. Statistical analyses were performed in R 3.24 (58). Plots of residual normality distribution can be found in the annexes (**Figure S2**).

RESULTS

We note that the farmer's management of cattle usually involved the transfer of young individuals. Young individuals are usually dominated by older ones in cattle (Pearson correlation test for our data: df = 176, $r = -0.37$, $p < 0.0001$). Moreover, individuals arriving in a new group have fewer familiar individuals and initially have a lower dominance rank than their resident counterparts (Pearson correlation test for our data: df = 111, $r = 0.41$, $p < 0.0001$), not because of their low number of familiars but because resident individuals are usually dominant over new arrivals to the group. This phenomenon is considered in the discussion.

Do Dyadic Spatial Associations Depend on Shared Characteristics Among Dyads?

Table 3 indicates the results of correlation tests between the dyadic association matrices and those of differences in characteristics. **Figures 2A–F** shows six instances of Highland cattle social networks. A relatively high variability is observed according to the group composition. There is a significant correlation between matrices of dyadic associations and those of dominance rank differences. Most correlations are negative, indicating that close-ranking individuals have stronger associations than individuals with distant ranks. This is illustrated by the social networks in **Figure 2C**. Dyadic associations were only dependent on the sex of individuals in the Rolbing group, where individuals of the same sex had stronger associations (**Figure 2C**). However, dyadic associations are mostly negatively correlated with age difference, indicating that individuals of the same age have stronger associations than cattle with greater age differences (greatest difference represented in **Figures 2A,D**). The results for age and dominance led us to make correlations between dominance and age difference matrices. Results show that individuals of a similar age also share similar ranks; VIF analyses based at the individual level do however show that these two factors are not collinear (see Statistical Analyses in the Methods section).

How Does a Change of Group Composition or Enclosure Affect Dyadic Spatial Associations?

The correlation coefficients concerning periods before and after a change ranged from −0.03 to 0.69, with an average of 0.47. This average is lower than we expected and means that 47% of relationships are stable after a change, whilst 53% change significantly. This correlation coefficient is not significantly affected by the number of transferred individuals ($r = -0.49$, $z = -1.4$, $p = 0.169$). This result was then detailed for each group. After the removal of the male, the dyadic spatial associations of the Niedersteinbach group did not change significantly ($r = 0.52$, $p = 0.0002$; **Figures 2A,B**). Dyadic spatial relationships in the Robertsau group seemed to stay stable after a change, regardless of whether if it is a change of enclosure or of group composition ($0.69 > r > 0.52$; $p < 0.0001$). Finally, results in the Sturzelbronn group are quite different from the two previous groups with no significant stability of dyadic spatial relationships (**Figures 2D–F**). The correlation coefficient after the removal of juveniles is −0.03 ($p = 0.812$), and indicates the strong instability of mothers' relationships after the removal of their offspring. Similarly, the dyadic spatial relationships after the addition of the bull into the group are not significantly correlated to relationships prior to this addition ($r = 0.14$, $p = 0.426$), and could mean that the male has a strong impact on the relationships of females (**Figures 2E,F**).

How Do Sociodemographic Factors Influence Individual Centralities?

The model selection for eigenvector centrality is indicated in **Table S1**. The three variables retained in the best models are dominance, familiarity and age. However, the relative importance of these variables is low (RVI(dom) = 0.23; RVI(famil) = 0.04; RVI(age) = 0.01) and after permutations, none of these variables have a significant influence that could explain the variance of the eigenvector centrality (**Table 4**).

The model selection for the strength of associations is indicated in **Table S2**. The variables retained in the best models are dominance, familiarity, sex and age. Familiarity (i.e., the number of familiar individuals in the group) has a strong and

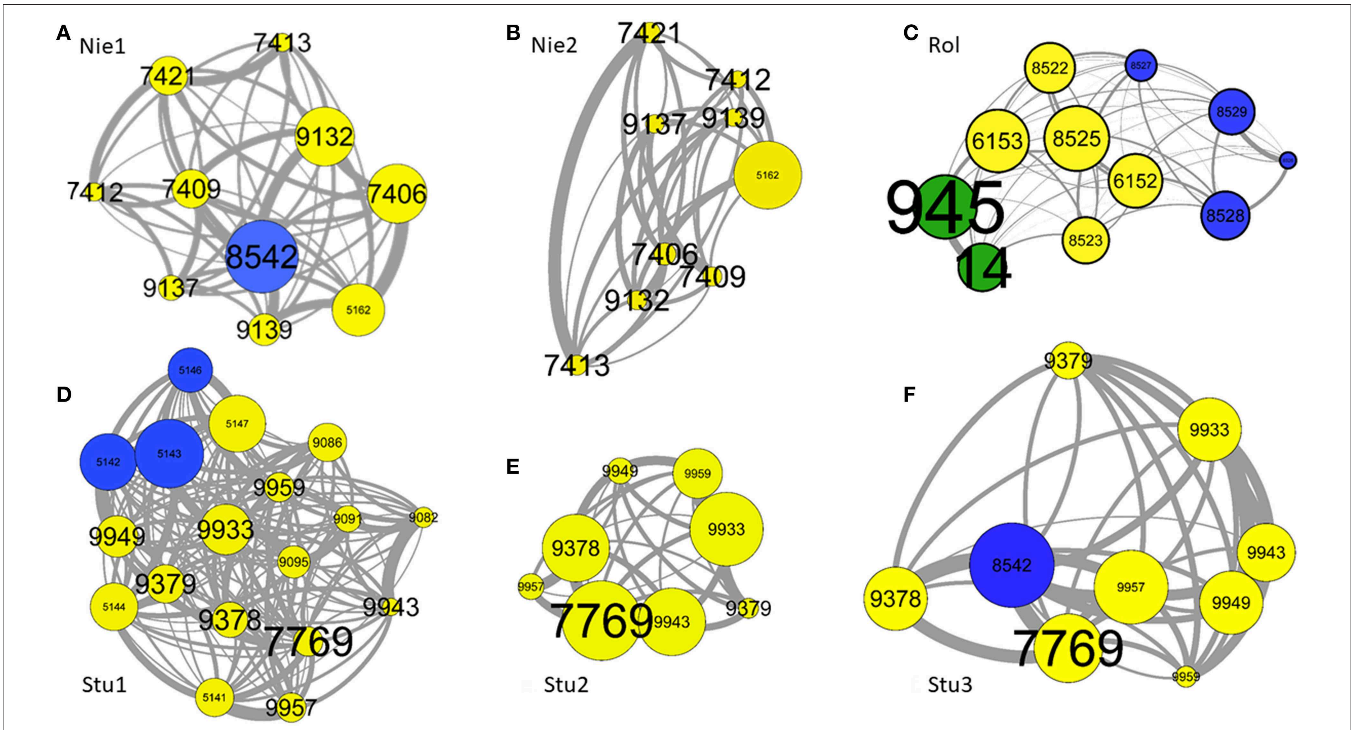

FIGURE 2 | Examples of six group compositions: **(A)** Nie1, **(B)** Nie2, **(C)** Rol, **(D)** Stu1, **(E)** Stu2, and **(F)** Stu3. One node represents one individual, each identified by a number (label). The links between nodes are dyadic associations. The size of nodes depends on the strength of associations but are relative to each group composition (the strengths are not comparable between networks). The thickness of links depend on the weight of dyadic associations. The size of labels increases with the age of individuals. Yellow, blue, and green node colors indicate females, males and castrated males, respectively. Individuals are positioned according to their weight of associations: two individuals located close to each other share a stronger dyadic association than distant individuals. Graphs were created using Gephi 0.91 (59) with the "ForceAtlas" spatialization package.

TABLE 4 | Values of the variables retained in the best models to explain the variance of the eigenvector centrality.

	Estimate	Std.Error	*z*-value	Pperm left side	Pperm right side
(Intercept)	0.388	0.0157	24.571	0.00	1.00
Dominance	−0.051	0.0204	2.463	0.199	0.801
Familiarity	−0.029	0.026	1.105	0.298	0.702
Age	0.004	0.002	1.531	0.664	0.336

significant influence on the strength of associations (RVI = 0.99, **Table 5**, **Figure 3**), i.e., the more familiars an individual has, the stronger its strength of association will be. Females also have significantly lower strengths of association than castrated males (RVI = 0.89, **Table 5**, **Figure 4**). Finally, age has a significant influence on the strength of associations (RVI = 0.12, **Table 5**), with higher strength values in older individuals than for younger ones.

How Do Changes in Group Composition Affect Individual Centralities?

The model selection for the difference of eigenvector centrality after a transfer is indicated in **Table S3**. The three variables retained in the best models are dominance, familiarity and age. However, only age has a significant influence (RVI = 0.05, **Table 6**), with the eigenvector centrality of older individuals increasing whilst that of younger individuals decreases (**Figure 5**).

The model selection for the difference of strength of associations after a transfer is indicated in **Table S4**. The variables retained in the best models are dominance, familiarity in the new group, age, and the number of transferred individuals. However, only the number of familiar individuals in the new group had a significant influence on the difference of strength of associations (RVI = 1, **Table 7**), with individuals that had greater numbers of familiar individuals showing stronger strengths of association (**Figure 6**).

DISCUSSION

This study shows how individual and dyadic social network metrics are shaped by sociodemographic factors and composition

TABLE 5 | Values of the variables retained in the best models to explain the variance of the strength of associations.

	Estimate	Std.Error	*z*-value	Pperm left side	Pperm right side
(Intercept)	0.915	0.138	6.578	0.65	0.35
Familiarity	**0.259**	**0.065**	**3.94**	**1.00**	**0.00**
SexF	**–0.301**	**0.161**	**1.83**	**0.003**	**0.997**
SexM	−0.156	0.141	1.09	0.175	0.825
Age	**0.001**	**0.005**	**0.341**	**0.98**	**0.02**
Dominance	−0.004	0.19	0.211	0.357	0.643

Bold values indicate significative results.

TABLE 6 | Values of the variables retained in the best models to explain the variance of the difference of eigenvector centrality after transfer.

	Estimate	Std.Error	*z*-value	Pperm left side	Pperm right side
(Intercept)	−0.010	0.016	0.629	0.344	0.656
Age	**0.009**	**0.003**	**2.38**	**0.985**	**0.015**
Familiarity	−0.018	0.548	0.33	0.438	0.562
Dominance	0.017	0.042	0.392	0.398	0.602

Bold values indicate significative results.

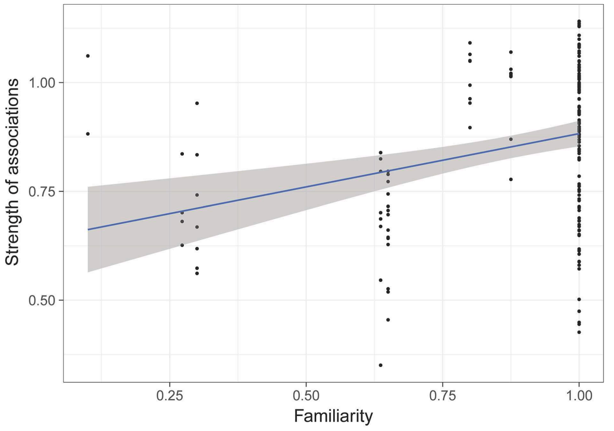

FIGURE 3 | Strength of associations according to familiarity of individuals (i.e., proportion of familiar individuals in the group). GLMM highlighted a significant effect of familiarity on strength of associations.

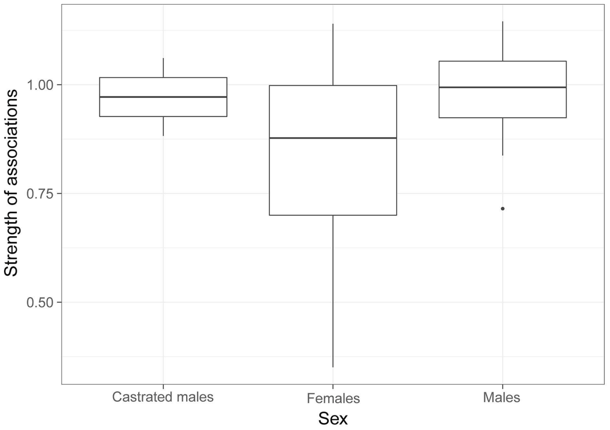

FIGURE 4 | Strength of associations according to the sex of individuals. GLMM reveals that only the strength of associations of castrated males is different to those of females.

changes in several groups of Highland cattle. Analyses of dyadic associations and individual centralities highlighted correlations between spatial proximity, age and dominance, an influence of familiarity, age and sex on individual centralities, and finally an impact of transfers that mainly varied according to the number of individuals with which the transferred animal was already familiar. These results have strong implications for animal welfare.

Do Dyadic Spatial Associations Depend on Shared Characteristics Among Dyads?

Matrix correlation tests revealed that individuals of similar age and dominance rank develop stronger associations and are located closer to each other than individuals of different age and sex. However, the tests also showed a correlation between age and dominance rank similarities. For instance, individuals 951, 949, and 947 in the Robertsau 6 group composition are approximately the same age, are the top-ranking individuals and form a triad with strong associations. This configuration has also been reported in female mouflons (*Ovis gmelini*) where the most dominant females form triadic relationships (60, 61) and is reminiscent of "triadic closure," a mechanism that may facilitate the development of cooperation for social alliances or access to food. However, it is not clear whether triadic closure is a by-product of socio-demographic characteristics (i.e., individuals that share the same characteristics also share the same needs), or if it is a social strategy leading to better cooperation between multiple partners (62, 63). Other examples also show this homophily according to age and dominance (53, 64). Many authors have confirmed homophily (tendency of individuals to associate and bond with similar others) in ungulate

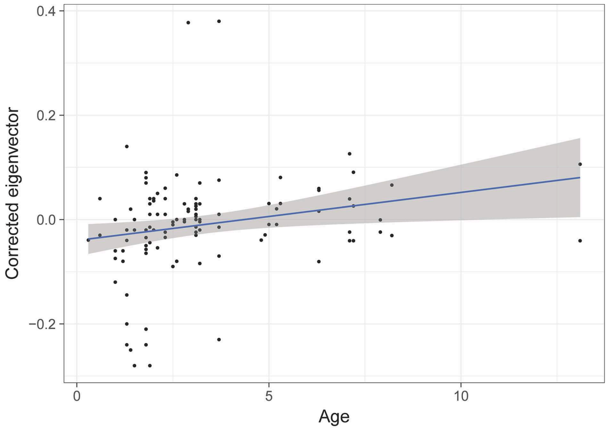

FIGURE 5 | Difference of eigenvectors after a transfer, according to the age of individuals. GLMM highlights a significant effect of age of individuals on the change in strength of associations after a transfer.

species (65–69), and underline that animals with the same socio-demographic characteristics may also share the same social or physiological/nutritional needs. Indeed, younger individuals show strong associations, as observed in the Rolbing and Sturzelbronn 1 group compositions (**Figures 2C,D**, respectively). This tendency of individuals to associate and bond with similar others means that animals feel better by doing this. This increases their welfare and could be use in this way: associating individuals having same age. This homophily seems to help young individuals to learn how to live in groups and acquire sociality without risk of injury, particularly when in contact with adults (70). The same reasoning about reducing risk of injury could be applied for homophily between individuals that have the same dominance rank. Risk of injury prevents subordinate individuals from having strong associations with dominant individuals [as described in ungulates (71) and in primates (46, 72, 73)]. This dominance-related homophily may also result from competition between individuals seeking to associate with top-ranking individuals on order to obtain tolerance or access to resources. However, as high-ranking individuals are already associated among themselves, low-ranking individuals might not gain access to them (46). The results we obtained were not observed in all group compositions, and this could be explained by intra-group age variance. The difference in dominance and the strength of homophily increase with differences in age. This was seen in the Niedersteinbach group, where the maximum age difference between individuals was 2 years (individuals aged 7 yo and 5 yo, with the exception of one juvenile). Unlike the other compositions, no age-related homophily was observed in this group.

Individuals of the same age also have more similar dominance ranks than individuals of different ages. Age affects dominance through the association of individuals, meaning that individuals of the same age are likely to develop the same dominance rank because of their strong and close associations. Social status such as dominance increases with age through different processes such as increases in body weight, experience and knowledge or social power (29, 73–76). In the Niedersteinbach 1 group composition (**Figure 2A**), the male, which was also the oldest and highest-ranking individual, played an important role in the correlation with dyadic associations. The correlation was no longer significant when this individual left the group (Niedersteinbach 2, **Figure 2B**). This is either simply because it had been removed from the statistics, or because the group's social structure had been perturbed. When this male arrived in the Sturzelbronn 3 group composition (**Figure 2F**), it was no longer the oldest in the group but it became the highest ranking individual, making the correlation with dominance and associations significant. This link between age and dominance is advantage for livestock. It means that associating individuals of same age decreases the range of dominance between them and the rate of aggressions.

Whilst age and dominance have a strong impact on dyadic relationships, we found that age was the only variable affecting strength of associations. Older individuals obtain stronger strengths of associations, but dominant individuals do not. There does not appear to be any competition for the central positions in the groups we studied. Dominant individuals are usually expected to develop strong associations because they occupy central positions in the group for better protection against predators or increased access to other resources. This affords higher centrality to these dominant individuals than to others. Other resources are used in this system, such as small clumps of trees that protect from the sun and high temperatures. These spots are appreciated by animals for thermoregulation, and dominant individuals have been seen to occupy them and prevent others from entering them (77–79). Whilst some such areas were present in our study groups, no correlation of this type was observed between strength of associations and dominance.

How Do Sociodemographic Factors Influence Individual Centralities?

Centrality is also linked to age, with the oldest individuals having the highest strength of associations. With age, individuals become more and more selective (80) in their social relationships. Young individuals interact unselectively with many partners in order to learn social rules (70). With time, they develop more stable relationships and become more and more central (73). In our study, this effect was amplified because young individuals, juveniles or young adults were also those the farmers chose to transfer. They therefore had to develop new relationships each time they were transferred, accentuating the link between age and centrality. Juveniles usually have strong relationships with their mothers, yet few juveniles were still in the presence of their mother in our study. They were not easily accepted on their transferal and remained on the periphery of the new group, forming strong dyadic associations among themselves as already shown in previous studies (8, 32). This result for age is emphasized by that obtained for familiarity. Indeed, in our study, familiarity was linked to age as older individuals stayed in their enclosure whilst younger ones were transferred. This is not the best way to avoid stress for juveniles. Transferring adults instead of juveniles or transferring a mix of juveniles and adults could be better for the group integration and the welfare of juveniles. Individuals with a greater number of familiar individuals in the group showed

TABLE 7 | Values of the variables retained in the best models to explain the variance of the difference of strength of associations after transfer.

	Estimate	Std.Error	Adjusted SE	*z*-value	Pperm left side	Pperm right side
(Intercept)	−0.730	0.145	0.147	4.975	0.00	1.00
Dominance	0.120	0.143	0.144	0.831	0.90	0.10
Familiarity	**0.816**	**0.151**	**0.153**	**5.323**	**1.00**	**0.00**
Age	0.001	0.005	0.005	0.213	0.812	0.188
N	−0.0003	0.002	0.002	0.159	0.112	0.888

Bold values indicate significative results.

higher centralities. In bovines, group members form subsets of familiar individuals, accentuating dyadic relationships and increasing centralities (12, 81). In sheep (*Ovies aries*), familiar individuals are attracted to each other, whilst non-familiar individuals are not (69). In our study, resident cattle rebuffed new individuals and were more aggressive toward them around coveted spots (personal observations). The same result has been found in barnacle geese (*Branta leucopsis*) (82) and in mallards (*Anas platyrhynchos*) (83).

Sex also affected the strength of associations in our study, with castrated males showing stronger strengths of association than females. We did not observe any difference between males and females, and this is mainly due to the social organization of bovines. Bovines show sexual segregation, and females usually develop stronger and more stable dyadic associations than males, resulting in a higher centrality for females (17, 28, 84). However, this sex-centrality link in our study is influenced by the fact that male juveniles remain closer to their mother and other young individuals. The stronger centralities of the two castrated males in our study are mainly explained by the group composition. These two individuals were the two only adults in a group of juveniles, which seek group cohesion more than adults. The sex variable was therefore not dissociated from age in the Rolbing group, which probably explains this result in our study. However, the presence of the castrated males seemed to be important for the juveniles and could replace females or non-castrated males, with a lower rate of aggressions. The two castrated males did not show aggressions toward juveniles, which is a good way to manage excess of juveniles.

Eigenvector centrality was not affected by any of the factors we studied. This is probably because eigenvector centrality takes not only direct connections (i.e., how an individual is connected) into account, but also indirect connections, i.e., how its neighbors are connected to other individuals (52). Our studied groups were quite cohesive with a low sample size, which may have led to a low variance of eigenvector centralities between group members and an absence of correlations with socio-demographic factors.

Together, these results allow us to identify which factors affect the social relationships and thus the centralities of group members; the combination of these factors as a management tool could reinforce group cohesion by giving a key sociality role to one specific group member or decreasing aggressiveness during group transfers: juveniles need adults and age similarities increase cohesion and decrease centralities differences and aggressions between individuals.

How Does a Change of Group Composition or of Enclosure Affect Dyadic Spatial Associations?

Our results showed that social relationships are more affected by the identities of transferred individuals than by the number of individuals transferred. Indeed, the addition or the removal of young or adult individuals that were not related to other group members does not seem to significantly affect the social relationships of resident individuals, except for the addition of the male in Sturzelbronn (**Figures 2E,F**). However, the removal of offspring seems to strongly destabilize the relationships of the mothers (**Figures 2D,E**).

How Do Changes in Group Composition Affect Individual Centralities?

The difference in the eigenvector centralities between two transfers is explained by age alone. Results show that the centrality of young individuals tends to decrease during transfer, whilst older individuals obtain higher centrality. During transfer, most young individuals leave their original group for a new group without their mother. These individuals are then isolated and placed at the periphery of the group until they form new and stable relationships (21). Conversely, adults benefit from the transfer of young individuals as they are residents, and newly transferred individuals seek cohesion to alleviate their stress. Indeed, stress increases social cohesion and proximity with partners (85–87). Moreover, the eigenvector centrality coefficient takes into account not only the connections of a group member, but also how these connections are connected to other individuals in the group (52). If the relationships of an individual change but those of its connected individuals do not, then little change will be seen in eigenvector centrality, whilst the strength of association will increase or decrease. In this respect, the eigenvector centrality coefficient is more stable than coefficients that are solely focused on the individual, such as strength of associations or degree (88).

Strength of associations was only affected by the number of familiar individuals in the new group. Individuals with a stable number of familiar individuals in the new group composition showed frequent interaction with them, whilst the individuals that had been separated from familiar individuals interacted less with other group members and needed time to develop strong and stable associations. Researchers (21) showed that the presence of familiar individuals during transfer is indeed less stressful. Familiar individuals have a stronger

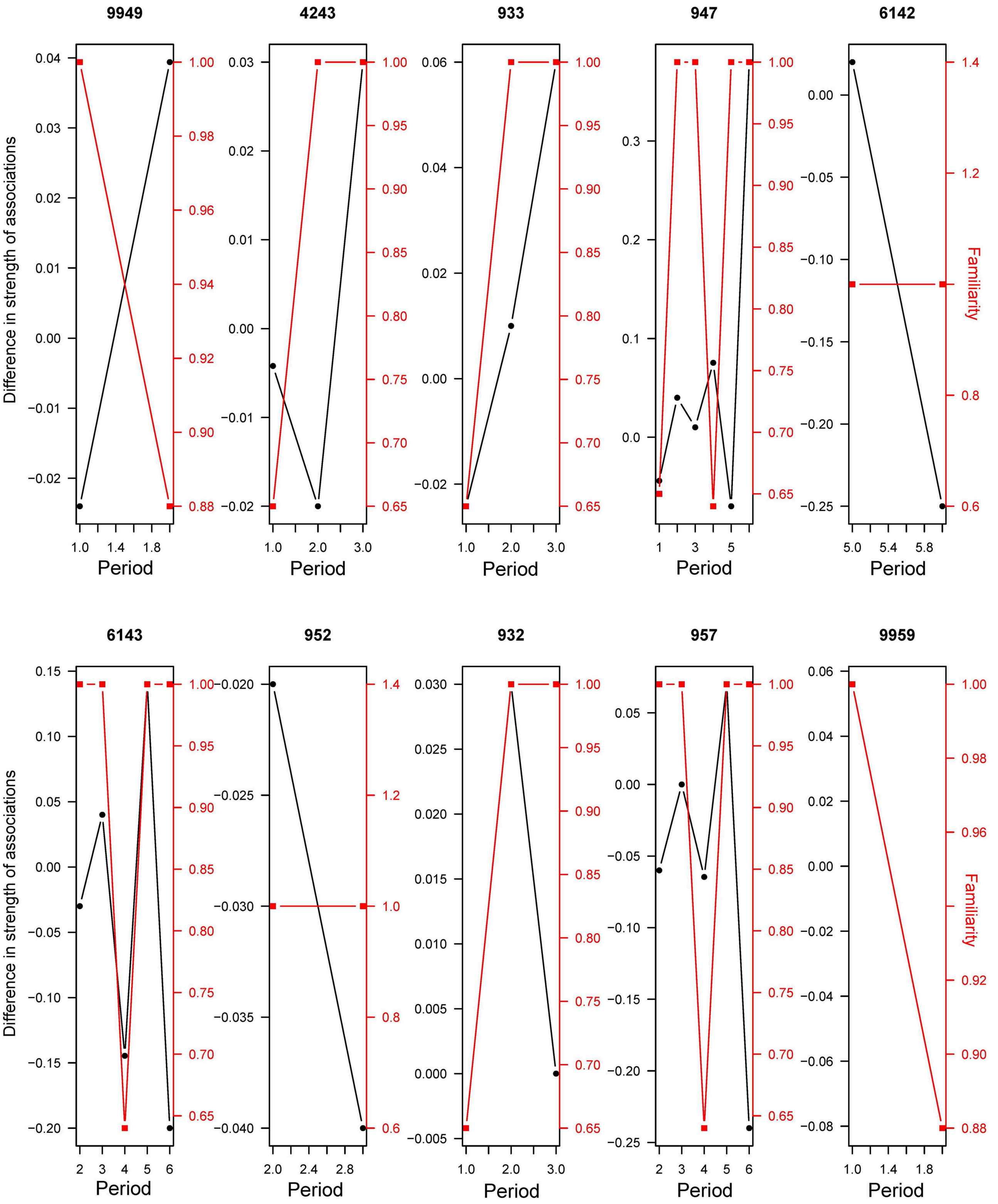

FIGURE 6 | Difference in strength of associations (black line) and familiarity (red line, as proportion of familiar individuals in the group) between different periods of transfer (Periods exclude any transfer activity). Examples for ten randomly chosen individuals.

strength of associations due to increased group cohesion (85, 86). Finally, and surprisingly, the number of transferred individuals did not lead to a change in strength of associations. Mathematically, as there are more partners to associate with when the number of individuals increases in a group, there is less possibility and less time for each partner to associate. We should therefore observe a global decrease in the strength of associations per individual. Another study (89) showed that an increase in group size may lead to decreased space availability and therefore result in a higher occurrence of agonistic behaviors. The fact that we did not observe such an effect in our study, at least after removing the first 8 days after a transfer, could be explained by the large size of the enclosures. Indeed, aggressive interactions are at their highest when the groups are first mixed. In most cattle groups, aggression is rarely seen once the dominance rank is established, as groups operate more through affiliative than agonistic behaviors (31). Newly transferred individuals in this study have usually all been removed from the same group, which may lead these individuals to stay together (resident vs. transferred) and thus exclude any change in their relationships. However, this hypothesis remains to be tested as even if they stay amongst themselves, the stress entailed by the change should lead to a greater cohesion of individuals, and this was not observed in our study.

Implication for Animal Welfare

Our results show that a group is structured according to age, dominance and familiarity. Favoring specific age differences between individuals and subsets of familiars may be a tool to control cohesion and stability and decrease aggression in a group. The individual centralities of cattle decrease during transfers and changes in group composition. This occurs mainly in young individuals and is due to the loss of familiar individuals. During stressful events, animals seem to prefer interacting with familiar individuals and avoid interacting with unfamiliar group members (69). When transferring individuals, it is therefore preferable to select a certain number of familiar individuals to transfer as a group in order to decrease stress. Juveniles have to been transferred preferentially with a familiar adult, the best is the mother. We may also prevent this stress by transferring first a castrated male (or a female) that will be transferred later with the juveniles. Although it is true that animals should adapt to their new environment after a certain time (11) an optimal group composition will permit a more rapid integration of new individuals. This is particularly important in view of the fact that stress can impact the behavior, cognition, reproductive performance and health of individuals (14, 90, 91). It would also be preferable to transfer juvenile individuals aged around 3 yo with an adult, and avoid transferring juveniles that are less than 1 year old. This would be the best way to decrease the stress of juveniles, related or not to aggressions, to a minimum during transfer. On the other hand, forming stable pairs of individuals before and during transfers may increase food intake and weight gain, particularly in calves (20). Following these rules, then the optimal group composition should be composed of at least four pairs of adults of about same age (mostly females but castrated males is working) and four pairs of juveniles, i.e., sixteen individuals. This will allow transferring two to four adults and two to four juveniles and the same time, preferentially kin. This study has highlighted some interesting results for the improvement of livestock welfare, but other factors could be studied to further enhance animal wellbeing during changes in group composition, notably the personality of the individuals chosen for transfer (92, 93).

ETHICS STATEMENT

This study was based on the observation of animals, and no handling or invasive experiments were involved. Our study was approved by our research institution (IPHC). It was carried out in full accordance with our university ethical guidelines and complied with European animal welfare legislation. Every effort was made to ensure the welfare of the animals and minimize disturbance by researchers present in the field.

AUTHOR CONTRIBUTIONS

All authors scored data and read the final version of the manuscript. CS and MP designed the study and wrote the manuscript. CS and SS analyzed the data.

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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.2019.00183/full#supplementary-material

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An Appraisal of Methods for Measuring Welfare of Grazing Ruminants

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Although disturbances in body function of animals can be measured to determine whether a state of stress may exist, there is growing interest in finding ways to assess their emotional status as an indicator of good or bad welfare status. Generally it is easier to determine poor states of well-being than positive ones. For grazing ruminants some indicators of well-being include absence of illness, good growth and productivity, and longevity. Motion detectors can provide automated remote monitoring of behavior and it is likely that there will be advances in the interpretation software to increase the utility of this technology for assessing well-being. Cortisol levels in body fluids, feces and pelage are prominent as a marker of poor animal welfare, but like many of the other objective measures that are used, are not wholly reliable at the individual animal level. These other measures include: plasma serotonin, heart rate variation, infra-red thermography, cytokines, salivary alpha amylase, and acute phase proteins. Use of automated facial expression recognition may supplement electrophysiological recording as means to quantify the pain experience of animals. Although the measures described in the literature do not necessarily provide the final answer for determination of welfare in grazing ruminants, they all have some merit and deserve further investigation.

Keywords: animal welfare, ruminants, HPA axis, assessment of pain, animal behavior

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INTRODUCTION

One of the initial tasks that arises from attempts to quantify the well-being of grazing animals is that of deciding what their well-being, or welfare, actually is. Although this issue is dealt with in some depth in nearby papers, it needs to be addressed here as well to provide context for the measures under consideration. Confusion stems from differing views about what constitutes animal welfare. For instance Moberg, (1) described disturbances of stress in animals as the development of a pre-pathological state, specifically as "a stress-related change in biological function that threatens the animal's well-being," this being the onset of poor welfare status of an animal. Broom and Johnson (2) provided a wider view that all biological responses represent states of welfare, very good and poor, with the development of pathology as a manifestation of excessive stress and, thus, a poor welfare status. The latter authors (2, 3) also pointed out that most of the quantifiable measures of animals that were being used principally determined poor welfare. What is really needed is a clearer view of the mental state of animals. It is argued that welfare is fulfilled when animals experience positive emotions and do not feel prolonged negative emotions (4–6). This has led more recently to consideration of "animal happiness" where emphasis has shifted from concern about negative aspects of animal welfare to the positives (7). However, the problem remains about how we can interpret the responses of animals in terms of their emotional experiences. It has been addressed by Safina (8) but further discussion of this question and of these definitions lies outside the scope of this paper, which is primarily confined to an examination of the methodology used to evaluate measures of welfare.

The focus of concerns about the welfare of grazing ruminants must center on farmed animals. The great bulk of wild, free-ranging ruminants are only loosely managed by humans and are generally able to experience their normal behaviors. However, they must encounter all manner of situations where their welfare is poor, for example: from predation, during droughts, from wildfires, during blizzards, presence of uncontrolled infectious diseases, etc. Nevertheless, we tend to view this group of animals as beyond our immediate concern in terms of management of their welfare. Apart from farmed animals (and some held as pets or in zoological parks, circuses, etc.) for whose management we are fully responsible, the other group of grazing ruminants for whom welfare is a major concern are those utilized in research projects or in testing procedures. There is an underlying belief amongst animal rights proponents that scientific research involving use of animals is wrong on philosophical grounds, but this belief is often associated with concerns about welfare of the animals. In recent years, many research projects involving ruminant animals have been conducted just for purposes of evaluating measures of their welfare (see later). It is likely that some of these are motivated by the possible need to set welfare standards for the farming of ruminants, as has been experienced by the poultry and pig farming industries—largely in response to concerns raised by the general public.

Physical Health and Productivity

One generalized view about the determination of a farm animal's well-being is that it can be obtained from their health status and productivity. This concept has been carefully explored by Professor Marian Dawkins in her book "Animal suffering: the science of animal welfare" (9). She pointed out that although the concept that a physically healthy animal must be in a state of good welfare seems attractive, this measure does not provide any evidence about mental well-being of such animals. Nor does high productivity necessarily mean that the animals are experiencing high levels of welfare. In spite of these caveats, the presence of healthy animals with acceptable growth rates and levels of meat, milk or fiber production, and normal reproductive performance must be considered both as sound indicators of good animal welfare and as ones that are readily measured.

One measure that probably integrates both the health status and productivity of farm animals is longevity. It is likely that shortened longevity represents poorer reproductive performance or health status of animals, possibly associated with selection and management practices aimed at high levels of productivity. This appears to be evident in the dairy industries where there has been a reduction of about 50% in the life expectancy of cows in Denmark between 1960 and 1982 (3) and the current life expectancy for dairy cows in Sweden is only 60.5 months (10). These Scandinavian data are likely to be representative of dairy industries globally. It is generally argued that nutritional strategies which improve longevity of ruminants will impact favorably on their welfare (11). Intuitively, longevity of production animals must be fairly easy to determine. This makes it a simple measure that provides a potentially useful indicator of well-being that should be placed high on the list of measures available for assessing animal welfare on farms. Nevertheless, there is a counter argument. Sometimes longevity of animals may come at cost in terms in terms of their welfare and it would be important to recognize when this is the case.

The Good Life

Another concept of a good animal welfare scenario is that they "are healthy and have what they want" (12). This statement obliges us to determine animals' wants and presupposes that we can determine positive states of emotion. It moves away from the notion that good welfare is simply the absence of negative experiences and forces us to find ways to demonstrate that animals are enjoying positive emotional experiences. This has proven to be difficult, as most studies in the literature for humans and other mammals have focussed on negative emotions such as fear; the reason being that negative experiences are more intense than positive ones and are therefore easier to measure (13). Their review (13) indicated a need at that time for more research on positive affective states in animals, however it seems that this area of study still continues to lack attention (7). Evidence of pleasure can be based on behaviors such as social interaction, reproductive activity, play, self-grooming, anticipatory hyperactivity, and exploration. In many cases these activities are associated with a reward, such as food [e.g., see (14)], environmental enrichment or shelter from inclement weather, and these rewards can be akin, in physiological terms, to those provided by addictive drugs. As with addictive drugs, the underlying physiology of pleasure experience is manifested in activation of specific neural and neuroendocrine pathways which are quantifiable and likely to provide complementary information to that determined from behavioral studies. Some of these are addressed below.

Threats to Welfare

In all circumstances grazing ruminants experience a wide realm of threats to their welfare. As well as the obvious threats such as inadequate feed or water, inclement weather associated with inadequate shelter and infectious diseases, grazing ruminants may have to contend with competition for space and feed, gastrointestinal parasitism, unsuitable surfaces, lack of feed diversity or variety, toxic plant compounds, predation, and inadequate care from their human minders. In most cases, these are easily identified and can be quantified and managed. The problem occurs when the threats are either not obvious to the observer or when they are below levels of detection, or not considered important. It is in these cases and where we cannot determine whether a threat exists, e.g., limited feed diversity or negative social interactions amongst individuals, that we are fully dependent on the availability of reliable measures of animal well-being.

Behavioral Assessment to Determine Welfare of Animals

Welfare of animals can be assessed from ethograms of behaviors. This is where the activities of animals are recorded throughout a monitoring period and the amount of time performing each activity provides a spectrum of behavior that can differentiate individuals who are behaving abnormally or even indicate they are undergoing stress. Alternatively, external indicators of behavior, such as skin lesions to quantify aggression in pigs (15), can provide useful assessments of welfare.

The major limitation to behavioral assessment of animals has been the workload demand on the observers, whether conducting direct observations or interrogating hours of closed circuit television recordings. Automation of behavioral monitoring is a rapidly expanding technology that offers much promise for monitoring the welfare of animals, as well as providing measures of their physical health and reproductive status. Use of accelerometers with automated data capture is providing information about activity of animals from devices placed on a leg (16, 17) and/or on a neck collar (17). However, the reliability of the information as an indicator of pain, inflammation or stress does not appear to be very precise. For instance, use of these devices showed that primiparous cows with clinical metritis spent more time on average lying than their metritis-free counterparts although there was no such effect in multiparous cows (16). Likewise, Williams (17) showed a failure of activity-sensing devices to record all potential oestrous events in dairy cows. In the case of a short-term removal of dietary supplement from dairy cows that was insufficient to cause clinical signs of metabolic disorder, these devices revealed a compensatory increase in mean eating and rumination times (18). It is possible that these changes would be more pronounced when the metabolic disturbance reached a point where welfare is compromised, but the current picture emerging from these studies is that the type of information obtained with the automated devices lacks the degree of resolution that would make it reliable for assessing the welfare status of individual animals. Nevertheless, there is promise of increased sophistication of such devices and of data analysis software in this area. For example, linking these recording devices to a real-time location system has enabled cow behavior to be classified more accurately (19). This is an area of technology that is likely to advance rapidly.

Another promising avenue of study is the analysis of farm animal vocalizations (20). Vocal expression by mammals is linked physiologically to their emotions (21) and it is suggested that vocalizations of cattle can be interpreted to assess how they are coping with their farming environment (22).

An interesting behavioral measure that has been applied to sheep is ear posture. Their ears can be scored as "forward," "backward," "asymmetric," or "passive" (23). These authors and Reefman et al. (24) have reported changes in ear posture according to different emotional states in sheep, which makes this measure attractive because of its ease of detection.

Qualitative Behavior Assessment and Cognitive Bias

An holistic measure of animal welfare based on behavior can be obtained by applying the process of qualitative behavior assessment [e.g., for sheep-(25)]. This involves an initial group assessment to determine whether animals are: relaxed, dejected, thriving, agitated, responsive, dull, content, anxious, bright, vigorous, distressed, then scoring the predominant behavior. Following this, a follow up examination is conducted to count prevalence of several physical indicators of health and welfare (e.g., coughing, lameness, soiling). Collins et al. (26) found this approach useful for evaluation of sheep transport stressors. However, measures of animal behavior are susceptible to the mood of animals at the time of assessment and this is the basis for consideration of cognitive bias, sometimes termed attention bias, during behavioral evaluation of animal welfare. It is not surprising that an animal experiencing a negative affective state, based on its current emotional experiences (or mood), will display different judgment about a stimulus to that of an animal in a positive affected state. This has been ably reviewed recently by Clegg (27) and several studies have examined this topic in respect of studies with sheep (28–31) and calves (32).

Cortisol

A major component of the stress response of mammals is activation of the hypothalamus-pituitary-adrenal (HPA) axis which manifests as an elevation of circulating levels of β-endorphin, vasopressin and, particularly, cortisol. The stress-induced elevation of β-endorphin levels in blood is related to the stress modulating activity of this and other endogenous opioids (33) and a similar role is performed by vasopressin, in addition to its direct effects on cardiovascular and kidney function (34). Cortisol is a glucocorticoid with an important role in the mobilization of energy stores during activation of the stress process (35). However, cortisol has almost reached "silver bullet" status as the answer to our need for a simple, quantifiable, measure of lowered welfare status for an individual animal. Its measurement in blood plasma has proved useful as a tool to compare various, potentially noxious, farm procedures such as the various techniques for castration of calves [e.g., (36)] and castration and tail docking of lambs [e.g., (37)]. In spite of its universal appeal as a monitor of negative animal welfare status, caution needs to be applied to conclusions based on measurement of cortisol levels in body fluids (38). One factor is the blood sampling procedure itself. Red deer stags blood sampled by jugular venepuncture during manual restraint had a mean plasma cortisol concentration of 56.5 ng/ml which is in stark contrast with the values obtained with a remote blood sampling backpack whilst the stags were on pasture and undisturbed – 8.4 ng/ml (39). These plasma cortisol values obtained from undisturbed animals are low in comparison with other figures in the literature and indicate that even where blood samples are obtained via an indwelling cannula that has been placed intravenously some days prior, the animals are still susceptible to human presence at the time of sampling. Although, gene transcription and eventual synthesis and secretion of *de novo* hormone product may take several minutes, there are ready releasable sources of cortisol—as seen in blood samples collected at 10 min following administration of adrenocorticotrophic hormone (ACTH) or corticotrophin releasing hormone (CRH) in young (3 weeks) and older (26 weeks) calves (40). This means that the arrival of operators to collect blood, albeit remotely, may be a sufficient stimulus to elevate cortisol in the resultant samples. The same will be true for saliva samples. The study by Van Reenen (40) also revealed a lack of consistency between the responsiveness of cortisol to exogenous CRH or ACTH and behavioral tests, and an age-related increase in responsiveness in the calves. It is thus very unlikely that much emphasis can be placed on a single cortisol measurement in a circulating body fluid as a measure of an animal's state of welfare.

Measurement of glucocorticoid metabolites in the feces of mammals provides a non-invasive approach for determination of recent adrenal cortex activity. The methodology for dairy cows has been nicely validated by Catherine Morrow and her co-authors (41). The lag intervals between elevation of plasma corticosteroids and subsequent elevation of metabolite levels in feces approximated digesta intestinal transit times (41). Although the magnitude of the elevations of the metabolites in feces is much lower than that of the corresponding steroid plasma levels, the method is sensitive enough to detect changes on exposure of cows to a new environment and following their transportation (41, 42). Whilst the data obtained from the numerous published studies have been very encouraging, many authors still consider that it is necessary to use this methodology in concert with other monitoring measures to provide reliable indicators of stress.

It can be argued that the information provided by measurement of cortisol, or its metabolites, in blood, saliva, urine or feces is relevant only to the previous few minutes and up to a few days of retrospective experience of the animal. A longer-term picture of HPA axis activity, for instance a period of chronic stress, may be afforded from measurements of these compounds in the hair or wool of animals (43). However, there are several considerations that must be borne in mind regarding cortisol levels in hair. Firstly, skin (melanocytes) and hair follicle cells contain all elements of the HPA axis including signal molecules (pro-opiomelanocortin, corticotrophin releasing hormone, adrenocorticotrophic hormone) and their receptors, plus the steroid synthesis machinery (44). Thus, there is an HPA axis homolog in skin tissues that can produce corticosteroids independently of the central stress axis. Secondly, incorporation of locally derived corticosteroids and those passively acquired from blood into the growing hair shaft takes place at the follicle bulb (45)—several millimeters below the skin surface (46)—so there is considerable delay before they can be located in shaved hair and this is further complicated by variation, especially seasonal, in hair growth rate and skin blood flow. Also, there is possible "washout" of steroids from hair caused by chemical degradation, grooming, ultraviolet radiation, rainfall, etc., and possible contamination from sweat. However, the ease of collection of hair or wool and the stability of its corticosteroid levels during storage makes this an attractive approach to assessment of stress in animals (43). Results from studies of hair cortisol content of cattle have shown significant elevations when stocking density was markedly changed (47) but not when the change was minor (48) and similarly inconsistent findings have been reported for castration of calves [e.g., (49, 50)]. It seems that when there is a major source of stress, e.g., heat and water deprivation in sheep (51), there is an elevation in hair cortisol content and, likewise, hair cortisol content was associated with clinical disease and pregnancy (52) and with the duration of clinical disease (53) in cows. However, Tracy Burnett and her co-authors (52) pointed out that this parameter did not differentiate lower magnitudes of stress or sub-clinical disease in cattle. Hair cortisol content does show promise as an indicator of animal welfare status but clearly there is a need to develop sampling protocols (such as those suggested by 42) and to be aware of its possible limitations.

Serotonin

Serotonin, which is actually 5-hydroxytryptramine (5-HT), is derived from tryptophan. It is a neurotransmitter, produced by the serotonergic neurons, but is also formed in a variety of tissues and appears in the circulation from which it is readily removed by platelets or endothelial cells in the lungs and liver. In mammals, there have been numerous studies relating plasma serotonin levels to stress and other disorders. However, as shown in the extensive review of their relation to various pathological states in horses (54), the picture in relation to stress is confusing. Nevertheless, a study of plasma serotonin levels in dairy cows showed the values to be elevated by the stress of negative energy balance (55). This seems to be a measure with potential for detecting the existence of stress conditions in farm animals although the involvement of platelets in metabolism of serotonin means that measurement of free serotonin concentrations is best done with platelet-poor plasma.

Cardiac Function

The acute response to stressors is an elevation of activity of the sympathetic-adrenal medulla (SAM) axis, most readily detected as an increase in heart rate. Heart rate is easy to measure with electronic recording devices and can be stored on data loggers attached to the animal or transmitted to distant recorders. Heart rate *per se* probably does not provide useful information about long-term welfare status but there is interest in heart rate variation (HRV) as a measure of welfare. HRV is simply obtained by a Fourier transformation of data from any continuous (preferably at least 5 min) heart rate recording. It is alleged that HRV provides information about the balance of activity between the two divisions of the autonomic nervous system: sympathetic and parasympathetic. Or, simply, the balance between sympathetic and vagal activity. Use of HRV for assessment of welfare of farm animals has been comprehensively reviewed (56) and the evidence obtained from about a decade of investigation provided a strong case for continued development of this technology for use in farm animals.

Infra-Red Thermography (IRT)

Infra-red thermography (IRT) is based on photography of the external surfaces of animals using an infra-red camera. The thermal image can be reproduced in color to reveal the surface heat transfer and blood flow. Initial users of the technique were able to obtain early detection of clinical disease (57). The technique can be used on any region of the body surface, however the eye and surrounding skin tissue provide an image that may reflect the sympathetic-vagal balance of the animal (58). In general, disturbances in thermal radiation from the various surfaces of animals indicate the presence of inflammatory processes, although the genitalia may provide indicators of reproductive status. An overview of use of IRT in farm animals has been provided by (59). Although it is non-invasive and simple to perform, IRT currently shows most promise mainly as a tool for early detection of disease. One of the problems encountered with the technique is standardizing the positioning (angle and distance) of the camera whilst minimizing the need for restraint of the animals.

A variant of IRT is use of functional near infra-red spectroscopy (fNIRS) probes to determine differences in oxy-hemoglobin and deoxy-hemoglobin between right and left cerebral hemispheres of the sheep brain. This has been used to detect a bilateral increase in cerebral activity in response to anticipation of a food reward together with a greater haemodynamic response in the right hemisphere compared with the left (23).

Other Measures of Sympathetic-Vagal Activity

In addition to IRT other non-invasive measures of sympathetic-vagal activity include proportion of eye white and eye temperature. Percentage of visible eye white in cows increased with increasing frustration (60) however, Gómez et al. (61) found no relation between this measure or eye temperature of cows experiencing either non-stressful (feeding) or stressful (claw trimming) experiences. Likewise, there were no emotion-related effects on percentage of eye white in sheep (62), although the latter authors suggested that eye aperture (possibly related to eyelid muscle tension) may be meaningful (24). Another measure that comes under this heading is body surface humidity which also seems to vary, particularly in concert with the level of sympathetic activity (24, 62).

Markers of Immune Function

As well as the obvious involvement of immunological mechanisms in response to the presence of pathogenic antigens, the immune system has important functional links with brain function [e.g., emotional limbic system activity (63)] and with the HPA axis (64), the latter particularly through the immunosuppressive activity of glucocorticoids. Brain function is impacted directly by neuroinflammation arising from associated immune dysregulation (65). Markers of immune function include immunoglobulins (e.g., immunoglobulin-A) and the cytokines. The cytokines have been grouped into eight families: interleukins, tumor necrosis factors, interferons, chemokines, haematopoietins, colony stimulating factors, neurotrophins, and growth factors (66). Immune activation may be considered as a stress response in its own right and because many of the markers mentioned above can be measured in samples of blood or saliva, this has become a potentially rich avenue for monitoring well-being of farm animals.

Salivary Alpha Amylase

There is much interest in the concept, particularly in the human-related literature, that levels of alpha amylase, a digestive enzyme that is present in saliva, provide a marker of sympathetic nerve activity (67). Among farm animals, this measure of stress has been applied particularly to pigs [e.g., (68)] and a study of sheep (69) showed a significant elevation in concentration of salivary alpha amylase 15 min after exposure to a barking dog stimulus. Nevertheless, both of the studies cited above reported considerable individual variation in the responses and indicated that the measure needs further investigation to confirm its reliability as a monitor of stress in farm animals.

Acute Phase Proteins

Acute phase proteins are a group of approximately 30 mainly liver-derived proteins present in blood that experience a change (25% or more) in concentration in response to inflammation, or specifically in response to altered activity of pro-inflammatory cytokines—particularly interleukin-6, but also interleukin-1, tissue necrosis factor alpha and interferon gamma. Members of this group of proteins include C-reactive protein, serum amyloid A and haptoglobin. Their functions include: enabling entrapment of microorganisms and their products, activation of the complement system, binding cellular remnants, neutralizing enzymes, scavenging free hemoglobin and radicals, and modulation of the immune response (70). Specifically, the acute phase proteins are useful indicators of animal ill health and tissue damage, thus providing information about the severity of the condition and on the degree of recovery or healing that is occurring. Such information is likely to be relevant to assessment of the level of stress experienced by an animal.

Assessment of Pain

It is generally accepted that the definition of pain in humans, i.e., "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage," has to be applied equally to other animals. The occurrence of pain experienced by animals is clearly a welfare concern that can be managed effectively only if there are sound means to recognize and quantify it. Presently, for both humans and animals, there are no universally accepted methods for achieving this. Management procedures for farm animals that are perceived (by humans) as being painful usually require use of analgesic drugs or local anesthetics to block potential pain. However, the monitoring of grazing animals to determine when they are experiencing pain "in the field" is largely dependent on assessment of their behavior.

Facial Expression

Although it is assumed that the apparent 'stoicism' of grazing animals may have been acquired to protect injured animals from the attention of prey species, it has not been a complete impediment to behavioral assessment of grazing animals as a means for detecting pain. Automation of behavioral assessment and its application has been briefly discussed above. Analysis of facial expression has been applied successfully with sheep to determine the effectiveness of this approach for animals with footrot or mastitis (71). Likewise a 'sheep grimace scale' correlated well with the occurrence of post-surgical pain in sheep (72). There has been some solid progress in advancing the automation and sophistication of this technology [see work with sheep by Lu et al., (73)] and the topic has been recently reviewed in the wider context of farm animal welfare by McLennan (74).

Electroencephalography

Because pain is a sensory experience, it manifests at the level of the cerebral cortex so that any technology that provides information on brain function at this level could be used to assess the magnitude of pain (75). Currently, non-invasive imaging of the brain based on computed tomography (CT) or magnetic resonance (MRI) does not appear to provide sufficient resolution for this type of assessment. However, neurophysiological techniques do show promise for assessment of pain in animals (76). These include electroencephalography (EEG) and magnetoencephelography (MEG) (76). EEG has proven useful as a tool for monitoring depth of anesthesia to ensure that the patient is unaware. However, it has been applied also to the identification of nociceptive, i.e., painful, stimuli. Considerable variation occurs with data from EEG recording in animals and this has to be countered by use of highly standardized procedures in association with halothane anesthesia (76). These concerns have limited the usefulness of this technology for monitoring pain. However, when used in conjunction with somatosensory evoked potentials that are generated by various stimuli applied to the skin or other peripheral tissues, especially those evoked by lasers, there has been useful progress in understanding of pain pathways and of the processing of painful stimuli in animals (76).

Domain-Based Assessment of Animal Welfare

A device for quantifying an animal's overall state of welfare is the so-called Five Domains Model (77). This is a systematic scoring of welfare-significant internal states, labeled as Domains 1–3 (e.g., Nutrition, Environment and Health) plus welfare-significant external circumstances (Domain 4–e.g., Behavior). Once these are identified, any associated affective experiences are accumulated into Domain 5 (e.g., Mental State). A nice account of how this approach was used to evaluate adverse effects of husbandry and other interventions in horses has been provided by McGreevy et al. (78). The authors indicated that the model requires some effort to refine the scoring parameters but it can certainly be extended to various species of animals and could provide a more holistic assessment of welfare than previous approaches.

CONCLUSIONS

There is no perfect remedy for providing objective measures of welfare in animals generally and this obviously applies equally to grazing ruminants. It is likely that the methodology for assessing welfare will utilize a variety of tools, rather than being reliant on a single measure. All of the alternative measures or approaches mentioned in this review show promise for this role and are undergoing further refinement and development, but their reliability is currently generally confined to situations where the degree of compromise to welfare is already severe. All is not lost however. In many cases the particular measure can be applied to studies where data from groups of animals can be ranked to provide information about aversions and unfavorable environments or circumstances that reduce animal welfare or, similarly, about preferences or environments that enhance their welfare.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REVIEW
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A Case for Eustress in Grazing Animals

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Herbivores grazing in extensive systems are exposed to a series of challenges, rooted in the inherent spatial and temporal variability of their environment that potentially constrain their health, nutrition, and welfare. Nevertheless, in this review, we argue that challenges induced by some biotic (e.g., vegetation) and abiotic (e.g., terrain) factors may also be viewed as "positive" sources of stress or eustress, since they present complex problems, that when solved successfully elicit a greater degree of behavioral plasticity and adaptability in grazing animals. Chemically and structurally diverse landscapes require animals to display complex behaviors and exhibit adaptive capabilities, like building a balanced and safe diet or finding shelter, which ultimately lead to positive emotional states. Thus, maintaining or enhancing the diversity occurring in natural systems represent a management approach that can be used to improve welfare and prepare the animal for an efficient adaptation to future, and potentially unknown, environmental challenges.

Keywords: eustress, grazing, herbivore, animal health, adaptive behavior

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INTRODUCTION

Animal welfare is an essential element of modern animal production. First and foremost, animal welfare is grounded on ethical concerns that derive from the fact that animals are sentient beings, i.e., able to suffer and experience emotions, but improving animal welfare may have additional benefits. As many welfare problems have a detrimental effect on production, improving the welfare of farm animals very often has positive effects on performance (1, 2). In addition, improving animal welfare is one of the strategies that potentially contributes to reduce the use of antimicrobials in farm animals (3).

An in-depth discussion of the concept of animal welfare is well-beyond the scope of this review paper, and several reviews are available on the topic [e.g., (4–6)]. However, it is important to mention that animal welfare encompasses not only the physical health of the animals (i.e., the absence of diseases and injuries) but also their behavior and emotions (6–8).

For many years, the Five Freedoms (9) have provided a useful framework to identify the welfare problems of farm animals. These freedoms, which represent ideal states rather than actual standards for animal welfare are (a) freedom from thirst, hunger and malnutrition, (b) freedom form thermal and physical discomfort, (c) freedom from pain, injury and disease, (d) freedom to express most patterns of normal behavior, and (e) freedom from fear and distress.

More recently, the Five Freedoms have been criticized since they can be misunderstood as aiming at eliminating all negative experiences (which is not realistic or even desirable, as we argue in this review), but also because they fail to capture our current understanding of the biological processes underlying animal welfare (5). As an alternative to the Five Freedoms, the so-called Five

Domains Model for assessing animal welfare was developed to address these problems. The Model incorporates four physical domains of "nutrition," "environment," "health," and "behavior," and a fifth "mental" domain. Each physical domain has an impact on the affective state of the animal (i.e., on the fifth domain), and the net outcome in the mental domain resulting from the combination of the four physical domains represents the animals' overall welfare state.

It is clear that the Five Domains Model applies to animals kept in confinement under intensive livestock production systems, but the approach is equally relevant to herbivores grazing in extensive systems, as all states identified in the model are crucial for the maintenance of their welfare, even when animals evolved and are presumably adapted to their "natural" grazing environments. In several instances, the natural environment negatively impacts animal welfare due to its inherent temporal and spatial variability, which may lead to unsuccessful coping responses to unpredictable and ever-changing challenges (10, 11). With regards to the grazing process, climate variability has significant negative effects on herbivores, particularly for dryland regions with low and variable precipitation and high temperatures in the growing season (12). Under these conditions, forage abundance and quality may be limited during certain periods, negatively affecting the nutrition domain, which in turn compromises animal welfare. Clear seasonal patterns have been observed for fecal cortisol levels (an indicator of chronic stress) in Pyrenean chamois (*Rupicapra pyrenaica*) grazing in rangelands of northeastern Spain (13). Concentration of fecal cortisol tracked the levels of nutrient and other environmental stresses experienced by these animals throughout the year (13). The effects of season are compounded with the impacts of thermal stress on ruminants in the face of future heat and cold waves of greater frequency, intensity and duration (14). As an example, heat stress reduces feed intake in ruminants while increasing maintenance requirements, a trend that is aggravated by the predicted decrease in the quality and productivity of feed resources available to herbivores grazing in rangelands (15). All these effects have the potential to promote reductions in animal welfare and productivity given that long-term declines in food availability lead to poor nutrition and stress (16). Another response related to the unpredictability observed in rangelands involves fear to predation and the indirect effects that predators impinge on prey by negatively affecting foraging patterns (i.e., reduced grazing time, increased vigilance), and as a consequence animal nutrition and reproduction rates (17, 18).

The aforementioned inherent variability of rangelands and the potential negative impacts on the nutrition and welfare of herbivores has been extensively reviewed elsewhere [e.g., (19) and papers in that book]. This review was developed with the aim of looking at variability in rangelands from a different angle, i.e., as a force that may potentially bring about greater adaptation and resiliency for animals grazing in the complex chemical and structural realm of rangelands. It is clear that welfare depends not only on whether the animal succeeds at coping with the challenges emerging from its environment, but also on whether coping attempts lead to negative consequences for the animal (20). On this regard, animals have evolved mechanisms to cope with different environmental challenges such that if they are to survive and reproduce, they should maintain a fitness level >0. Innate behavioral strategies and learning play a key role in the ability of animals to cope and adapt to stressful situations imposed by an ever-changing environment (21).

STRESS AND EUSTRESS IN EXTENSIVE SYSTEMS

The concepts of "animal welfare" and "stress" are closely linked, partially because many welfare problems cause stress (22). The term "stress" has been widely used in biology to describe a set of physiological and behavioral changes elicited by aversive stimuli. Cannon (23) described stress as the sympatho-adrenomedullary (SAM) system's attempt to regulate homeostasis when threatened by a variety of aversive stimuli or stressors. Later, Selye (24) conducted some of his classic studies on the response of the hypothalamic-pituitary-adrenal (HPA) axis to noxious stimuli and suggested that the organism reacted in a non-specific manner to a wide variety of aversive stimuli, mainly with an increase in the HPA axis activity. Some forms of stress such as the chronic activation of the HPA axis caused by long-term factors are typically viewed as impinging deleterious effects on natural populations, inevitably resulting in maladaptation and pathology (25). Both the HPA axis and the SAM system are generally considered to be the two main elements of the stress response and levels of glucocorticoids in different animal tissues (e.g., blood, hair, saliva) and excretions (e.g., feces) have been widely used as measures of stress. The problem with this approach, however, is that the HPA axis and the SAM system have a crucial function in energy mobilization and redistribution of nutrients to active tissues and both aversive (e.g., fighting) and rewarding situations (e.g., play and mating) may elicit a similar physiological stress response (26). Therefore, if stress is perceived as potentially negative, it may be misleading to consider stress as a synonymous of the HPA axis activation. On the other hand, there is now enough evidence showing that it is not the physical nature of an aversive stimulus that has negative consequences on the animal but rather the degree to which the stimulus can be predicted and controlled (26). As a result, it has been suggested that the term "stress" should be restricted to conditions where an environmental demand exceeds the regulatory capacity of the organism, mainly when such conditions include unpredictability and uncontrollability (26). Thus, when animals have available the conditions or "tools" to control or predict their environment, a challenge may represent a stimulus that results in improvements to their welfare, provided that the animal overcomes the challenge. Interestingly, research in zoo animals has shown that giving animals the opportunity to choose between two different environments (which presumably increases the animals' perception of control) reduces several behavioral and physiological indicators of stress and poor welfare (27, 28).

The coping process to a stressful situation (i.e., an animal being exposed to a certain uncomfortable environment) may lead to fitness costs (i.e., searching activities that increase energy expenditure). Nevertheless, the net result of this response needs to be adaptive (i.e., finding a more comfortable environment) if the animal is to survive and reproduce, and if improvements in animal welfare are expected. Thus, under circumstances when the animal is able to fully cope to a challenge, this may have a positive impact on animal welfare. Under this context, the word "eustress" was coined (24) to refer to the idea that there is a "correct or optimal stress level" that is adaptive (29). For instance, problems that emerge during the grazing process such as building a nutritious and safe diet may be stressful (e.g., overcoming food neophobia, or preventing the ingestion toxic plants or excessive amounts of nutrients), like the example described above in zoo animals exposed to a choice between different environments, but ultimately beneficial if the individual possesses the skills and resources needed to meet such challenge. Problem-solving opportunities presented during enrichment programs for captive animals potentially enhances welfare, as individuals may be motivated to participate in problem-solving activities when there is an optimal level of challenge, which depends on the individual's cognitive and behavioral skills to solve the presented problem (30). Contrafreeloading, the choice to work for resources when identical resources are simultaneously available in free form, also entails an enrichment of the captive animal's environment as it provides opportunities for general exploration and cognitive challenge that may result in a positive outcome (i.e., "earning food"), even when such activities represent departures from optimal foraging strategies (31).

Our thesis in this review is that the inherent variability in structure, taxonomy, and chemistry provided by rangelands represent stimuli that enhance the adaptability and resiliency of herbivores grazing in these dynamic environments. This is a novel approach to managing animals in rangelands as it suggests that preserving and promoting rangeland diversity is crucial for "providing the training grounds" that will prepare animals to better respond and adapt to future challenges that compromise their welfare. We also submit that management interventions can contribute to foster flexibility in animals grazing in variable environments by providing the means and facilitating the acquisition of skills that optimize the prediction and control of the coping response to the problems presented in the context of a changing world.

FORAGE DIVERSITY AND EUSTRESS

It is known that a variety of plant species enhances the nutrition of mammalian herbivores because no single plant provides all the nutrients or proportions needed by the animal (32). In addition, plant secondary compounds (PSC) ingested as a dilute mixture of plants are less toxic to herbivores because they are less concentrated and potentially detoxified by different pathways [i.e., the Toxin Dilution Hypothesis; (33)]. Anatomical, physiological and experiential differences among individuals lead to specific needs, and thus individual animals can best meet their needs for nutrients and medicines when offered a multiplicity of forages, instead of receiving a single food, even if that food is balanced to meet the "average" needs of the "average" animal (34).

When herbivores engage in the process of building a diet from an array of different foods from a diverse plant community they are faced with solving a problem. This is because they need to balance the ingestion of required nutrients and potential medicines (35) from an array of nutritionally unbalanced and potentially toxic foods. The solution is achieved by the application of a suite of complex behaviors that require cognitive and non-cognitive mechanisms for their efficient execution in time and space (35, 36). For instance, locomotion activities position the individual in space, within the preferred patch and feeding station (36), followed by handling and ingestive activities that consummate a preference for particular plants and parts. Such preference is triggered by learning mechanisms that integrate the plant's orosensorial characteristics with its post-ingestive consequences (37, 38). The challenge of building a balanced diet from a diverse array of alternatives may "breed" innovation and exploratory behaviors in herbivores that when successful, foster positive emotional responses and allow for better adaptations to future unpredictable conditions of the environment (30). Good welfare is not simply the absence of negative experiences; positive affective states play a significant role in providing animals a better quality of life (39). Under this analysis, forage diversity could be interpreted as an "eustressor" that gives individuals the challenge but also the opportunity to execute behaviors efficiently across contexts and solve problems with potential to improve their welfare. In support of this idea, lambs faced with the problem of building a diet from a diverse array of food items with or without PSC, showed greater acceptance of novel foods and flavors in familiar (40) and novel (41) environments, and showed lower levels of stress-induced hyperthermia and ambulation scores in open field tests than animals exposed early in life to a single ration (41). Although the initial reaction to novel feeds in all treatments was similar (i.e., low food intake; neophobia), neophobia was attenuated at a quicker rate in animals that had the task of building a diet from single foods relative to those previously exposed to a single ration (40). In another study, lambs challenged to build a diet from an array of foods with different energy to protein ratios showed lower blood cortisol levels and neutrophil to lymphocyte ratios than lambs fed a single ration (42). Additionally, lambs under the diet-building task also spent a lower proportion of time eating and showed greater intake rates and greater proportion of time lying and greater activity than lambs under a single diet (42). Heifers grazing 2- or 3-way choices of different legumes showed greater body weight gains, forage intake (43) and hair cortisol levels than heifers grazing monocultures of these species. Some non-overwhelming challenges that increase cortisol levels, with the subsequent decline when the task is mastered or the stimulus removed, have been linked to the development of resilience and the fostering of adaptations that enhance emotional processing, cognitive control, and curiosity in monkeys (44).

Being able to solve a foraging problem, in addition to the reward provided by the nutrients harvested in the process (nutrition and behavior domains in the Five Domains Model), represents an intrinsic reward and positive emotional state (mental domain in the Five Domains Model) inherent to being successful at solving the task performed (30). Thus, the process of building a balanced and safe diet from a diverse array of nutritionally unbalanced alternatives may be interpreted as an achievement [a sense of "victory;" (24)] that leads to a positive emotional state that improves welfare. More research is clearly needed to better understand the effects of forage diversity and diet building on positive emotions in grazing animals. Nevertheless, since the display of behaviors concerning essential activities such as foraging are considered self-rewarding (39), it is plausible to speculate that successful diet building activities are also linked to positive emotional states. Food seeking (motivational states of wanting) and consummatory behaviors (liking or the hedonic pleasure felt during food consumption) have rewarding properties (45) and they are clearly involved in the process of diet selection in mammalian herbivores (35, 37). In addition, it has been shown that controlling an event *per se* can be perceived as rewarding or at least as less stressful (46). In contrast, foragers may experience negative states (i.e., frustration) when exposed to monotonous rations that may not satisfy all their individual and specific nutritional and medicinal requirements, as well as the need to experience a diverse array of flavors during the foraging process (47, 48). Under the context of single feeds or rations, foraging opportunities are limited since the only responses possible are to eat or to stop eating. Single foods/plants may elicit frustration as the animal's response does not lead to a solution, i.e., there are no single plants/forages that provide all the nutrients and proportions required by the animal (32, 34) and generalist herbivores evolved consuming a diversity of flavors from diverse plant communities instead of single flavors in monotonous foods. In addition, the initial stress (i.e., neophobia) promoted by exposing animals to diverse novel foods may be attenuated by the presence of a familiar model such as mother (35) or experienced companions (49), which allow for a prompt selection of a diversity of food items from the array of novel foods presented. Finally, forage diversity allows for the expression of foraging preferences that may not be able to be expressed under monotonous diets resulting in some animals expressing abnormal and stereotyped behaviors, considered an indicator of poor welfare (20).

FORAGE DIVERSITY AND GENERALIZATION TO OTHER CONTEXTS

As expected from an evolutionary point of view, the ability of animals to compensate for the variability imposed by their environment will be a function of the individuals' phenotypic plasticity (50). Such plasticity may also be interpreted as behavioral flexibility, allowing for a rapid pathway for adjusting to environmental changes that exceeds the rate of evolutionary genetic change (51). Behavioral flexibility may be acquired when animals become familiar with solving foraging problems, generalizing their problem-solving abilities to other contexts, and situations imposed by a changing environment. There is evidence for this process to occur under natural and artificial settings. For instance, models with hummingbirds suggest that environmental heterogeneity (e.g., changes in temperature, water and food availability) are linked to problem-solving abilities, innovation, and exploration that allow individuals to better adapt to the unpredictable conditions of their environment (52). Environmental enrichment programs that allow farm animals show a more flexible foraging behavior lead to reductions in chronic stress due to confinement (53). Sheep have been found to predict and form expectations about the amounts of food that they are receiving, and to control an aversive event in order to access food, showing problem-solving abilities that allow for adjustments to new situations stemming from challenges experienced and solutions achieved in previous tasks (46). This plasticity acquired by the appraisal of novel situations relative to the individual's abilities and past experiences suggests that animals challenged by less predictable environments may be more likely to show a broader range of coping strategies in response to changing environmental conditions than animals living in more stable and predictable conditions (54). Behavioral flexibility may be also influenced by early life conditions as individually housed calves had learning deficits relative to calves housed in a dynamic group with access to their mothers (55).

VARIABLE LANDSCAPES AND EUSTRESS

Access to pasture for animals kept in confinement provides some health-related welfare benefits to cows, even when diets in confinement are nutritionally balanced and cover all of the animals' physiological needs (health domain in the Five Domains Model). For example, at least in some circumstances, cows on pasture have a lower incidence of lameness (56) and mastitis (57) than cows kept indoors. If given the choice, cows will spend a significant proportion of time on pasture, mainly at night (58). Moreover, by using operant responses to assess motivation, it has been shown that cows value access to pasture as highly as fresh feed (59). It might be suggested that access to pasture provides an opportunity to experience a more diverse, stimulus-rich environment than indoor housing. Although boredom in animals has received little empirical study, research done in several species suggests that monotonous environments caused an increased motivation for diverse stimuli, consistent with the hypothesis that animals kept in barren environments may experience boredom or something like it (60). Cattle in pens with *ad libitum* access to a monotonous forage displayed contrafreeloading, spending energy (they pushed a gate) to obtain a forage which was simultaneously available in a feeder and in abundance (61). This behavior could be interpreted as a form of environmental enrichment, given that the housing environment was barren and the animals had limited social contact (61). Alternatively, pushing a gate may have been perceived as rewarding if this behavior attenuated boredom, created a sense of control over the environment or allowed the animals to experience "a sense of victory" by handling a doable challenge (61).

Consistent with the research described for cows in confinement, and for zoo animals exposed to a choice between different environments (27, 28), the welfare of ruminants grazing in extensive systems may benefit from the opportunity to choose across different locations in the landscape. Nevertheless, preference in dominant animals within a social group may overcome spatial preferences by subordinate animals, which may lead to frustration. Food preferences and social interactions both influence choice of foraging location by sheep (62), although when animals experienced toxicosis after eating certain foods, dietary preferences overrode social influences (62).

Access to locations that require complex tasks like moving across rugged terrain or uphill may represent challenges that elicit a higher degree of behavioral diversity in grazing animals (environmental domain in the Five Domains Model). Foraging enrichments in confinement are in general designed to facilitate the physical expression of feeding behaviors such as food-searching and food consumption, but not to facilitate complex tasks related to food acquisition (30), although recent research on cognitive enrichment shows positive effects on animal welfare (63). We propose that the equivalent of complex task-solving processes for animals grazing in extensive systems entails building a diet from a diverse and complex landscape with different biotic (e.g., plants with diverse chemistries and structures) and abiotic (e.g., slope, rough terrain, rocky outcrops) challenges. Foraging across different spatial scales, from regions and landscapes to plant communities and patches could be viewed a "natural cognitive enrichment program" that enhances animal welfare by providing a form of enrichment, creating a sense of control over the environment, or allowing for the realization of a task that leads to a positive emotional state. In contrast, lack of biotic and/or abiotic challenges, similar to those observed in barren environments lead to boredom, frustration, helplessness, and depression (5). Supplying grazing animals with the opportunity to interact with a more sophisticated environment by challenging their cognitive abilities with chances to gain environmental control or to anticipate rewards represents an appealing approach to enhance their welfare, supported by the positive results observed for animals living in captivity (64). These conditions at the spatial scale may facilitate the acquisition of positive emotional states, induced by a successful coping with a complex cognitive challenge rewarded by the formation of a balanced diet. Consistent with this idea, complex behavioral tasks rewarded by food improve the welfare of intensively housed pigs by providing adequate cognitive challenges that generated successful coping and positive emotional states (63, 65). In addition, structural (climbing racks) and cognitive enrichment (drinking water as a reward for a correct choice) improved different aspects of behavioral competence (e.g., visual four-choice discrimination tasks and reactions to external challenges) in goats exposed to stressful situations relative to animals exposed to barren environments with easy access to water (64). It has been hypothesized cows remain longer at feeding sites in rugged heterogeneous pastures, with more diverse vegetation and nutrient profiles, than in homogeneous pastures as variability in biotic and abiotic factors reduce satiety and increase residence time at the more complex feeding sites (66). In contrast, monotonous landscapes of uniform topography promote satiety and reduce the time spent at individual feeding sites (67). Cows born and raised under the environmental challenges of the Chihuahuan Desert were farther from water and spent less time at water than naïve cows of the same breed grazing at the same location, but born and raised in a humid environment with gentle topography and lush vegetation (68). During winter and early summer (drought conditions), naïve cows selected diets with lower crude protein content than cows born and raised in the desert (68). No welfare parameters were measured in this study, but it is likely that "desert cows" experienced a sustained cognitive enrichment by the association of successful coping with a demanding behavioral task (i.e., moving in rugged terrain, uphill and away from water in a dry environment) rewarded by food. Interestingly, cows born and raised in the desert, moved to lush pastures for 3 years and then returned to the desert, displayed behavioral patterns similar to cows that spent their whole life at the desert (68).

VARIABLE LANDSCAPES AND THE THERMAL ENVIRONMENT

Trees, shrubs, or long grass, as well as abiotic factors such as topography also provide a diversity of structural arrangements in the landscape that contribute to reduce the incidence of thermal stress in animals living under natural conditions. Thermal stress is a direct welfare problem, as it causes discomfort and it can significantly reduce access to pasture in grazing animals (58). Use of shade is likely to be the most feasible strategy for grazing ruminants. Depending on the quality of shade, provision of shade will reduce radiant heat load by 30–70% (69). Even in temperate climates, provision of shade has positive effects on heat load and production in grazing ruminants (70). The thermal environment plays an important role in determining livestock distribution (71), and thus factors in the landscape such as aspect, slope, type of terrain, type of vegetation also provide "tools" that allow animals gain environmental control or anticipate rewards (i.e., approach to their thermoneutral zone). Such tools are absent in flat terrains without shelter. On sunny summer days, cows have been observed spending considerable time (8 h) under shade trees near water (72). Contrastingly, during winter cattle exhibited heat seeking strategies of grazing south slopes during the day and not resting under shade trees and laying down at night on warmer ridges (72). On cold days cattle would move to lower, sheltered areas that were warmer (72), and on windy days cattle rest in sinkholes sheltered from the wind, possibly creating a more thermally neutral microclimate (71). Effective shelter during cold weather may also entail dry grass or shrubs when sheep graze in areas with such structural diversity in the vegetation, leading to substantial improvements in lamb survival under cold stress (73). Thus, biotic and abiotic factors that lead to successful coping such as efficient thermoregulation could be viewed as "natural" enrichment elements with potential to enhance animal welfare.

RANGELAND DIVERSITY, MANAGEMENT, AND PREPAREDNESS TO THE UNKNOWN

Social and psychological research has placed emphasis in recent years on positive outcomes to stress-related experiences that breed resilience in organisms (74), instead of negative emotions and chronic stress that promote illness. A similar approach could be followed in natural systems by maintaining or enhancing their chemical and structural diversity, which in addition to services like improvements in the efficiency of resource capture, nutrient cycling and stability (75, 76), may promote improvements in animal welfare. Providing new chemicals to the landscape like medicinal PSC (e.g., with the introduction of herbs, shrubs or trees) will benefit the nutrition and health of grazing animals (77, 78), thus addressing the nutrition and health dimensions of the Five Domain Model. The benefits of plant diversity on animal welfare may also need to be pondered in relation to the nature of the assemblage of plant species presented to herbivores. For instance, when subjects are offered a choice of foods under experimental settings to understand their specific nutrient requirements through their diet-building abilities [e.g., the geometric framework of diet selection; (79)], the foods presented are unbalanced but designed in such a way that allows for the construction of a mixed balanced diet that meets the specific nutrient requirements of the individual. Extrapolating from this controlled setting to natural systems, the foods presented in the plant community should be such that different individuals under different physiological states should be able to build a balanced diet. If mixing diverse foods does not lead to meeting specific nutrient and medicinal needs, animals may experience negative emotions such as frustration in response to suboptimal diets, in addition to the direct negative impacts of unbalanced diets on fitness. Thus, it is important to consider the herbivores' foraging preferences when managing grazing environments for improvements in animal welfare and productivity. As an example, it has been shown that beef heifers grazing in natural grasslands use low-quality tussocks in order to harvest strategic amounts of dry matter during the day, given that the high productivity of these plants offer large intake rates (80). Intake of tussocks is then complemented with the consumption of high-quality herbs and grasses (of lower productivity) present in inter-tussock areas (80). Originally, farmers tended to eliminate low-quality tussocks, regardless of their abundance in the plant community, as they were considered problematic for animal production. After exploring the animals' feeding preferences, tussocks are now considered beneficial when present in the plant community below a certain frequency threshold such that animals build a diverse diet, where tussocks optimize the amount of biomass harvested daily and herbs and grasses in the inter-tussock areas provide the nutrients needed to optimize the digestibility of tussocks in the rumen (81). Managers also offer proportions of grass and clover in the landscape that match the grazers' preference for these forages in order to foster an optimal use of food resources by herbivores (82); such arrangement may also contribute to animal welfare improvements as it offers the conditions needed to successfully complete the task of building a balanced diet. Thus, fostering the "right" diversity, i.e., arrays of complementary plants that when mixed lead to the realization of a balanced diet is essential.

Appropriate challenge is a key concept in environmental enrichment, referring to the problem that is potentially solvable through the application of an animal's cognitive and behavioral skills (30). Under this scenario, managers may be able to "enrich" a certain landscape by providing the supplements or plant species that complement the chemical composition of the existing vegetation such that animals are able to build "optimal diets" that provide an appropriate mix of nutrients and medicinal PSC. Environments of low nutritional quality reduce the fitness and welfare of grazers and browsers (13), but the same is true when plants are high in nutrients (38). Non-complementary plant species that provide excesses of nutrients (e.g., crude protein) lead to stress and food aversions because excessive or frequent ingestion of such foods produce high levels of byproducts of fermentation that are toxic [e.g., ammonia or acid loads; (38, 83)].

Animals faced with the problem of building a diet from an array of diverse and complementary alternatives may be more adapted to maintain their fitness and welfare in response to future challenges triggered by shifts in vegetation like the predicted reductions in crude protein content in grasses in response to increased ambient temperatures (15). It has been proposed that as climatic warming reduces grass protein concentrations, woody species increase in abundance and grassland habitats decline for growing populations of herbivores, wild, and domestic species may need to compensate by relying less on grass and more on browse, which contains greater concentration of PSC (15, 84). In addition, the pattern of protein reduction in grasses may also increase the reliance on protein-richer eudicots, with greater potential for toxicity due to the greater concentration of PSC in these flowering plants (85). Plant secondary toxicity may also be exacerbated with the predicted increases in ambient temperature because toxins interfere with thermoregulation (86, 87). Detoxification pathways are thermogenic and toxins uncouple mitochondrial oxidative phosphorylation, which also generates heat (88). The imbalanced nature of herbivore diets and the presence of toxins, which affect the thermal balance, may increase the likelihood of heat stress at high ambient temperatures (88). These emerging problems may require building diets of lower PSC content in landscapes where the concentration of protein is declining. Finding appropriate locations in the landscape to dissipate heat under warmer conditions and the challenge of temperature-dependent toxicity will be more relevant for future generations of herbivores. Adaptability in these predicted scenarios is expected to be greater for animals previously exposed to solving the problem of building balanced diets from complex arrays of unbalanced alternatives and complex landscapes with a diversity of biotic and abiotic factors that foster cognitive enrichment. Trans-generational diet-building abilities of offspring as observed in cattle (89) and sheep (90) may also contribute to more efficient adaptations to future environmental challenges.

CONCLUSIONS

We submit that chemical and structural diversity breed animal resiliency and adaptability to current and future challenges imposed by the inherent dynamic conditions of rangelands. Positive outcomes to stress-related experiences may enhance behavioral competence and lead to positive emotions that benefit animal welfare. Using this concept, managers should promote resilience and plasticity in animals by enhancing chemical and structural diversity in rangelands (i.e., through targeted grazing treatments, revegetation efforts that increase plant species diversity, or by strategic distribution of water points that enhance animal distribution in the landscape), thus creating "natural cognitive enrichment programs" that enhance animal welfare and better prepare animals for future challenges inherent of living in these dynamic and variable landscapes.

AUTHOR CONTRIBUTIONS

JV drafted the manuscript. JV and XM wrote it in collaboration and read and approved the final manuscript.

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REVIEW
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On the Search for Grazing Personalities: From Individual to Collective Behaviors

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While grazing lands can offer a diverse range of forages, individuals within herds prefer to graze some habitats and not others. They can have consistent differences in grazing patterns and occupy specific spatial domains, whilst developing tactics and strategies for foraging that are specific to their grazing personalities. In this review, we explore the development of our understanding of grazing personalities, as we move away from the search for an "optimal animal" toward designing behavior-customized herds with an arrangement of individual grazing personalities that enhance ecosystem services and productivity. We present a "grazing personality model" that accounts for the personality of individual animals and for collective behaviors of herds. We argue that grazing personalities of grazing ruminants and other large herbivores are in part genetically determined, and that they can act at the individual and collective level. The social and biophysical environments as well as the emotional state of animals regulate the expression of "grazing genes" that are observed phenotypically as distinct grazing personalities. The reproductive and sexual successes of individuals and herds filter for allele variants of grazing genes and in turn determines their relative frequency. While the selection of one grazing personality may be adequate for homogeneous pastoral systems, the design of herds with a range of grazing personalities that are matched to the habitat diversity may be a better approach to improving the distribution of grazing animals, enhancing ecosystem services, and maximizing productivity.

Keywords: grazing patterns, behavioral syndromes, conceptual model, genotype-to-phenotype associations, heritability, social environment, personality plasticity

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INTRODUCTION

We picture foraging animals distributed throughout grazing lands. Individually or in various sized groups of one or more species, herbivores explore and graze a diverse range of habitats including riparian areas, open flat plains, gentle or steep hills and mountainous lands. Even when considering herds of one single species, individuals show divergent dietary tactics and foraging site preferences resulting in consistently and regularly repeated grazing patterns, like for example in cattle or sheep (1–4).

Grazing has been described as a process composed of short-term ingestive tactics, and mid- and long-term digestive strategies (5), and its pattern is defined as a cluster of decisions that lead to ingestive actions and digestive strategies that are motivated by the interaction of both internal and external stimuli (6). Differences in grazing patterns are far from being trivial or random, with individual animals behaving consistently and adopting specific grazing strategies across situations and over time; such that animals are said to display recognizable grazing personalities (7–10). The diversity of grazing personalities within herds modulates the intensity and frequency of forage defoliation achieved with recommended stocking rates, the fitness of animals and other production traits such as reproduction success, survival, and live-weight changes (11, 12). As a consequence, differences in grazing patterns and personalities affect ecosystem functions such as speeding up nutrient cycling (13), increasing productivity of grasslands (14), and preventing loss of plant diversity (15).

The concept of animal personality, also referred to as behavioral syndrome (10), copying style (16), and temperament (17) among other closely related terms (18, 19), was developed by integrating correlated traits of behavior with other traits. For example, Carere and Maestripieri (20) defined animal personality as correlated behavioral and physiological traits that differ among individuals of the same species, and that are temporally stable across different contexts or situations. Gosling and John (21) suggested this concept should not be restricted to differences observed within-species, but rather these are behaviors and patterns that are consistently displayed by individuals regardless of the species identity. Some authors also argue that personality should include traits that account for consistent patterns of feelings and thoughts that affect behavior (22). In this way, the concept of animal personality includes emotional and cognitive traits, which can influence animal decision-making and well-being. In line with Maderspacher's (23) arguments and Biro and Post's (24) speculations, we have chosen to include morphological traits in our definition of grazing personality, as evidence showed correspondence between behavioral polymorphisms and morphological polymorphisms. Accordingly, we define grazing personality for grazing ruminants and other large herbivores as "suites of traits of different nature (e.g., behavioral, cognitive, physiological, and morphological), which are correlated and often concatenated, to result in specific grazing patterns displayed consistently across contexts and over time."

Regardless of the species identity, differences in grazing personalities are observed at the individual (8, 21, 25, 26) and collective level; that is in groups, herds, and populations of animals (27, 28). Consequently, we argue that grazing personalities are the result of evolutionary processes that filtered alleles and established allele frequencies of key genes related to behavioral patterns, tactics, strategies, and decision-making in the grazing process, hereafter referred to as "grazing genes." In addition, interactions with social and biophysical environments, the emotional state of animals and their experiences early in life, might modify the epigenome of grazing genes, thereby modulating their expression.

We support the contention that grazing personalities are observable at individual and collective levels, and suggest that divergent grazing personalities result in distinct grazing patterns and attributes; such as the ability to explore, define a home-range, display a habitat preference, and fragment into groups. These all affect the ecological functioning of grazing systems.

We also propose a "grazing personality model" (GP-model). The purpose and context of the GP-model is to represent the genetic elements, the regulatory systems, and the phenotypic elements that encompass individual and collective personalities in a context of herds of grazing ruminants and other large herbivores. The objective of the GP-model is to further develop our understanding of distribution of grazing animals following the initial "Ecological-Hierarchical grazing model" (29) and the additional concepts of the "Distribution Patterns and Mechanisms" model (30). The GP-model represents grazing personalities, which are genetically determined (genotypic personality) and epigenetically modulated through systems that regulate the expression of grazing genes (personality plasticity) via interactions with the social herd environment and the biophysical features of the grazing environment. The emotional state of animals influences the regulatory systems that modulate gene expression and affects grazing decision-making. In this review, we first deal with grazing personalities at the level of the individual animal, then we deal with collective personalities and finally we illustrate GP-model implications based on movement ecology, genetics and animal personality.

INDIVIDUAL GRAZING PERSONALITIES

> *"...from the population optimum perspective [...] natural and sexual selection may favor the evolution of multiple responses to environmental challenges, thus resulting in within-population variation in the same behavioral trait, and in whole suites of behavioral traits"* (31).

This section describes the GP-model at the individual level (**Figure 1**, left side): from individual genotypes of grazing personalities at the top, through regulatory systems that modulate the gene expression and confers the personality plasticity in the middle, to individual phenotypic grazing personalities at the bottom. Thus, in section The Genetics of Behavior and Grazing Related Genes we present evidence about grazing genes and its heritability. We then investigate regulatory systems that modulate the expression of grazing genes in variable responses to stimuli conferring the personality plasticity (see section The Effect of Personality Plasticity and Regulatory Systems on Grazing Patterns). Finally, in section Grazing Traits of Individuals we present examples of phenotypic grazing personalities and traits at the individual level (**Table 1**).

Individual animals exhibit repeatable differences in their grazing behavior within populations, within species and across species. These personality differences arise for many reasons, such as differences in permanent environmental effects (e.g., familial, parental, and epigenetic contributions) and the effect of genetic variation. In ruminants, personality differences can influence eating tactics and ingestive behaviors (9, 54). For example, Gregorini et al. (9) studied a group of 16 dairy cows that were selected as calves (6–8 months old) based on divergence in residual feed intake (i.e., having high and low residual feed intakes) and measured their individual grazing behaviors, eating patterns, and ingestion tactics as milking cows. From a grazing behavior viewpoint, low residual feed intake individuals prioritized grazing and ruminating over idling. They typically took fewer steps when walking during grazing and had a higher

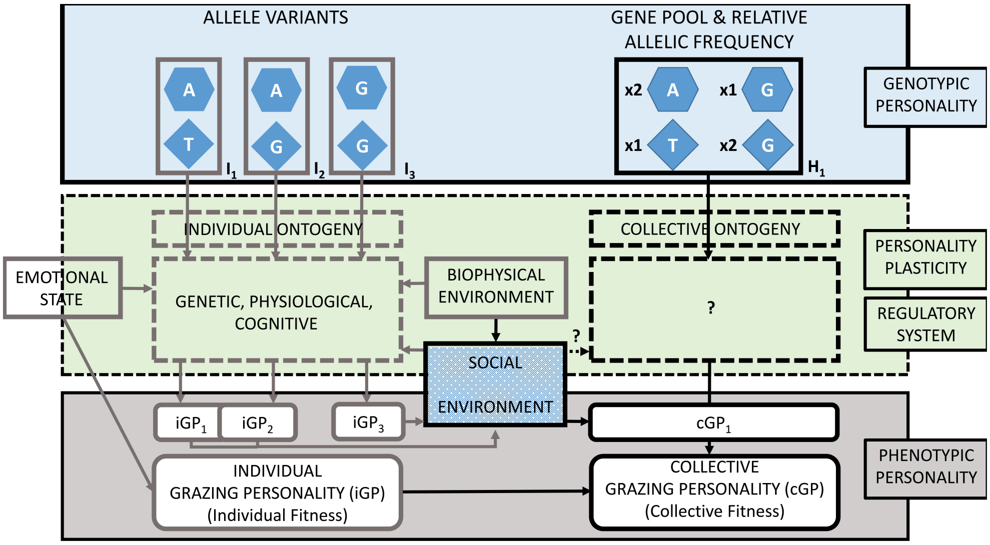

FIGURE 1 | The Grazing Personality Model (GP-model) for ruminants and other large herbivores described by three main aspects: the genotypic personality (top), regulatory system conferring personality plasticity (middle), and the phenotypic personality. In the example, three hypothetical combinations of allele variants (I_1, I_2, and I_3) applied to two grazing genes represented at the individual level (left side). The genotype of individuals constitutes the gene pool and the relative allelic frequency (H_1) of grazing genes at the collective level (right side). Individual and collective grazing personalities (iGP and cGP) are phenotypically represented with corresponding fitness. The example shows two grazing genes in beef cattle (32): the glutamate receptor 5 in chromosome 29 (hexagons) and the mastermind-like 3 in chromosome 17 (diamonds). Allele variants specified by nucleobases adenine [A], guanine [G], and thymine [T]. The interactions between genes and environment regulates the expression of grazing genes and confers personality plasticity. Phenotypic grazing personalities of individuals (iGPs) may overlap (e.g., iGP_1 and iGP_2) or diverge (e.g., iGP_3). A group of individuals coexisting and displaying distinct grazing personalities constitutes a grazing herd with its own collective grazing personality (cGP). Adapted from Bengston and Jandt (33); with concepts from Koolhaas and van Reenen (34), Robinson (35), and Sih (10).

ratio of grazing to non-grazing steps when compared with cows with high residual feed intake. From an ingestive viewpoint, low residual feed intake individuals masticated less, but ruminated more intensively, and they had feces with 30% less quantity of large particles size than their counterparts with high residual feed intake. Wesley et al. (39) also pre-classified 18 beef cattle heifers from within 80 animals in two consecutive years ($n =$ 36) based on the rate of consumption of supplementary feed (another trait related to the eating tactics). Similarly, the authors reported divergent grazing behavior and ingestion tactics; for example, cows with faster rates of consumption of supplement tended to spend less time at water, cover larger areas and exhibit less concentrated grazing search patterns than cows with slower consumption rates. These two studies speculated a link between the divergent phenotypic behaviors (i.e., eating tactics) displayed by the selected animals and their genotype. In the following section, we present the genetics of behavior and genes related to grazing patterns.

The Genetics of Behavior and Grazing Related Genes

Van Oers and Sinn (53) undertook a meta-analysis of studies on animal personality to quantify the heritability of personality in wild, captive and domesticated populations of a range of animals. The statistical meta-analysis included 209 estimates of heritability on 14 taxonomic groups such as Ruminantia, Equidae, Canidae, and Hymenoptera, just to mention a few. The authors reported an average heritability of 0.26 for animal personality traits with a cumulative size effect ($E = 0.18$) significantly different from zero. The average heritability was higher in wild populations than in domesticated populations ($0.36 > 0.24$), and unweighted heritability estimates for exploration behavior were 0.58 and 0.21, respectively. These authors concluded that selection of animals based on their personality could be expected in wild populations.

At a more general level, a more recent meta-analysis of behavioral studies on non-human animals reported estimates of heritability and repeatability of animal personalities (55). After screening 306 relevant articles, they selected 10 research studies and 71 pairs of estimates for analysis. Their analyses suggested that the repeatability of behavioral responses has a substantive genetic component, with the study revealing that 52% of the phenotypic variation in general behaviors such as aggression, antipredator, foraging, parental effort, and mating, was attributable to additive genetic variation (i.e., genotypic personality in the GP-model). The authors also reported a greater and large mean heritability for animal personality (0.52) than for behavioral variation (0.14). Animal personality heritability being inclusive of additive genetic variation, dominance genetic variation and permanent environmental effects, while behavioral variation includes in addition the temporal environmental effects. If genetic dominance (i.e., non-additive genetics) plays a minor role in determining animal personality (55), then one can potentially attribute about half of personality variation to the effects of the social and biophysical environment (e.g., parental care and vegetation characteristics, respectively) and to epigenetics. In the GP-model, these effects are referred to as

TABLE 1 | Dichotomous and multiple classifications of animal behavioral types in grazing ruminants and other large herbivores.

Species	Behavioral types	Behavioral categorizing criteria	Continuous and categorical variables	Genetically explained	References
Beef cattle	1. Riparian areas users 2. Uplands users	1. Home-range fidelity	Categorical (dichotomous) and continuous	Probably	(2, 36)
Beef cattle	Breeds better suited for mountainous terrain	1. Slope 2. Horizontal distance 3. Vertical distance	Continuous	Probably	(37)
Beef cattle	1. Bottom dweller 2. Hill climber	1. Terrain-use indexes	Continuous	Probably	(1)
Beef cattle	1. Dominant 2. Subordinate	1. Dominance	Continuous	No	(38)
Beef cattle	1. Fast-eater 2. Slow-eater	1. Supplement intake rate	Continuous	Probably	(39)
Beef cattle	1. Bottom dweller 2. Hill climber	1. Terrain-use indexes	Continuous	Yes	(32)
Beef cattle	1. Bottom dweller (?) 2. Hill climber (?)	1. Terrain-use indexes	Continuous	Yes	(40–42)
Beef cattle	1. Favorable distribution 2. Unfavorable distribution	1. Terrain-use indexes (?)	Categorical (dichotomous) and continuous?	Yes	(43)
Beef cattle	1. Highly exploratory/bold 2. Slow-exploratory/ shy	1. Response to novel object	Categorical (dichotomous) and continuous		(44)
Dairy cattle	1. Low residual 2. High-residual	1. Residual feed intake	Continuous	Yes	(9)
Highland beef cattle	1. Initiator 2. Follower	1. Leadership 2. Dominance	Continuous		(45)
Multiple species (mice, rats)	1. High-aggressive 2. Low (medium)-aggressive	1. Aggressiveness	Categorical (dichotomous) and continuous	Yes	(46)
Multiple species (foragers).	1. Leader 2. Trailer A. Speeder B. Laggards	1. Walking speed 2. Accelerations to conspecifics 3. Length of decision zones 4. Sense of orientation	Categorical (dichotomous) and continuous		(47)
Sheep	None specified	1. Sagebush consumption/ dietary selection	Continuous	Yes	(3)
Sheep	1. Bold 2. Shy	1. Shyness-boldness	Categorical (dichotomous) and continuous		(48)
Sheep	1. Bold 2. Shy	1. Shyness-boldness	Categorical (dichotomous) and continuous		(49)
Beef cattle	None specified	Consumption of several species of grasses and forbs	Continuous	Yes	(4)
Beef cattle (Nellore)	None specified	1. Crush score 2. Flight speed 3. Movement score 4. Temperament score	Categorical (nominal) and continuous	Yes	(11)
Deer	Several combinations of multiple dimensions	1. Boldness 2. Dominance 3. Flexibility	Categorical (nominal) and continuous		(50)

(Continued)

TABLE 1 | Continued

Species	Behavioral types	Behavioral categorizing criteria	Continuous and categorical variables	Genetically explained	References
Multiple species (foragers) with whole spectrum of personality types	1. Superficial explorer/ bold/aggressive 2. Thorough explorer/shy/ non-aggressive	1. Exploration strategy 2. Boldness 3. Aggressiveness	Categorical (dichotomous) and continuous	Yes	(51)
Multiple species (cattle, horses, pigs)	1. Proactive/bold 2. Reactive/docile 3. 15 combinations of three-dimensions personalities	1. Coping style 2. Emotionality 3. Sociality	Categorical (dichotomous and nominal) and continuous	Yes	(34)
Multiple species (foragers) with whole spectrum of personality types	1. Fast-explorer 2. Slow-explorer	1. Area-restricted search (fractal movement) 2. Sense of direction 3. Home range size and structure 4. Aggressiveness	Categorical (dichotomous) and continuous		(52)
Multiple species (African elephant, Galapagos tortoises, mule deer)	1. Central place foraging 2. Migration 3. Nomadism	1. Node-level (local) metrics 2. Graph-level (system) metrics	Continuous		(27)

See Réale et al. (19) and van Oers and Sinn (53) for studies with genetically-associated behaviors; see Smith and Blumstein (12) for single personality dimension related to fitness. Behavioral types, behavioral categorizing criteria, type of variable, and if behavior has been explained genetically.

the regulatory systems and the personality plasticity. However, it remains unclear how much of the non-additive genetics [i.e., allelic interactions at the same locus (dominance) or at different loci (epistasis)] can explain the phenotypic behavioral variation (56). For example, in humans, non-additive effects could be as significant as the additive effects in explaining several dimensions of personalities (57).

Results of Dochtermann's et al. (55) meta-analysis are promising but provisional and need to be taken with caution. They also reported that foraging behaviors had a much weaker genetic component (<0.2) than aggression and antipredator behaviors (up to 0.6).

Recent studies supported the premise of grazing personality being under genetic control. Howery and Bailey (43) described both genome regions and gene markers associated with grazing distribution patterns in beef cattle. As an example, using collared cows ($n = 87$) that carried global positioning systems (GPS), Bailey et al. (32) investigated the association of several quantitative trait locus (QTL) and genetic markers with the phenotypic variation of grazing patterns of cattle displayed along gradients of steep-sloping terrain, elevation and distance to water sources. These cows were grazed in mountainous and extensive grasslands at five ranches in New Mexico, Arizona and Montana in the United States of America (USA). A high-density single nucleotide polymorphism (HD SNP) array was used to genotype DNA samples from these cows. The study then ascertained whether associations existed between variation in the SNP markers and variation in grazing distribution based on indexes of terrain use. Two QTLs overlaying the glutamate receptor 5 (GMR5) gene accounted for up to 24% of the phenotypic variation in the use of vegetation patches on steep slopes and at high elevations, while another QTL overlaying the mastermind-like 3 (MAML3) gene accounted for 23% of the phenotypic variation (**Figure 1**). These genes have been reported to be involved in locomotion, motivation, and spatial memory as well as in the regulation of neurogenesis, myogenesis, vasculogenesis, and other aspects of organogenesis.

Studies conducted by Pierce et al. (40, 41) validated the previously reported genotype-to-phenotype associations between specific SNPs overlaying grazing genes and indexes of terrain use (32). While these results are promising and point toward the possible integration of grazing personality into selection programs, Howery and Bailey (43) suggested these studies need to be replicated and/or extended to larger number of animals of different origin and which are grazed in diverse environments, if robust and conclusive conclusions are to be reached. For example, the extended study of Pierce et al. (42) including 330 beef cows from 14 ranches in the western USA reported limited genotype-to-phenotype associations and pointed toward different candidate genes.

There are two outstanding explanations for the correlation of behavioral traits defining grazing personalities. The first one is pleiotropy, in which one gene could act on two or more traits, which further determine the displayed grazing patterns. If pleiotropy occurs, one single gene would effectively control several traits simultaneously. For example, phenotypic studies corroborated the correlation of distinctive grazing patterns (e.g., fast-explorer cows), growth rates and boldness within relatively small groups of cattle [i.e., 16 and 36 individuals in Gregorini et al. (9) and Wesley et al. (39), respectively].

Kern et al. (58) suggested that pleiotropic effects could explain the correlations between personality, morphological and performance traits on zebrafish (*Danio rerio*), but also did not confirm this possibility. A study with bighorn sheep from Ram Mountain, Alberta, Canada could not find pleiotropic effects at major locus because of the lack of genome-wide QTL overlap on genes related to docility and boldness (59). Instead, the authors concluded that small pleiotropic effects could have been missed and therefore, results did not confirm pleiotropy. Future studies might give insights of pleiotropy controlling grazing personality traits.

The second explanation for the correlation of traits as observed in grazing personalities is because of a non-random association of alleles at different loci that produce a combination of traits that confers advantage under a specific set of biophysical and social conditions, as is the case of linkage disequilibrium. For example, individuals with certain association of alleles tend to achieve higher reproduction rates than individuals with a different combination of traits. Such allele associations become common and more frequent in a population than other combinations, although traits are controlled by alleles at different loci (53).

Studies suggested that genetic variation might explain different eating tactics linked to distinctive grazing behaviors exhibited in groups of beef heifers (39) and dairy cows (9). For the latter example, Davis et al. (60) previously confirmed the different genetic basis found on nearly 200 dairy cows that, within a large herd of 3,359 milking cows, displayed extreme residual feed intakes. These genetically tested cows were mother dams of the 16 calves used on Gregorini's et al. (9) research. Future research on grazing personalities and its genetic variation might help to elucidate whether grazing traits are correlated because of genetic pleiotropy, or because of a linkage disequilibrium between grazing traits, or because of both mechanisms acting simultaneously. Both, pleiotropy mechanisms as well as linkage disequilibrium were represented in the hierarchical conceptual model "Organization of Behavioral Traits" (19) and have implications for the regulation and expression of grazing personalities.

The discovery of genetic associations with grazing personalities and thus the identification of specific grazing genes has the potential to assist in breeding programs. However, despite the high heritability of grazing patterns found in cattle, there are other factors controlling them. For example, interactions with the social herd environment (e.g., parental and familial effects), the biophysical environment, and the emotional state as well as the large number of range management practices that influences such interactions. In the next section, we discuss whether non-genetic factors can modulate the expression of grazing genes and if such effects over gene expression are transferable to offspring.

The Effect of Personality Plasticity and Regulatory Systems on Grazing Patterns

The section The Genetics of Behavior and Grazing Related Genes and the section Gene Pools and Allele Frequencies focused on alleles of grazing genes, their variation and frequency at two levels, individual and collective, respectively. In the GP-model, allele attributes of grazing genes are the ultimate determinants of grazing personalities. These attributes constitute the individual and collective genomes, respectively, and account for the specific sequence of nucleobases of each gene; that is the genome code. The gene products expressed into RNA and subsequent amino acids and proteins are the ones executing the observed phenotypic traits, such as behavioral traits. In this section, we focus on gene expression and regulatory systems that modulate the expression of behavioral genes related to grazing personalities. Here, we present the ontogeny, the epigenetic inheritance system, and the animal emotional state as the main modulators of behavioral gene expression. These three components of the GP-model create the interface between the genomic determination of grazing personalities and the external and internal stimuli that modulate its gene expression. The expression of grazing genes is variable and responds to changing environmental conditions and emotional states; regulatory systems modulating the gene expression and thus conferring the personality plasticity of the GP-model.

Regulatory systems are an integral part of the pathways between grazing genes and the observed grazing personalities. In the GP-model, grazing personality pathways originate from specific alleles of grazing genes and result in specific phenotypic grazing patterns. Grazing personality pathways involve hierarchical levels of intermediate and concatenated traits with multiple mechanisms that consistently respond to external and internal stimuli modulating the observed grazing patterns. The "Organization of Behavioral Traits" (19) conceptualized genes-neurophysiology-behavioral pathways in a hierarchical model where a few genes are involved in determining a few neurological, physiological, and morphological traits. These neurological, physiological and morphological traits further shape the expression of a number of behavioral traits that ultimately result in biological functions, such as herbivore grazing patterns. As the gene expression of intermediate traits is variable in response to stimuli, each adjusted response of intermediate traits is added up and further transferred along pathways of grazing personality (19).

The variable expression of grazing genes modulated by regulatory systems is referred to as grazing personality plasticity. The reaction norm of behavioral traits are examples of behavioral trait plasticity changing along environmental gradients (61). As the phenotypic response along environmental gradients differs from one individual to another, the grazing personality plasticity might be a trait by itself and even have its own heritability (62). However, even if environmental conditions stay unchanged, the behavior of an individual changes as it ages, which is known as ontogeny, and that leads to behavioral development (63).

Ontogeny

Here we discuss two aspects of animal ontogeny related to grazing personalities. Firstly, the ontogeny itself and the changes in behavior observed in animals over their lifetime. Grazing personalities are consistently observed across situations and over time. However, the behavior of an animal changes along its behavioral development or maturation. For example, Van Moorter et al. (64) conducted a study at contrasting locations in France to compare the exploration behavior of yearling (8–15 months old) roe deer (*Capreolus capreolus*) prior to the settlement phase of dispersal against the exploration of adult individuals (>2 year old). Young roe deer had larger exploration behavior than adult deer. The results proved that yearling roe deer leave their natal home range and display a period of exploration in spring and summer as part of their natural maturation process. Adult individuals settle down later in life and explore smaller areas. The example above shows that individuals display changes in grazing behaviors along their ontogenic development. Furthermore, within behavioral development phases, behavioral differences among animals are maintained from early life and along their lifetime. Finally, behavioral differences among individuals detected early in life can be used as predictors for divergent grazing personalities displayed at mature life phases.

The second aspect regarding animal ontogeny affecting grazing personalities is the importance of environments and emotional states experienced early in life (including experiences of predecessors in preconception) to influence the gene expression of behavioral and personality traits. Maternal effects early in life that induce changes in gene expression and thereby of phenotypic behavior have been documented in birds. For example, wild females of the altricial canary (*Serinus domesticus*) regulates the use of androgens when laying eggs in a way that late born chicks have higher levels of testosterone (65). Thus, chicks from late laid eggs showed faster embryonic development, increased muscular development and more begging behavior than chicks of early laid eggs. All these traits made the younger chicks of the clutch to be more competitive than older (i.e., earlier born) chicks. Different hormonal environment experienced early in life can induce changes in the expression of genes controlling physiological and behavioral traits, conferring a social hierarchy, which is maintained later during adulthood (65). To our knowledge, no study had documented changes of gene expression due to early-in-life experiences in large herbivores [but see study of Candemir et al. (66) with mice].

In the following paragraphs, we explain and exemplify how adaptive responses to early life experiences are determinant in shaping the gene expression of an individual and how such responses can be inherited epigenetically.

Epigenetics

The epigenetic inheritance system of the GP-model is a set of mechanisms that modifies DNA arrangement and that affects the expression of genes related to grazing personalities without causing alterations to the nucleotide sequence. Epigenetic mechanisms stimulate, discourage, or inhibit the expression of genes through DNA folding and transcriptional activities. Most known epigenetic mechanisms are DNA methylation and histone alterations (67). Such mechanisms mediate the interface between the genomic control over grazing behaviors, and responses to stimuli such as the social and biophysical environments and the emotional state of animals. Adaptive and maladaptive responses to stimuli are reflected in the phenotypic grazing personality of individuals that undergo changes to their epigenetic state and thus modulate their gene expression. Thus, alterable epigenomes—i.e., facilitated epigenotypes (probabilistically controlled by the genotype) and pure epigenotypes (not controlled by the genotype outside the affected locus)—depend on stimuli signals and is modified according to each individual's experiences (68). The transgenerational epigenetic inheritance is the transference to offspring and following generations of adopted epigenetic states in response to stimuli. Steroid hormones mediate a particular case of epigenetic modifications in response to stress (68). The study conducted by Howery et al. (2) in an extensive grazing allotment in Idaho (USA) reported that the majority of individual beef cows (78%) showed high-fidelity to home range and habitats, returning to these feeding areas in consecutive years. The study was carried on for another 4 years (1990–1993) to test if offspring and cross-fostered offspring maintained fidelity to the home range and habitats where they were reared and whether grazing behavior of dams and foster dams influenced their grazing behavior (36). These authors reported that home range and habitat fidelity was displayed by dams and foster dams as well as by yearlings and cross-fostered yearlings. They concluded that grazing behaviors experienced early in life conditioned the behavior in adulthood, and this was observed independently from yearlings being reared by their dams or by foster dams. Habitat fidelity decreased however with a severed drought and in response to the grazing behavior of other peers. These studies showed that grazing behavior was consistent over time and it was transferred to the progeny and foster-progeny. While parental effects of dams and foster-dams were corroborated, at that time, genetic heritability of grazing behavior was not tested and remained unknown. The grazing behavior of dams and yearlings was affected by a severe drought in 1992, which illustrates the plasticity of grazing behaviors responding to changing biophysical environment. Parental effects and peer effects modulated the grazing patterns of yearlings accordingly to the social herd environment experienced early and in subsequent stages of life. Howery and Bailey (43) attributed these results to a combination of nature (genetic) and nurture (learned), although, the latter could also be attributed to epigenetic inheritance. In the following section, we present examples showing how the emotional state of animals can induce changes on the expression of behavioral genes.

Emotional Operating System

Conscious and unconscious internal states of the brain dictate the mental well-being of mammals. While fulfilling their physiological needs, animals can react to external and internal stimuli to attempt to minimize negative emotions and to seek positive emotions (69). For example, grazing actions and reactions of ruminants and foragers in general are conditioned by their current emotional state, past experiences, and expectations (70, 71); referred to as cognitive mechanisms in the GP-model. Emotions modulate the expression of grazing genes through epigenetic states (inheritable emotional states) and/or affect the observed grazing behaviors directly (i.e., see the two arrows of emotional state in **Figure 1**). For instance, domestic chickens (*Gallus gallus*), under a social environment of intermittent isolation early in life developed a lowered response to corticosterone, which restrained stress (72). Using microarrays immediately after the treatment, treated chickens have upregulated the function of genes related to stress. Later in life, chickens treated with social isolation displayed a decreased reactivity of the hypothalamic-pituitary-adrenal axis, increased growth and improved associative learning in comparison with untreated chickens. The study provided evidence of transgenerational inheritance triggered by the chickens' emotional state. The emotions and the emotional state of animals affected their immediate behaviors; also experiences early in life might have underpin lifetime "conditioning" that altered the epigenetic environment of specific genes. Such effect was transferred to the progeny. Negative and positive emotions may affect (non-heritable) and modulate (heritable) the behavior of animals. For example, among these emotions, stress has been studied extensively because of the relevance to animal welfare, health and fitness. As individual animals display different coping styles while facing stressful situations, their emotions, emotional state, and ultimately their welfare, depends upon their individual personalities (46).

Grazing Traits of Individuals

On the one hand, quantitative and continuous traits are commonly used to describe grazing behaviors along continuum gradients (28, 32). On the other, grazing personalities as categorical attributes of consistent behaviors may emerge because of the existence of trade-offs among correlated traits. Thus, animals may adopt contrasting strategies (52) such as the contrasting proactive and reactive personalities, *sensu* "life-history theory" (51) or the fast and slow metabolisms, *sensu* "pace-of-life syndromes" (73).

Behavioral studies on foraging animals are commonly limited to describe two types of grazing animals, which account for the extreme behaviors observed at the opposite ends of a continuum axis. For example, the residual feed intake was estimated for nearly two thousand dairy cows and a continuous gradient of this parameter was obtained. Then, individuals displaying the lowest and highest residual feed intake within this gradient were selected for further research [i.e., 183 and 16 selected individuals (74) and (9), respectively]. Similarly, animals of several species have been classified into two contrasting types (**Table 1**). For example, ruminants have been categorized as either riparian or uplands users (2, 36), bold or shy explorers (48, 49), bottom-dwellers or hill-climbers (1, 32, 40, 41).

Alternatively, a diverse range of discrete personalities can be depicted by integrating multiple behavioral "dimensions" (e.g., grazing traits) to describe and classify animals that show distinctive behaviors (17, 75). A multi-dimensional approach applied to grazing behaviors allows the conceptualization (and description) of consistent movement patterns both within species and across species. For example, studies have investigated a large diversity of foraging species and thus clustered individuals into four major types of so-called movement syndromes (25), movement strategies (26), or functional movement classes (27). These studies included, thirteen species of several vertebrate taxa of herbivores and carnivores (25); large herbivores such as the African elephant (*Loxodonta africana*), giant Galapagos tortoise (*Chelonoidis* spp.), and mule deer (*Odocoileus hemionus*) (26); and 92 species of marine life with feeding habits of carnivorous, zooplankton and algae feeders (27). The four movement types of these three studies were described and similarly named as: centered home-range, territorialists, nomads, and migrants under movement syndromes (25); as resident, multi-patch, nomadic and migration under movement strategies (26); and as resident, occasional, irruptor, and roamer under functional movement classes (27). The studies found four common movement patterns across several taxa that have different modes of movement (e.g., terrestrial locomotion, swimming, flying) and different feeding habits. For example, there were herbivores [e.g., African elephant, plains zebra (*Equus quagga*), springbok (*Antidorcas marsupialis*), mule deer, and several algae feeding marine species] and carnivores [e.g., African wild dog (*Lycaon pictus*) and several fish feeding marine species]. Furthermore, the authors observed these common movement patterns consistently across situations and over time, a condition for behavioral personalities. We anticipate that grazing ruminants and other large herbivores consistently display such common grazing patterns within herds, populations and species and even across species (i.e., regardless of species identity).

Finally, another alternative would be if grazing patterns and behaviors of grazing ruminants and other large herbivores are displayed as normally distributed variables and genetically independent traits that show no phenotypic correlations (37, 76). In such a case, conceptualizing categorical grazing personalities might be challenging or even inappropriate.

COLLECTIVE GRAZING PERSONALITIES

> *"The social environment and interactions have a lifelong influence on what an animal eats and where it goes [. . .]. In herbivores, social organization leads to culture, which is the collective knowledge and habits acquired and passed from generation to generation about how to survive in a particular environment"* (77).

In this section, we focused on the collective grazing personalities of the GP-model (**Figure 1**, right side): from collective genotypes (at the top), through regulatory systems modulating plastic responses (middle), to phenotypic grazing personalities as observed in herds of grazing ruminants and other large herbivores (at the bottom). In section Gene Pools and Allele Frequencies, we hypothesize that the allelic variation and frequency of grazing genes determine the emergence of grazing personalities at collective level. Section The Social Environment of the Herd presents the collective social environment as the main regulatory system that shapes grazing personalities at collective level. In section Grazing Traits of Herds, we present examples and discuss the emergence of collective grazing patterns as consistently observed across contexts and over time.

Gene Pools and Allele Frequencies

The existence of distinctive grazing personalities among individuals and the coexistence of divergent personalities within populations (so-called behavioral polymorphic populations) are both products of evolutionary processes. Selection acts over phenotype through differential fitness (e.g., individuals achieving different rates of survival and reproduction), which is then reflected in the gene pool of the group (12, 51). Animals that achieve longer lives, and/or greater reproduction rates under certain social and biophysical conditions, will produce more offspring. In this way, outperforming phenotypes with greater fitness get larger representation within the herd, making their alleles more common in the gene pool. Inversely, phenotypes with lower fitness are less represented in the population and in turn, their alleles become less common. Changes in social or biophysical environments may affect the fitness of distinct grazing personalities and lead, over generations, to changes in the allele frequencies of genes. Despite their lower performance, low fitness phenotypes still reproduce and therefore, their genes are maintained (78). Mating success of behaviorally distinct individuals would influences the allele frequencies of the population. Populations may have different behavioral morphs that exist at specific ratios. Here are two examples.

Lampert et al. (79) reported genetic associations with divergent behavioral strategies of mating in panuco swordtail fish (*Xiphophorus nigrensis*). Divergent mating-strategy and morphs of panuco swordtail fish are genetically associated with specific alleles and therefore, these populations seems to be genetically and phenotypically polymorphic. The small male morphs have relatively smaller swords, have a female appearance and are less ornate than large males, which are gifted with larger swordtails and are much more decorated. Females prefer mating with large males, which are territorial and court them. The apparent reproductive disadvantage of small swordtail fish morphs does not stop them mating, and instead of undertaking courtship, small males chase and force females to copulate. By adopting a different behavioral mating strategy, small fish morphs successfully passes their genes ensuring the persistence of this morpho-behavioral phenotype. In the second example, Pruitt and Goodnight (80) reported that natural populations of communal spider (*Anelosimus studiosus*) have behavioral polymorphic individuals labeled as aggressive and docile. Populations of spiders growing under contrasting environmental conditions such as high and low availability of resources have different ratios of the aggressive to docile phenotypes. The phenotype ratio largely explained the reproductive success of the colony and determined the behavioral attributes of the colony. The authors concluded that aggressive:docile behavioral ratio would ensure long-term survival at the collective level. The phenotype ratio was site-specific and was the result of a collective-driven selection. On artificially made populations, switches of the phenotype ratio toward the ratio of spiders' origin (and regardless of the environmental conditions i.e., maladaptive responses) can be attributed to collectively controlled inheritance.

To our knowledge, there have not been any studies looking at genotypic diversity, composition and relative frequency of grazing genes in ruminant herds. Since the very beginning of animal domestication, herders are selecting individual animals by their behavior (e.g., docility). But it is only in the last 30 years that scientists started to recommend culling individual animals that display undesired grazing patterns (2, 81). Certainly, the behavioral selection conducted in the past over domesticated herbivores has shaped the gene pools of present-day herds. However, it is unknown how this selection has affected their grazing patterns. Similarly, environmental changes, such as fragmentation of natural ecosystems, limited animal migration or selective hunting, has affected the gene pools and relative frequency of grazing genes of herds of wild animals and in that way, may have modified their collective grazing personalities. This has been exemplified by the selective capturing of fish with nets over wild fish populations (24). As seen with the artificially-made colonies of communal spiders (80), we speculate that the ratio of genotypic grazing personalities within a herd of ruminants might be regulated collectively to ensure long-term survival of the group. As the ratio of genotypes within a herd might be site specific, it is possible to speculate that such collective traits are inherited epigenetically.

The recent discovery of nucleotide variation in grazing genes and their association with the grazing patterns of individual animals opens the opportunity to search for an ideal grazer; one that displays the "best" grazing personality (32, 40–42). However, large herbivores do not graze alone but in herds of interacting animals, where individuals display a range of distinct grazing personalities that shapes the grazing personality of the herd. In this way, herds have unique attributes of grazing behavior (see section Grazing Traits of Herds). At collective level, genetically similar herds may display different personalities because of the plastic expressions of grazing patterns. This is discussed in the following section.

The Social Environment of the Herd

The interactions among conspecifics constitutes the social environment of herds. Such interactions establish the social status occupied and the behaviors adopted by each individual. For example, the roles of leader and follower (45), dominant and submissive (38), and producer and scrounger (82), are extensively documented in ruminants, birds and other foraging species. Socially responsive individuals adjust their behaviors according to the social context and within the limits of their personality plasticity (83). Thus, the social herd environment is a major factor of behavioral variation that affects the phenotypic expression of grazing personality and its plasticity at the individual and collective level (61). In section The Effect of Personality Plasticity and Regulatory Systems on Grazing Patterns, we provided examples of how the social environment (e.g., social isolation and parental care) affects the behavior of individuals. Similarly, the emergence of socially central individuals (e.g., leader and dominant animals) conditions collective grazing behaviors. For example, in Highland cattle (*Bos taurus*), Sueur et al. (45) reported that castrated mature males provided leadership and promoted group cohesiveness to juvenile cattle. These authors suggested using trained matured castrated males to increase grazing intensity of targeted areas. In another experiment with groups of fallow deer (*Dama dama*), Stutz et al. (84) showed that high aggregation and cohesiveness working toward increasing safety against predators have reduced the individual and collective exploitation of preferred and more nutritious diets. Thus, the collective perceived risk of predators influences collective exploration and utilization of feed sources. Another way to study the effects of collective behaviors is by replacing (or removing) socially central individuals. Vital and Martins (85) removed the key individuals from a group of zebrafish (*Danio rerio*) and reported reduced learning of foraging skills. In bottlenose dolphins (*Tursiops truncatus*) the presence of certain individuals was crucial to maintain interactions between subgroups (86). However, in beef cattle the effects of socially central individuals might be only relevant in small size herds, for example <40 individuals, where the fidelity of individuals to the group they belong to is relatively high; on the contrary, social bonds in larger herds are expected to be weaker (87, 88). In the collective context of colony living organisms, the social environment is crucial for the survival and fitness of the group as well as for the relative success of each individual (33); to a certain extent, this is also the case for collective grazers such as grazing ruminants and other large herbivores.

The GP-model establishes that stimuli from the social and biophysical environments and the emotional state of animal affects the displayed grazing personalities of individuals, which in turn are transferred to the grazing patterns displayed collectively (see section The Effect of Personality Plasticity and Regulatory Systems on Grazing Patterns). Similarly to the case of individuals, the social environment of the herd might influence the gene expression of collective grazing traits and therefore modulate the phenotypic grazing personalities as observed collectively. However, until now, it is unknown whether there are genes controlling collective behavioral traits in ruminants and, if so, whether the social environment controls its expression. A combination of social learning and a segregation of leader and followers could also explain collective behaviors (85). We posed these unresolved aspects using question marks in the GP-model (**Figure 1**). In the next section, however, there are examples of grazing traits measured at collective level.

Grazing Traits of Herds

Based on behavioral genetics, Gross (89) described three main pathways to explain phenotypic polymorphism of behavior displayed by individuals within animal populations. Firstly, the so-called "alternative pathway" which considers a frequency-dependent selection of animals that maintains genetically polymorphic populations with individuals displaying behavioral polymorphism and achieving similar fitness. Secondly, the "mixed pathway" occurs in genetically monomorphic populations with individuals displaying mixed behavioral tactics. Finally, the so-called "conditional pathway" occurring in genetically monomorphic populations where individuals display a set of behavioral tactics according to state-dependent conditioning. For the GP-model and for any study of grazing herds in general, it is crucial to bear in mind that herds of ruminants are phenotypically behaviorally polymorphic. Within a herd of ruminants, individuals coexist displaying a range of distinctive grazing personalities. While the alternative pathway attributes the phenotypic behavioral polymorphism to genotype variation (i.e., personality genotype in the GP-model), mixed pathways and conditional pathways apply to populations comprised by genetically monomorphic individuals. As previously presented in section The Effect of Personality Plasticity and Regulatory Systems on Grazing Patterns, the personality plasticity at collective level accounts for the variable gene expression and therefore, different phenotypic outcomes from genetically identical individuals may take place. We hypothesize that the mixed pathway may correspond to variations attributable to the epigenetic system (heritable), and that the conditional pathways may correspond to direct effects over the emotional state. For the previous, adopted behaviors might be transferred to offspring and therefore show transgenerational epigenetic inheritance; for the latter, behavioral polymorphism may be observable only in the animals that adopted such behavior as a direct response to their emotional states.

We set the GP-model using an individual-based approach of grazing personalities to explain distributional grazing patterns as observed in real herds of ruminants. Gueron et al. (47) presented a model that simulated distributional patterns of grazing herds based on a set of behavioral traits that were applied to individual agents. The authors applied a hierarchical decision-making algorithm, with rules-of-thumb establishing individual sensitiveness to crowding and attraction to conspecifics that applied respectively according to a repulsion zone (animals getting too close), an attraction zone (animals getting too far), and an intermediate buffer zone called neutral zone without response. Simulations were ran for a thousand time-steps of individuals that displayed different behavioral traits, such as walking speed and sense of orientation toward a targeted direction. Gueron's model showed differences in herds distribution and fragmentation as it happens in real herds. The model showed that integrating behavioral, physiological and individual decision-making traits could reproduce attributes of interacting "grazing" animals. From individual differences in grazing traits emerged collective behaviors of herd fragmentation and distributional patterns.

Gueron's mechanistic simulations were later tested and validated in a similar model using groups of sheep of variable number (two, four, six, or eight sheep) of either exclusively bold individuals or exclusively shy individuals (90). In support of individual-based approaches, the findings of these authors showed that the grazing patterns observed in interacting animals derive from individual behavioral traits and interaction rules; however, behavioral traits at the group level, such as the strength of social attraction, seems to control emergent decision-making mechanisms at collective level. A further step on the simulation of grazing herds was achieved by Spiegel et al. (52). These authors simulated grazing agents with divergent movement traits in variable contexts of vegetation patchiness. With some similarity to the simulations done by Gueron et al. (47), Spiegel et al. allocated divergent behavioral traits to groups of individuals "grazing" along increasing levels of vegetation patchiness, i.e., from low patchiness where pixels of nine different vegetation resources were uniformly mixed (patch size equals pixel size), through medium patchiness with randomly mixed pixels (mid-size patches), to high patchiness where pixels of each resource are highly aggregated forming large and discrete vegetation patches. Comparing divergent personalities such as slow and fast explorers, these authors concluded that under low patchiness, fast explorers would achieve higher foraging efficiency than slow explorers. This would be reversed, however, in grazing lands with discrete vegetation patches. Such results are consistent with real experiments in dairy cows (9). Spiegel's et al. (52) scenarios showed that seasonal dynamics of vegetation would alternatively benefit one or another grazing personality at different times of the year, highlighting the temporal variation of animal performance in support of the existence of herds with behavioral polymorphism. Finally, these authors pointed out the emergence of a complex group-level structure displaying collective grazing patterns with its own attributes (e.g., clustering of similar phenotypes, home range size, and structure), which changed along environmental gradients (e.g., vegetation patchiness). Interestingly, individual-based simulation models set behavioral rules and traits to be repeated over time [i.e., 1,000 and 2,000 time steps in Gueron et al. (47) and Spiegel et al. (52), respectively] and even across different contexts such as a gradient of vegetation patchiness (52). By allocating different values of behavioral traits to individuals that coexist and interact with each other, simulation models recreated real ruminant herds as mixed behaviors displayed consistently over time and across situations; therefore complying with conditions of grazing personalities used in the GP-model.

Individuals displaying divergent personalities comprise herds of ruminants, which are recognized and described as extended families that maintain cohesiveness and display unique identities (77). So, how can we characterize and compare the unique identities of ruminant herds (i.e., collective grazing personalities)?

One way to value behaviors at collective level is by using grazing traits measured in individuals while performing within the herd and by integrating these individual values into an averaged and/or weighted value. Additionally, the statistical dispersion of behavioral traits (e.g., coefficient of variance) within herds can be used for comparisons among herds. To our knowledge, there are not many studies with such examples. Partially, this might be because of the challenge of measuring grazing behaviors in all members of the herd while grazing as a herd. However, this might be also because of the lack of conceptualizing collective measurements of grazing behaviors, although, this has been proposed for other social living animals such as foraging insects (33). Sueur et al. (45) studied leadership within four Highland cattle groups (groups ranging from 8 to 21 individuals), but did not compare collective behaviors among groups. Rudin et al. (91) compared behavioral traits on two groups of over 500 Australian field crickets (*Telogryllus oceanicus*) growing under contrasting social environments of "silent" or "signing" individuals. Based on statistical differences in the mean value and standard error on distance traveled and speed measured in individuals, these authors concluded that the social environment significantly affected "the repeatable aspect of behavior (i.e., personality)," and that behavioral changes were heritable. However, Rudin et al. (91) measured traits in individuals pulled apart from the group rather than on individuals performing within a group. Several studies in the past compared distinct behaviors displayed in ruminants (9, 32, 39) and authors commonly conclude that "individuals" pertaining to a certain group behave differently to "individuals" pertaining to another group rather than assessing collective behaviors. We advocate for comparisons of different groups that display collective grazing personalities with their unique attributes.

Another way applicable to certain scenarios and for certain traits is by representing collective grazing behaviors with the behavior of one or a few animals of the herd. For example, Liao et al. (28) studied the grazing behavior of 20 herds of beef cattle in five different study sites of Southern Ethiopia. These authors derived collective behavioral traits such as daily allocation of time to travel, grazing, and resting by averaging the behavior monitored in three cows of each herd with GPS collars. Pastoral people herded their animals to daily foraging areas and brought them back to their camps for overnight. The herd was moved as a relatively compact group, thus, monitoring of any three cows of each herd would be sufficient to provide comparative information among herds. These authors reported different daily patterns of grazing behavior of monitored herds and provided insights on the different foraging habitats used by different herds with details on greenness, elevation and terrain slope.

Here, we mention attributes of ruminant herds and grazing traits relevant to collective grazing personalities. For example, home range was defined as the spatial expression of behaviors [that individual] animals perform to survive and reproduce (92) in a defined timescale (93). Thus, a certain number of individuals that comprise a herd occupies, needs or is allocated to an area with features of size, shape and biophysical conditions. Similarly, one could compute the area utilized by a herd, for example, on a daily basis. Fragmentation (47), cohesiveness (94) and assortativity (52, 95) are examples of group-level traits that in a future can be used to study collective and individual grazing attributes as well as the impact of grazing herds to ecosystem functions of grazing lands or to animal welfare. For example, Foister et al. (96) used phenotypic attributes of social interactions measured at group-level (i.e., social network properties) to predict consistent aggressive events (i.e., a personality dimension) among pigs reared as a group in pens. In beef cattle, the centrality of individuals as a specific collective measurement rather than the number of individuals determined the group composition and affected the social stability and stress of the herd (97).

ILLUSTRATIONS AND IMPLICATIONS

Figure 1 presents a hypothetical example with individuals (left side) differing in the allelic variations of two grazing genes, which comprise a herd of ruminants (right side). Grazing personality pathways between an individual's genotype (I_x) and its displayed grazing personality (iGP_x) involve several intermediate and concatenated traits, which have a regulatory system of the gene expression. Following the GP-model, we described this example starting on the individual genotype (top left), going through stimuli that influence the expression of grazing genes (middle left) to yield in the phenotypic grazing personality of individual grazers (bottom left). As ruminants graze in herds, individual genotypes were aggregated into the collective gene pool (top right), then, we discuss the modulation of the gene expression at group level (middle right) and finally describe the collective personality of the herd.

Grazing Personality Genotype

Individuals with allelic variations I_1 and I_2 display shy grazing personalities named iGP_1 and iGP_2, respectively. Individuals with shy personalities occupy relatively small home ranges, stay at relatively short distances from one another and prefer grazing flat terrain in low altitude habitats. As personalities are phenotypically plastic, under certain conditions, iGP_1 and iGP_2 cannot be differentiated because of phenotypic overlap. Individuals with allelic variation I_3 are associated with animals displaying a bold grazing personality named iGP_3. Such herbivores show relatively large home ranges, they graze alone or at relatively large distance from one another and show grazing preference for steep slope terrain in high altitude habitats. Regardless of conditions, IGP_3 always display discernible grazing patterns from the previously described personalities. For example, iGP_1 and iGP_2 could be similar to bottom dweller cattle and, iGP_3 to hill climber cattle, which display divergent indexes of landscape use and exhibit divergent grazing patterns (1). These cattle have genetic associations to gene markers overlaying the glutamate receptor 5 (*GRM5*) gene and the mastermind-like 3 (*MAML3*) gene (32). In the example, these genes are represented with hexagon and triangle shapes in **Figure 1**. For simplicity, only two of the five genes reported by Bailey et al. (32) are represented in the GP-model. Applying individual-based models, grazing patterns of herbivores can be simulated by using traits such as walking speed and sense of direction toward a preferred habitat and by applying variable responses to stimuli such as to vegetation patchiness, like variable walking acceleration or proximity to conspecifics (47, 52, 90). In our example, iGP_1 and iGP_2 have equal allelic variation as I_1 *GRM5*[A] = I_2 *GRM5*[A]. This genotype determines animals to have low concentrations of blood cortisol that makes them to display low walking speed and travel relatively short distances (39, 98, 99). For this example, we establish that *GRM5*[A] animals prefer grazing in flat terrains. Walking acceleration and attraction zone to conspecifics are also similar ($iGP_1 \sim iGP_2$) making them quickly accelerate toward conspecifics that get away and to do so at relatively short distances. These personalities differ in their allelic variation I_1 *MAML3*[T] $\neq$ I_2 *MAML3*[G], responsible of sense of orientation toward preferred areas. For example, *MAML3*[T] animals display a high sense of orientation and *MAML3*[G] express a low sense of orientation ($iGP_1 > iGP_2$). I_3 animals differ from both previous genotypes by having *GRM5*[G], which is phenotypically expressed with a high blood cortisol concentration. *GRM5*[G] animals display fast walking speed, and therefore I_3 animals travel relatively long distances. For this example, we establish that *GRM5*[G] animals prefer grazing in steep slope terrain in high altitudes. iGP_3 walking acceleration is low and attraction zone to conspecifics is long, therefore, iGP_3 individuals accelerate slowly toward conspecifics that get away and do so when conspecifics are relatively far away. iGP_3 has equal allelic variation to iGP_2 animals for the sense of orientation trait (I_2 *MAML3*[G] = I_3 *MAML3*[G]), therefore show low sense of orientation toward its preferred mountainous terrain.

In a herd of ruminants, allelic diversity is defined as the number of different alleles of a grazing gene present when accounting for all individuals. Allelic composition refers to which alleles in particular are represented. Finally, relative allelic frequency refers to the proportion of each allelic variant of grazing genes. While these two previous attributes do not necessarily depend of the number of members but on their genotype, the latter, depends on combining the genotype of members and their proportional representation. Finally, the total size of the herd, at equal proportion of individual grazing personalities, affects the collective personality (not considered in this example). In our example in **Figure 1**, two grazing genes, *GRM5* and *MAML3*, are shown in three grazing personalities I_1, I_2, and I_3 that comprise herd one (H_1). Each gene has two variants. Therefore, the allelic diversity for either of these genes in H_1 is two. The allelic composition of *GRM5* is Adenine and Guanine, while for *MAML3* is Thymine and Guanine. Note that the total existing allelic variation for these genes is much larger than in our example; Bailey et al. (32) reported four possible nucleobases (adenine, cytosine, thymine and guanine) at six different positions in *GRM5*, and the nucleobases thymine and guanine for *MAML3*. In **Figure 1**, we did not specify the number of individuals of each genotype nor total number of individuals comprising the herd. However, we represented the relative allelic frequency of grazing genes *GRM5* and *MAML3* establishing equal number of individuals ($n = 10$) of each genotype. For example:

If I_1 $n = 10$; I_2 $n = 10$; I_3 $n = 10$, then the relative allelic frequency in H_1 would be: *GRM5* x2[A]: 1x[G]; *MAML3* x1[T]: x2[G].

Personality Plasticity

Despite the genetic determination of cortisol concentrations in blood in individual animals, it has also been revealed that its expression is affected by stimuli, such as during experiments of social isolation [see Goerlich et al. (72) in section The Effect of Personality Plasticity and Regulatory Systems on Grazing Patterns]. For example, the use of low-stress herding techniques might reduce cortisol concentration in the blood of ruminants and foster the use of targeted areas because of emotional state of lower predation risk as in comparison with animals under "traditional" herding techniques (100). In our example, reduction of the concentrantion of cortisol in blood is established to reduce walking speed and also daily traveled distance. We represented personality plasticity on the phenotype of the hypothetical individuals. In **Figure 1**, iGP_1 and iGP_2 overlap each other and under certain conditions it will not be possible to distinguish them by simple phenotypic observation. On the other end, iGP3 is separated toward the right of the GP-model and representing therefore that differences in grazing personalities are phenotypically observable.

Grazing Personality Phenotype

The GP-model as shown in **Figure 1** represents genetically polymorphic individuals (i.e., individuals with different alleles) that comprise the collective gene pool and relative allelic frequency of grazing genes of a herd. Phenotypically, in such a herd coexist individuals that display distinct grazing personalities. iGP_1 and iGP_2 individual display slow walking speed and travel short distances. As soon as conspecifics move away a relatively short distance, these individuals will accelerate and reduce distance to conspecifics. These grazing personalities prefer flat and low altitude habitat, where they graze more intensively and spend more time than on steep slopes located in high altitude habitats. iGP_1 individuals will return quicker and more often to vegetation patches of their preferred habitat than iGP_2, because of the lower sense of orientation of the latter. Therefore, iGP_1 tends to utilize its preferred habitat for a longer time. Herds comprised purely of either iGP_1 or iGP_2 individuals are less fragmented, move slowly and have smaller home ranges [slow-explorer *sensu* (52)]. In grazing lands where patches of vegetation are small and homogeneously distributed, these two personalities may display similar grazing patterns (i.e., phenotypically similar) because the sense of orientation would not make a difference in distribution where non-conspicuous patches of vegetation exist. In grazing lands where significantly big patches of vegetation are heterogeneously distributed, iGP_1 will utilize more intensively its preferred habitat, taking advantage of its better sense of orientation in comparison with iGP_2 individuals (i.e., phenotypically dissimilar). Herds comprised purely of iGP_3 individuals are highly fragmented, move faster, and individuals graze at greater distances from one another. iGP_3 individuals graze alone or in relatively small groups that occupy larger home ranges than iGP_1 or iGP_2 individuals. iGP_3 individuals prefer steep slope areas in high altitude habitats and have low sense of orientation. As per their low sense of orientation, these animals will show similar grazing patterns in homogeneous and heterogeneous grazing lands. However, iGP_3 are "always" phenotypically dissimilar to the other grazing personalities.

Implications

The GP-model proposes a novel understanding of social foragers: grazing is a social activity performed by herds of interacting ruminants that display collective grazing personalities with their own unique attributes. Individuals that display distinct grazing personalities comprise behavioral polymorphic herds of ruminants. Grazing personalities of ruminants are controlled by their genetic composition and are modulated by their epigenetic states in response to the social herd environment, biophysical environment and the emotional state. Adaptive and inheritable epigenetic states confers plasticity to grazing personalities at individual and collective levels.

Selecting for Grazing Personality

Farmers, ranch managers and breeders may adopt the concept of grazing personalities and select for animals according to the desired and needed distinctive behaviors. By so doing, we forecast a genetic gain on herds to address major challenges faced by the pastoral livestock production industry. The identification of grazing personality genotypes and the development of the corresponding genetic markers can be used to determine the grazing personality composition of herds and to further assist in applying goal-oriented selection of animals using a relatively simple and inexpensive genetic test such as single-strand conformation polymorphism (SSCP) (101, 102).

Enhancing the Expression of Grazing Personalities

The GP-model establishes that grazing personalities of ruminants and other large herbivores are plastically displayed in response to stimuli (e.g., social herd environment, biophysical environment, and animals' emotional states). Such responses might be adopted and shown for the entire lifespan of animals and, can be farther transferred to their progeny through transgenerational epigenetic inheritance. This is particularly relevant for experiences occurred early in life. Exposing grazing ruminants and other large herbivores to the biophysical environmental conditions where they are targeted to perform may trigger epigenetic mechanisms and regulatory systems that foster the expression of grazing genes toward desired behaviors of individual grazers and herds. As per the GP-model, the social context in which an animal and its predecessor grow (i.e., the social herd environment) modulates the expression of grazing genes and therefore the displayed grazing personalities. For example, social environments of isolation, crowdedness, threats and fearfulness, as well as the aggressiveness of herds, affect the emotional states and modulates the individual and collective grazing behaviors and associated decision-making. Similarly, the biophysical environment might shape the expression of grazing genes.

Influencing Grazing Personalities Through Emotions

Grazing management practices such as fasting, supplementation or herding techniques alter animal internal states (e.g., hunger, emotions), influence animal decision-making and ultimately, modify their grazing patterns.

Designing Behavior-Customized Herds

The composition and relative frequency of grazing personalities of domesticated ruminant herds has been manipulated and shaped for millennia to produce docile and manageable individuals and herds suitable for living alongside and under management of humans. The GP-model proposes to apply behavioral-based selection for the design of ruminant herds matching the spatial diversity and the temporal variety of forages, foodscapes and landscapes. Pastoral livestock production systems are heterogeneous in space and time. Despite efforts to create "simple and homogeneous" systems, individualities and collective attributes of grazing patterns emerge. Herds are comprised of a mix of individuals displaying distinctive grazing personalities. Therefore, grazing patterns of ruminant herds can be manipulated through designing and deciding the relative frequency of individual grazing personalities along with the adoption of grazing management practices that foster the desired behaviors.

CONCLUSIONS

The GP-model proposes that genetic effects (allele diversity, composition and relative frequency) and epigenetic modulation (via regulatory systems that modulate the gene expression) conditions grazing behaviors of ruminants and other large herbivores, so that, animals display grazing personalities at individual and collective levels. The interactions with the social herd environment and the biophysical environment shape the phenotypic grazing personalities of individuals. Collective grazing personalities emerge from the social interaction of individuals and their grazing personalities. The social herd environment mediates between the individual and the collective grazing personalities. This is because interacting individuals constitute the herd and create its environment. In turn, the social herd environment influences both, the grazing personality of individuals and the grazing personality of the herd.

The allelic composition and the relative frequency of grazing genes characterize the collective genotypic of grazing personalities and, therefore, there is the opportunity to develop breeding programs aiming to influence grazing patterns of ruminant herds applying behavioral selection. Because of the genetic basis of grazing behavior, animal selection maybe a useful tool to improve grazing distribution of habitat-heterogeneous livestock systems.

The displayed grazing personality of herds of ruminants and other large herbivores results from their genome and the personality plasticity. Grazing management, herding techniques, feeding strategies, and rearing practices that affect animal welfare and the gene expression of grazing traits have the potential to foster desirable grazing personalities. Managers that account for the variety of individual grazing personalities naturally displayed in ruminants, and that manipulate its proportion, can enhance ecosystem services and improve animal welfare while maintaining the productivity of livestock production systems.

The grazing personality model presented here further develops our understanding of the distribution of ruminants and large herbivores by integrating discoveries from the past few decades into models of grazing distribution and behavior (29, 30). The GP-model was inspired from and supported with scientific works conducted with a diverse range of taxa from the animal kingdom, namely bees, birds, marine species, large herbivores, ruminants, and other ungulates. Future research on grazing personalities at the individual and collective levels may confirm the hypotheses posed in the "grazing personality model" and thus contribute to a better understanding of livestock production systems, grassland science and animal behavior.

AUTHOR CONTRIBUTIONS

CM wrote the manuscript. CM, PG, TM, and JH made substantial, direct and intellectual contribution to the work, and approved it for publication.

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Time of Grain Supplementation and Social Dominance Modify Feeding Behavior of Heifers in Rotational Grazing Systems

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Social hierarchy affects the access of animals to feed resources. On daily rotational pasture systems, supplementation time may influence feeding behavior. This trial was designed to test the effect of grain delivery time on the feeding behavior of heifers. Heifers divided into two groups according to breed ($n = 15$ Braford and $n = 19$ Jersey) were tested in a crossover design with two treatments: INITIAL—supplement at 8 am (entry time to a fresh paddock), and MIDDLE—supplement at 4 pm (middle time of paddock use). Animals entered a new paddock every morning, and grain supplement at 2 kg/animal/day was offered at the fence line (1 m/animal). Then, ingestive and other behaviors were registered by direct visual observation through scan sampling at 2-min intervals for 1 h after grain supply. Agonistic interactions were recorded continuously (instigator–victim) to build a social matrix whereby each heifer was defined as dominant, intermediate, or subordinate. Weekly pasture samples were collected according to the order that animals left the feeding area, using the hand-plucking technique, to determine crude protein and fiber content. Heifers spent more time grazing on the INITIAL treatment ($p < 0.0001$) but exhibited more behaviors on the MIDDLE treatment ($p < 0.0001$). Dominant heifers spent more time eating grain ($p = 0.0008$), whereas subordinate heifers spent more time grazing along the paddock ($p = 0.0067$), but not along the fence ($p = 0.0008$). The crude protein content of pasture samples was higher for the INITIAL treatment ($p < 0.0001$). Behavioral interaction occurred with respect to the order of leaving the feeding area, social rank, and crude protein consumed ($p = 0.04$). Subordinate heifers consistently grazed more and ate less grain supplement than dominant and intermediate heifers. However, when grain supplement was offered at the time animals entered the paddock, more grazing activity took place during supplement feeding, and subordinate heifers could select a high-protein diet. In the INITIAL treatment, this means that subordinate animals could benefit from the better pasture available, keeping a distance from dominant heifers, reducing agonistic interactions and likely improving their welfare.

Keywords: social hierarchy, cattle, resources, agonistic interactions, subordinate

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INTRODUCTION

Rotational grazing systems, as proposed by Andre Voisin, allow better use of the pasture, ensuring sufficient interval between two successive shearings for vigorous regrowth and ensuring that animals will forage at the optimal level (1). Despite the benefits of rotational grazing, farmers may need to offer feed supplements to the animals as a nutritional increment in times of pasture scarcity or as part of the diet of highly productive animals.

Supplementation at specific times of the day may supply the ruminant animal with an appropriate boost in energy and protein substrates, but may also alter grazing patterns (2, 3). Despite widespread use of dietary supplements, technical recommendations to farmers target a regular daily supply of feed to maximize weight gain or increase milk production. However, these recommendations mainly consider such aspects as animal category, nutritional requirements, stage of pastures, and cost of supplements, without taking into consideration the social behavior of the animals and the consequences of such behavior with respect to resource access. Cattle are social animals and organize themselves into hierarchies according to their willingness and ability to fight for resources (4). Social hierarchy affects individual access to resources, and dominant animals are known to exert pre-eminence over resources (4–6), especially when resources are limited (7). Social hierarchy thus affects drinking (8) and feeding behavior (9, 10).

Grazing behavior may also be related to diurnal changes in food quality (11). The circadian rhythm of forage increases soluble sugar concentrations during the day, which may explain why herbivores show a strong preference for afternoon, rather than morning, harvested forage (12). When instantaneous stocking rate is increased, more competition arises for food, and the forage availability per animal and animals' selectivity are reduced. In rotationally grazed paddocks, sward structure changes continually as grazing proceeds along the day, and as a result, changes in quantity and quality associated with the depletion of the sward have a detrimental effect on the bite mass and the intake rate (13). In this scenario, subordinate animals may have their access to feed limited, compromising their welfare.

Therefore, if dominant animals have priority over the use of resources, we raised to question how subordinate animals would behave under such conditions. We further asked what strategies might be used by both animals and farmers to mitigate the negative effects of social dominance to subordinate animals. In a rotational grazing system, we know that animals enter a new paddock every morning. Therefore, based on the animals' physiology, it would be logical to offer feed supplement in the late afternoon when pasture availability is decreased, and the animals are more motivated to obtain feed. However, we hypothesized that subordinate animals could graze the best patches, while dominant animals would eat feed supplement as long as it is offered when animals enter the new paddock. Thus, this study was designed to compare the effect of different delivery times (morning × afternoon) of grain supplement on the feeding behavior of heifers managed in a rotational grazing system.

MATERIALS AND METHODS

The study was conducted between June and August of 2016 (winter) at the Voisin's Rational Grazing (VRG) Unit of the Federal University of Santa Catarina Experimental Farm of Ressacada, Florianópolis, Brazil (17°40'25" S; 48°32'30" W). The VRG unit is a 24-ha pasture divided into 86 paddocks averaging 2,500 m^2 and mainly composed of plants of the genus *Axonopus, Paspalum, Brachiaria, Pennisetum, Melinus, Setaria, Cynodon, Panicum, Hemarthria, Desmodium, Trifolium, Lotus, Arachis, Stylosanthes,* and *Lolium.* The study was performed in accordance with the Ethics Committee on Animal Use of the Federal University of Santa Catarina (CEUA/UFSC) under the approved protocol number 1004100516.

Animals, Treatments, and Experimental Design

Before the study, the animals were routinely managed in two groups, according to breed: Braford and Jersey heifers without any feed supplementation. These breeds make up the herd of the experimental farm and are very representative of the herds in southern Brazil, where the Jersey breed is the most common breed for grazing milk production and the Braford breed is well-adapted to the region, being composed of zebu (3/8) and taurine (5/8) blood.

For the experiment, the separation between breeds was kept, and two groups were formed: 15 Braford heifers (group 1, averaging 316 ± 44 kg) and 19 Jersey heifers (group 2, averaging 232 ± 33 kg). Each group was first allocated to one of the treatments: INITIAL—supplement was offered at occupation time (8 a.m.); and MIDDLE—supplement was offered at middle occupation time (4 p.m.). The experimental design was a crossover. In each period, the animals had 5 days for habituation to observers and the experimental routine, followed by 35 days (each day in a new paddock) for data collection.

Animals were moved to a new paddock every morning with mineral salt and water *ad libitum*. Space availability per animal in the paddocks was ∼145 m^2/animal. Animals were identified by ear tags and individually marked with numbers on their bodies with black and green livestock markers (Raidex., Dettingen; Erms Germany). The supplement was a commercial ration for cattle (12% CP) and was offered on a daily basis of 2 kg/animal/day on the ground at the fence line in the morning or afternoon, according to treatment.

Measurements

Data collection included observations of agonistic interactions and ingestive behavior, recorded simultaneously and in the two groups, as to avoid any environmental influence in their behavior. The agonistic interactions were continuously recorded, and the ingestive behavior was recorded by instantaneous scan sampling with a 2-min interval (14) twice a week for one uninterrupted hour from the moment the grain supplement was offered, resulting in 20 non-consecutive days of direct visual observation. Six trained observers switched groups within and between periods so that every person could observe the same number of times, groups, treatments, and periods, completely

TABLE 1 | Description of behaviors observed during the study.

Behavior	Description
Grazing along the paddock	Animal grazing along the paddock, with head down and the mouth below or at the level of the forage making movements of forage apprehension or grabbing forage; stationary or moving forward to new grazing patches
Grazing near the fence	Animal grazing as described above but along the fence line where the grain supplement was offered (feeding area)
Eating supplement	Animal eating grain supplement, with head down on the fence line and mouth on the supplement or above it while chewing
Other	When the animal performed an activity, either standing or lying, with the exception of the behaviors described above

The ethogram was based on the definitions by Coimbra et al. (8).

TABLE 2 | Dominance score (Score) and respective social hierarchy (SH) of each individual animal within each group.

Group 1			Group 2		
Score	**SH**	**Animal**	**Score**	**SH**	**Animal**
13	D	8	16	D	19
10	D	4	14	D	33
8	D	13	14	D	34
6	D	9	10	D	35
5	D	1	8	D	16
4	I	2	6	D	28
0	I	5	4	D	31
0	I	10	4	I	27
−1	I	12	2	I	29
−2	I	14	0	I	25
−4	I	6	−2	I	17
−6	S	7	−3	I	23
−9	S	15	−4	I	30
−12	S	3	−6	I	32
−12	S	11	−10	S	37
	–		−11	S	20
	–		−12	S	26
	–		−12	S	36
	–		−18	S	24

SH: D for dominant, I for intermediate, and S for subordinate animal.

balancing the observations. The ethogram of the behaviors observed during the study is described in **Table 1**.

All agonistic interactions during the 1-h observation period were recorded—displacements, threats, and other behaviors associated with a conflict or fighting between two individuals that involved an instigator and a victim, including, or not, physical contacts, resulting in the physical displacement of an animal (15). Then, a dominance index was calculated according to Kondo and Hurnik (16). An "S" value was calculated for each heifer relative to the others. Therefore, if animal "I" won over animal "J" in Xij interactions, and animal "J" won over animal "I" in Xji interactions, then Sij would correspond to $Sij = Xij\text{-}Xji/|Xij\text{-}Xji|$, always resulting in a value of −1, 0, or +1. Then the dominance index for heifer "I" (Si) would be the sum of S that animal had in each dyad. The dominance value for each individual was calculated as a result of the sum of all relationships of each animal with all other animals within the group. When two or more animals had the same "S" value (for example, cow 17 = cow 36), the tiebreaker was the result of direct confrontation between both animals.

A dominance index was constructed for each group based on the difference between the maximum and minimum dominance value, and then it was divided into three social categories: dominants (D) in the upper stratum, intermediates (I) in the middle, and subordinates (S) in the lower stratum of the index. Social hierarchy of each heifer and its dominance score are shown in **Table 2**.

To estimate the quality of the consumed diet (crude protein/CP and neutral detergent fiber content/NDF), weekly samples of pasture were collected accordingly by hand-plucking. Grazing simulation can be defined as harvesting a forage sample in the areas where the animals were grazing and simulating the morphological composition of the forage consumed by the heifers (17). In each group, six focal animals were selected for pasture collection. The first three animals (FIRST3) starting to graze and the last three animals (LAST3) leaving the feeding area were chosen. Samples were taken along the paddock immediately after grazing started (SAMPLE1) and then 1 h later (SAMPLE2). Each sample was conditioned in a tagged plastic bag, taken to the laboratory and dried in a forced-air buffer for 72 h at 55°C until constant weight. Then, samples were ground to pass a 1-mm screen in a Wiley mill before analysis using near infrared spectroscopy (NIR/MPA, "Multi-Purpose Analyzer," Bruker Optics GmbH, Ettlingen, Germany). The FIRST3 and LAST3 data were also used for the analysis of the correlation between the order to leave the feeding area and start grazing and the social hierarchy of each individual.

Statistical Analysis

Descriptive statistics were calculated using Microsoft® Excel® for Windows, and all other statistical analyses were conducted using SAS 9.3. The percentage frequency of behaviors was summarized over the days per period yielding one value for each animal per period. The Shapiro test was used on the model residual information, as well as the examination of the normal plot to evaluate the dataset for the normal distribution.

The effect of treatment and social rank on the percentage frequency of eating grain supplement, grazing on paddock, and grazing near fence line and along the paddock was analyzed using mixed procedures (Proc Mixed of SAS). The effect of treatment and social rank on the frequency of other behaviors was analyzed using generalized linear mixed models (Proc Glimmix of SAS). Treatment and social rank were included in the model as fixed effect, period as random effect, and gamma as the type of distribution. The effect of breed and interactions between treatment and social rank were removed from all models as they were not significant ($p > 0.05$). Results of eating grain supplement, grazing along the paddock, and grazing near fence line are reported as the least square means ± standard error (S.E.) of the percentage frequency; results of other behaviors are reported as least square means (95% confidence interval).

The relation between treatment order (FIRST3; LAST3), forage sample (SAMPLE1; SAMPLE2), social rank (dominant, intermediate, subordinate), and pasture contents as crude protein, acid detergent fiber, and neutral detergent fiber were analyzed using mixed procedure (Proc Mixed of SAS). Treatment, sample, order, and social rank were included in the model as fixed effects and period as random effect. Interactions were included in the models when they were significant ($p < 0.05$). Results are reported as least square means ± S.E.

The number of agonistic interactions (either instigator or victim) for each animal was summed per period (morning, afternoon). The effect of period and social rank on agonistic interactions was measured through analysis of variance (Proc GLM). Period and social rank were included as fixed effect and animal as the experimental unit. Interactions between period and social rank were tested and excluded from the model, as they were not significant ($p > 0.05$). Data are expressed as least square means ± S.E. of the number of agonistic interactions/animal.

RESULTS

Treatment did not affect the time heifers spent eating grain supplement or the time they spent grazing near the fence (feeding area), but it did affect the total time dedicated to grazing along the paddock ($p < 0.0001$) and other behaviors ($p < 0.0001$), as shown in **Table 3**. Heifers spent more time grazing on the INITIAL treatment and performed other behaviors more frequently in the MIDDLE treatment.

Regardless of treatment, social hierarchy influenced the feeding behavior of the group. Dominant animals spent more time eating grain supplement compared to subordinate animals ($p = 0.0008$), which, in turn, spent more time grazing along the paddock ($p = 0.0067$), but not along the fence ($p = 0.0008$).

The order to leave the feeding area and start grazing was inversely related to social status. Of the first three heifers leaving the supplement site, most (53.3%) were subordinate, whereas of the last three heifers leaving the supplement site, most (48.3%) were dominants.

A significant effect of treatment was observed on the crude protein content (INITIAL: 11.27 ± 1.5 vs. MIDDLE: 8.27 ± 1.47; $p < 0.0001$) of the forage collected as grazing simulation. An interaction was also noted among the order to leave the feeding area, social rank, and crude protein content ($p = 0.04$) (**Table 4**). The NDF content was higher for LAST3 compared to FIRST3 (FIRST3: 70.26 % ± 1.52; $p > 0.05$; LAST3: 73.74 % ± 1.53; $p = 0.0367$). Treatment, sample, order, and social rank were not related to FDA (39.28% ± 0.96; $p > 0.05$).

Treatment and social rank affected the number of agonistic interactions. Heifers performed fewer agonistic interactions on INITIAL (182.1 ± 14.03) compared to MIDDLE (249.2 ± 14.03; $p < 0.01$). Dominant heifers (247.7 ± 16.26) performed more agonistic interactions than subordinate heifers (182.72 ± 19.15; $p < 0.05$). Intermediate heifers (216.6 ± 16.26) performed a number of agonistic interactions similar to that of the other two ($p = 0.2$).

DISCUSSION

Treatment and social status affected grazing along the paddock. Heifers spent more time grazing along the paddock when grain supplement was delivered at the time of paddock entry (INITIAL), and subordinate heifers grazed longer than dominant and intermediate heifers during grain supplement feeding. On the other hand, no difference was noted between eating grain supplement and grazing near the fence line (where supplement was placed) relative to treatment. However, dominant and intermediate heifers ate more grain supplement and grazed longer near the fence line when compared to subordinate heifers, regardless of treatment.

As seen in a number of works, supplemental feeding may affect the total grazing time (18), and the time of supplementation is likely to affect grazing. For example, beef cattle grazed for a longer period when corn supplement was offered in the afternoon (19). Steers receiving supplement had the highest forage dry matter intake when supplement was offered at noon compared to 7 am and 4 pm (2). On the other hand, Sheahan et al. (20) concluded that supplementing cows in the morning or in the afternoon does not affect the time spent in grazing or dry matter intake.

All these studies were conducted in extensive grazing systems, considered whole herd behavior, and were focused on the total grazing time. Our study was focused on the effect of social status on grain supplement access and grazing time during supplement feeding. Moreover, heifers were in a rotational grazing system, entering into a new paddock every morning with fresh pasture available. The major grazing events occur in the early morning and late afternoon/early evening; the later grazing event is the longest and most significant in terms of herbage intake (21); according to this author, the dusk grazing event seems to be an adaptive feeding strategy to maximize daily energy acquisition, providing a steady release of nutrients throughout the night. Grazing behavior and intake are a multifactorial phenomenon and interact strongly with the morphological characteristics of grazed plants and the environment such as climate, the feed supply–demand balance, pasture composition, and grazing method, and the challenge is to present feed to grazing animals in ways that allow them to meet their dietary preferences, while also allowing high rates of animal production per hectare (22). Grazing time is affected by the grazing system, with lower grazing times on rotational systems compared to continuous systems, which may be attributed to the ability of cows to anticipate the timing of the daily movement of the electric fence and, correspondingly, reduce the time spent grazing residual herbage (23).

In our study, dominant heifers spent more time eating grain supplement and grazing along the fence line than subordinate heifers, which, in turn, spent more time grazing along the paddock. Dominant animals are known to have priority of access

TABLE 3 | Effect of treatment (INITIAL; MIDDLE) and social rank (dominant, intermediate, or subordinate) on behavior: eating grain supplement, grazing, and other behaviors [normal data: least square mean ± standard error; non-normal data: least square mean (95% confidence interval)].

Behavior (%)	**Treatment**			**Social rank**			
	Initial	**Middle**	***p*-value**	**Dominant**	**Intermediate**	**Subordinate**	***p*-value**
Eating grain supplement	28.6 ± 0.96	30.8 ± 0.96	0.1113	32.9 ± 1.12^{a}	30.2 ± 1.12^{a}	26.0 ± 1.32^{b}	0.0008
Grazing on paddock	47. ± 7.15^{a}	26.1 ± 7.15^{b}	<0.0001	30.8 ± 7.27^{a}	33.6 ± 7.27^{a}	45.2 ± 7.45^{b}	0.0020
Grazing near fence line	16.1 ± 1.94	19.0 ± 1.94	0.2867	23.8 ± 2.26^{a}	18.9 ± 2.26^{a}	9.9 ± 2.66^{b}	0.0008
Other	2.1 (1.243–2.984)^{a}	2.9 (2.071–3.812)^{b}	<0.0001	2.4 (1.476–3.230)	2.6 (1.704–3.457)	2.6 (1.764–3.535)	0.1319

Means with different letters in a row indicate significant differences ($p < 0.05$).

TABLE 4 | Crude protein (%CP) content of hand-plucked pasture samples, according to the order of leaving the feeding area to start grazing (FIRST3; LAST3) and the social rank (dominant, intermediate, subordinate).

Content/order	**FIRST3**			**LAST3**		
	Dominant	**Intermediate**	**Subordinate**	**Dominant**	**Intermediate**	**Subordinate**
Crude protein (%CP)	12.3 ± 1.88^{a}	8.5 ± 1.55^{b}	10.2 ± 1.5^{a}	9.2 ± 1.65	9.3 ± 1.54	8.9 ± 1.78

Means with different letters in a row indicate significant differences ($p < 0.05$).

to feed resources (24), and for grazing ruminants, this is related to the priority of access to high-quality grazing areas (9). When the dominant heifers entered the new paddock, they went directly to the feeding area along the fence line and stayed there for a long time, even after all the grain had been consumed. This could represent a strategy to prevent subordinate heifers from eating grain supplement, as they were used to the experimental routine; that is, the grain was offered only once a day.

While the dominant heifers were eating grain supplement, the subordinate animals were grazing. Two key factors that influence the foraging behavior of group-living herbivores are feed availability and individual dominance status; therefore, they weigh the costs and benefits of both when making patch-joining decisions (25). Dominant sheep in heterogeneous flocks use the most preferred areas more intensively, and low-ranked sheep use less preferred areas. However, when high-ranking individuals were removed from the flock, low-ranking sheep shifted their selection patterns by increasing the use of the most preferred areas and strongly avoided using the less preferred sites (26). Manson and Appleby (27) found that cows of similar rank fed together compared with cows of dissimilar rank and that the greatest nearest-neighbor distance was found between animals of low and high rank.

The desire to ingest feed or to avoid disputes with other animals is variable and influences the animal's decision-making. High levels of competition and displacement in the feeder indicate that the access to feed is a priority for cattle (28). Nevertheless, this priority is dependent on its motivation to obtain it (29). Motivation is defined operationally as the tendency for an animal to perform a behavior, but understood as reflecting the animal's desire to do so (30), allowing us to estimate the value that an animal gives to a certain resource after weighing costs and benefits to obtain it. The value an animal gives to a resource is dependent not only on the quality of the resource, but on the need of the animal, as well. In a water restriction situation, subordinate non-lactating cows would drink water every other day, while subordinate lactating cows would fight to drink daily (31).

In this study, when grain supplement was offered at the time of paddock entry (INITIAL), with fresh pasture available, subordinate heifers were motivated to ingest feed, and they could choose to graze along the paddock instead of competing for grain supplement with dominant heifers. However, in the MIDDLE treatment, there were a higher number of agonistic interactions, compared to INITIAL treatment, probably due to the fact that heifers no longer had high-quality pasture available, but still motivated to obtain feed. Therefore, offering the supplement at the time of entering the paddock would reduce fights, giving subordinate heifers an opportunity to graze high-quality pasture, improving their welfare.

Since the subordinate heifers were the first to leave the grain supplement location to graze, they could ingest forage with the same crude protein content as the dominant heifers, while the intermediate heifers were left with pasture of inferior quality. In pasture-based systems, the amount of pasture consumed and its nutritive value may influence the between-cow variability in response to supplement and need to be considered as part of a dynamic model for calculating optimum supplementation rates (32).

In dairy cattle, the first animals moving to an allocation of fresh pasture after a milking session are offered feed of greater nutritive value compared with those arriving last, which is closely related to social hierarchy, as they show a consistent milking order (33). Highly dominant animals may obtain priority in resource access in intensive production conditions (34). Such information can be relatively easy for farmers to collect. Thus, the feeding order can be used as an on-site simple attribute of social dominance in intensive beef cattle production systems (24). Housing and management strategies may be implemented to optimize access to feed and feeding patterns, thus promoting good health, productivity, and welfare (35). Under pastoral systems, synergies between animals' and farmers' grazing decisions have the potential to offer greater benefits to our livestock, our landscape, and ourselves (36).

CONCLUSIONS

Subordinate heifers consistently grazed more and ate less grain supplement than dominant and intermediate heifers. However, when grain supplement was offered at the time animals entered the paddock, more grazing activity took place during supplement feeding, and subordinate heifers could select a high-protein diet. In the INITIAL treatment, this means that subordinate animals could benefit from the better pasture available, keeping a distance from dominant heifers, reducing agonistic interactions, and likely improving their welfare.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics Committee on Animal Use of the Federal University of Santa Catarina (CEUA/UFSC), under the approved protocol number 1004100516.

AUTHOR CONTRIBUTIONS

GB and LP contributed to the conception and planning of the experiment. GB, LP, and MH designed the experiment. GB and KS made the data collection and field work. DT did the statistical analysis. GB wrote the manuscript. LP and MH were advisors and revised and helped in writing. All authors have read and approved the last version of the manuscript.

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REVIEW
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How Dietary Diversity Enhances Hedonic and Eudaimonic Well-Being in Grazing Ruminants

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Ruminants evolved in diverse landscapes of which they utilized, by choice, a diverse arrangement of plants (grasses, forbs, and trees) for food. These plants provide them with both primary (carbohydrates, protein, etc.) and secondary (phenolics, terpenes, etc.) compounds (PPC and PSC, respectively). As no one plant could possibly constitute a "balanced-diet," ruminants mix diets so that they can exploit arrangements of PPC to meet their individual requirements. Diet mixing also allows for ruminants to ingest PSC at levels, acquiring their benefits such as antioxidants and reduced gastrointestinal parasites, without overstepping thresholds of toxicity. Meeting dietary requirements is assumed to provide satisfaction through achieving positive internal status and comfort, thereby a sense of hedonic (happiness through pleasure) well-being. Furthermore, choice including dietary choice is a factor influencing well-being of ruminants in a manner akin to that in humans. Choice may facilitate eudaimonic (happiness through pursuit of purpose) well-being in livestock. Nutritional status plays an integral role in oxidative stress, which is linked with illness. Several diseases in livestock have been directly linked to oxidative stress. Mastitis, metritis, hypocalcaemia, and retained placenta occur in animals transitioning from dry to lactating and have been linked to oxidative stress and such a stress has likewise been linked to diseases that occur in growing livestock as well, such as bovine respiratory disease. The link between physiological stress and oxidative stress is not well-defined in livestock but is evident in humans. As dietary diversity allows animals to select more adequately balanced diets (improved nutrition), take advantage of PSC (natural antioxidants), and allows for choice (improved animal well-being) there is a strong possibility for ruminants to improve their oxidative status and thus health, well-being, and therefor production. The purposes of this review are to first, provide an introduction to oxidative and physiological stress, and nutritional status as effected by dietary diversity, with special attention to providing support and on answering the "how." Second, to provide evidence of how these stresses are connected and influence each other, and finally discuss how dietary diversity provides a beneficial link to all three and enhances both eudaimonic and hedonic well-being.

Keywords: grazing, ruminants, animal welfare, taxonomical diversity, biochemical diversity

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INTRODUCTION

Dietary diversity in ruminants has recently received considerable attention in the literature (1–8). Much of this work has focused on how dietary diversity can improve animal production by providing animals with the opportunity to choose and mix their diets. By doing so, the animals are better able to meet their individual requirements and self-medicate, acquiring nutraceuticals, pharmaceuticals, and prophylactic benefits associated with the ingestion of specific secondary compounds (PSC) at self-regulated safe levels of intake (9). Here we use the term well-being when discussing the subjective mental state of the animal and welfare as the animal state including well-being, health and the animals experience with their environment. In a comprehensive review article on dietary diversity and welfare, Manteca et al. (4) concluded that the improved nutritional status given by appropriate supply of plant primary compounds (PPC) and the improved health benefits by the PSC are indicative of the intimate relationship between dietary diversity and animal welfare. However, the benefits of dietary diversity on animal welfare have been discussed only as they relate to hedonic well-being. The word hedonic stems from the Greek word *hēdone*, meaning pleasure, and thus hedonic well-being is the balance between positive and negative emotions (10). Emotions are clusters of experiences related to health, fear, nutritional comfort, nutrient supply, and familiarity, as a few examples. Animals integrate those experiences, at different time scales forming either positive or negative emotions (11). Another concept of well-being, commonly applied to humans, is eudaimonia, which was first proposed by Aristotle. Eudaimonia stems from the Greek words *Eu*, for good, and *daimon*, for guardian. There are several definitions proposed for eudaimonia, but the one which we propose can best be applied to ruminants is one of function. We propose that eudaimonic well-being is achieved in livestock and other animals when they are able to pursue their potential (10). To that end, eudaimonic well-being is achieved when a subject achieves its *telos*, which is defined as a given purpose (12). Eudaimonic well-being has rarely been applied to livestock welfare but (12) proposed that an animal's *telos* is enshrined in the species' uniqueness which is genetically coded [see also (13)]. We propose that *telos* may also be considered as an individual trait and this is supported by individual animal personalities, by genetically related grazing personalities in ruminants (8, 14), and by the reduction of stress when choice is allowed (4, 15). Improved well-being by offering choice to animals both facilitates and provides evidence in support for eudaimonia and *telos* in livestock, as it has been suggested that without choice one cannot pursue their *telos* and thus achieve eudaimonic well-being (12). Even if the available options (e.g., dietary options) provided will only allow the animals to choose the least-worse option available for their individual needs, we argue eudaimonic well-being will be improved.

We hypothesize that merely providing choice would improve eudaimonic well-being in livestock; however, for dietary diversity to improve hedonic well-being there must first be some subsequent actions to increase pleasure or reduce negative experiences and thereby emotions. Such actions constitute responses to environmental stimuli that provoke oxidative stress, physiological stress, or reduced nutritional state of the animal. These three features of animal state are of interest with regard to welfare and hence production. Oxidative stress influences the pathophysiology of diseases, and its management has received much attention (16–19). Physiological stress including cortisol release is often used as an index of welfare (20), which in turn is linked to production and economic return (21). Appropriate nutrition for each respective class of livestock is obviously a major feature of every livestock production system.

In this review we describe and explain how the influence of oxidative stress, physiological stress, and nutritional state influence well-being of grazing livestock as a response to taxonomic and biochemical diversity of the diet. We present a conceptual model (**Figure 1**) describing the interactive links between dietary diversity and animal state, resulting in positive effects on animal health and well-being (both hedonic and eudaimonic).

OXIDATIVE STRESS

Oxidative stress is a state of imbalance between oxidants (e.g., reactive oxygen metabolites) and antioxidants [both enzymatic [e.g., superoxide dismutase] and non-enzymatic [e.g., vitamin E and glutathione]; (22, 23)]. The circulating level of oxidants is subject to homeostatic regulation but situations may occur in which the animal is exposed to stressors, such as high metabolic demand, gastrointestinal parasites, heat stress, and diseases (19), which cause the rate of production of oxidants to exceed the capacity of the homeostatic regulatory system. The remaining oxidants damage important biological molecules [including lipids, proteins, DNA, and RNA; (19)], which then lead to metabolic and pathological disorder (24).

An example of this is isoprostane production, which has similar actions as prostaglandins (e.g., prostaglandin F-2α). Prostaglandins are involved in the regulation of many physiological functions (e.g., pregnancy maintenance) and also in inflammation and immune responses (25). The key enzymes involved in the conversion of arachidonic acid to eicosanoids (e.g., prostaglandins) is cyclooxygenases (25). In cattle, prostaglandin $F_{2\alpha}$ are an important part of the estrus cycle as they cause luteolysis [degradation of the corpus luteum; (26)]. Prostaglandins are also important in the pathological manifestation of chemical or physical injury, in fact nonsteroidal anti-inflammatory drugs function by inhibiting prostaglandin synthesis, by blocking the cyclooxygenases (27). Similar compounds to prostaglandins, the isoprostanes, are generated independently of cyclooxygenase enzymes through the peroxidation of arachidonic acid by oxidants (28). Isoprostanes have been identified as a promising *in vivo* marker for oxidative stress and they have been found to have negative biological effects as they can bind to many of the same receptors as cyclooxygenase derived prostaglandins (28). These effects include vasoconstriction and airway constriction, and therefore may be pathophysiological mediators of oxidative damage (28).

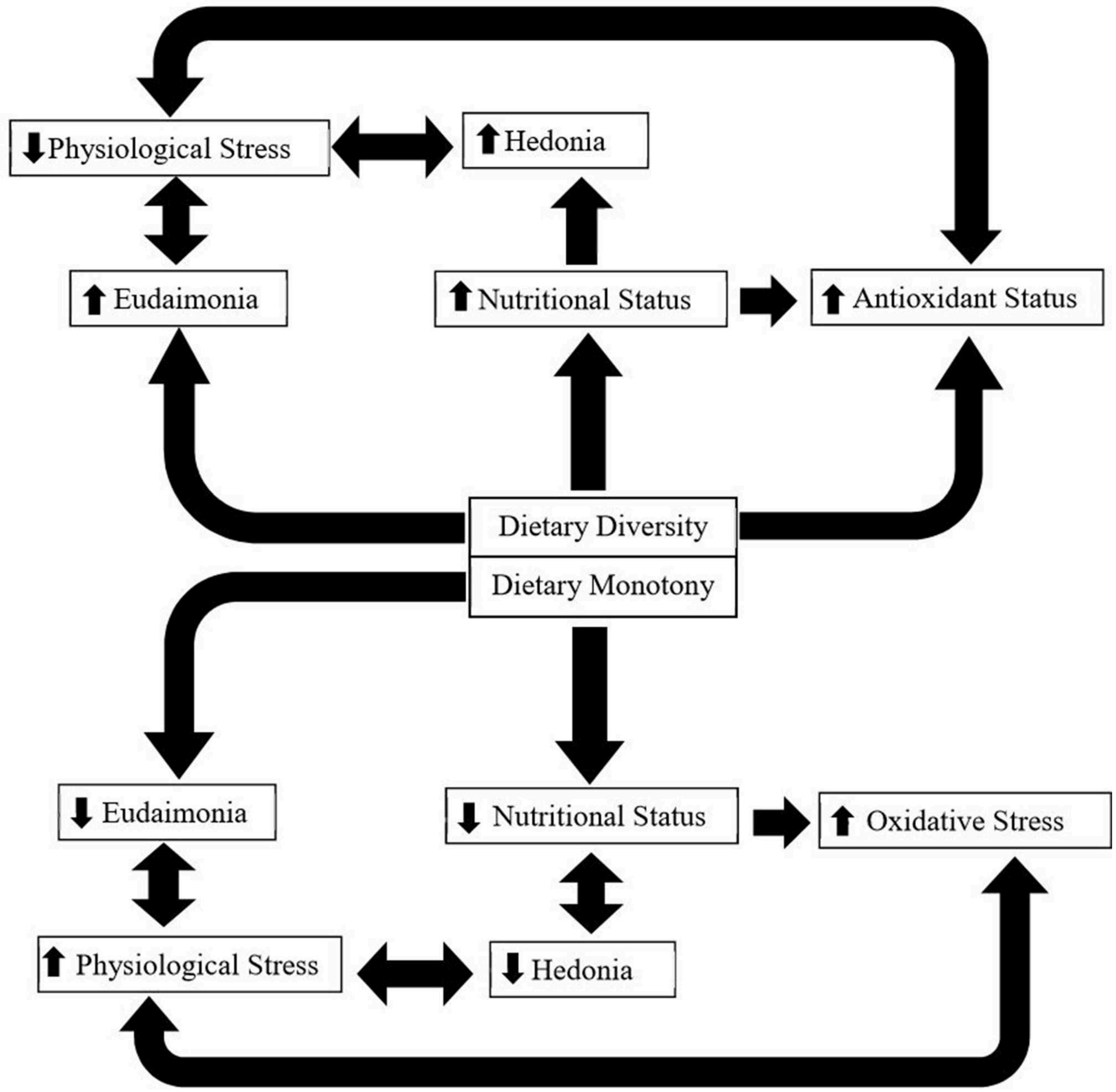

FIGURE 1 | This figure shows a conceptual model depicting how dietary diversity vs. monotony may affect ruminants. Dietary diversity improves nutritional status, through ingesting complimentary plant compounds (primary and secondary); improves hedonic and eudaimonic well-being; decreases physiological stress; and improves antioxidant status, through the ingestion of plant secondary compounds that exhibit antioxidant effects. It is important to note that both nutritional status and physiological stress can also impact antioxidant status directly. On the other hand, dietary monotony may reduce nutritional status (by not allowing animals to mix a diet containing a balance of plant primary compounds) and hedonic (reduced nutritional status) and eudaimonic (loss of choice) well-being.

Thus, isoprostanes are formed through oxidants oxidizing a biological molecule (arachidonic acid) and subsequently inducing inflammatory responses to oxidative damage. We postulate that the damage to biological compounds leading to metabolic or pathological disorders and inflammation, such as arachidonic acid, would result in discomfort and subsequently reduce well-being. This is supported by some works who found a positive correlation between blood cortisol and isoprostane concentrations (29, 30). The integration of uncomfortable experiences leads to negative emotions, thus reducing hedonic well-being. The link between oxidative and physiological stress is discussed further below.

Physiological Causes of Oxidative Stress

The following paragraphs provides a summary of the biochemical sources of oxidants, which is the "how" behind oxidative stress. This detail is important because it provides background information for understanding how biochemical (PSC) diverse diets reduce oxidative stress in grazing ruminants. We later describe how the improved antioxidant status of the animals would lead to enhanced hedonic well-being.

Oxidants are important in several physiological and biochemical reactions; consequently, they are well managed by the body. For example, Superoxide ($O_2^{\bullet -}$) is an oxidant produced in the mitochondria of mammalian cells, which is subsequently converted to H_2O_2 by mitochondrial superoxide dismutase (31, 32). This $O_2^{\bullet -}$ is generated in the electron transport chain, with the majority being produced by complex I, and a negligible amount by complex III (32). These $O_2^{\bullet -}$ are converted to H_2O_2, which exits the mitochondria to act as a redox signal to the cellular cytosol and the nucleus. Hydrogen peroxide, while still an oxidant, has a lower second order rate constant for reactions with biomolecules than other oxidants (e.g., hydroxyl radical or $O_2^{\bullet -}$) and is therefore appropriate for redox signaling (33). These redox reactions are important in regulation of enzymes and transcription factors (33, 34). This elicits various cellular responses such as enzyme activity, substrate supply, and mitochondrial biogenesis (32, 35). Redox signaling shows how oxidant production, when under normal physiological functions, is necessary rather than negative.

Another physiological source of oxidants come from phagocytic cells removing foreign organisms. Reactive oxygen species are toxic to many microorganisms. When phagocytic cells (e.g., neutrophils) engulf bacteria there is an initial oxygen consumption (called oxidative burst) where an NADPH oxidase complex transfers electrons from NADPH to oxygen in order to generate superoxide. As some works have reported that superoxide does not kill bacteria, it is believed that additional secondary oxidants are generated and responsible for bacterial death (36). One important example of phagocytic cells to ruminants is neutrophils involvement in removing pathogens related to pneumonia such as *Mannheimia haemolytica*. Removal of these pathogens is reliant on active immunity and the innate immune function, such as neutrophils (37). Therefore, oxidant creation in this context is required by the body to remove foreign organisms and to maintain health and comfort through the relief from pain, and thus hedonic well-being.

The final source of oxidants to be discussed in this review occurs in the gastrointestinal tract. Halliwell et al. (38) proposed several sources of gastrointestinal derived oxidants. Firstly, foods will generally contain iron, often in the insoluble Fe^{3+} salt form. Gastric acid can solubilize ferric and metallic iron. The Fe^{3+} is then reduced to Fe^{2+}, which is easier to absorb with stimulation by ascorbate. The oxidant, hydroxyl radical can be produced when ascorbate and Fe^{2+} are mixed, without H_2O_2 (Fenton Chemistry). Similar reactions can occur from Cu^{2+} and ascorbate (38). Other sources include: haem (from haem proteins), dietary lipids that undergo peroxidation, foods containing isoprostanes, oxidized cholesterol, nitrites, the gastrointestinal immune system, and oxidized phenolic compounds such as hydroxyhydroquinone [from coffee; (38)]. This may indicate that oxidized phenolic compounds from forage plants may act as oxidants. While information from the human literature is abundant, to our knowledge, few works have reported how important ruminant gastrointestinal derived oxidants are. One experiment measured antioxidant activity of rumen fluid and plasma from faunated or defaunated rumens [with or without protozoa; (39)]. It was found that faunated rumens had greater antioxidant activity than defaunated rumens in both ruminal fluid and in the plasma (39). Increased antioxidant capacity in the rumen leading to increased antioxidant capacity in the plasma would indicate that rumen fluid is important to the whole animal's antioxidant status. However, we are unaware of any research showing how this would translate to the lower gastrointestinal tract, but due to research on humans (see (38) for a review), we speculate that this is a significant source of oxidant production which requires further investigation.

Defense Mechanisms Against Oxidative Stress

Enzymatic Defense Against Oxidative Stress

Antioxidant enzymes are a major intrinsic, or endogenous, oxidant defense. Superoxide dismutase (SOD) is found in the cytoplasm and the mitochondria of cells in the Cu-SOD and Mn-SOD forms, respectively (40). This enzyme converts the superoxide anion (which is highly radical) to hydrogen peroxide (H_2O_2; which is less radical). Glutathione peroxidase (GPx) is then responsible for converting H_2O_2 to water and oxidized glutathione. Catalase is another important antioxidant enzyme which converts H_2O_2 to water and O_2. Glutathione reductase then "recycles" the oxidized glutathione by reducing it to its active form, reduced glutathione. This reaction occurs by oxidation of NADPH to $NADP^+$ by GR (41).

As these enzymes are important in maintaining homeostasis of ruminants, their quantification in biological samples have been identified as a marker of oxidative stress (42). However, their interpretation is not always straight forward. On one hand, when supplemented with selenium, GPx levels in ruminant erythrocytes increase, which is expected as GPx is a selenium dependent enzyme (43). These experiments interpret this result as an improvement in antioxidant status. On the other hand, there are greater levels of antioxidant enzymes in erythrocytes of dairy cows in the summer than in spring, which is due to increased heat stress (44). These increases are due to increased oxidative stress, as it is known that heat stress causes oxidative stress (44, 45). Due to these inconsistencies, we recommend implementing multiple markers of antioxidant status in order to assist with interpretation.

Intrinsic Non-enzymatic Defense Against Oxidative Stress

Non-enzyme antioxidants such as glutathione, uric acid, melatonin, bilirubin, polyamines, and metal binding proteins are also a part of the intrinsic oxidative stress defense system (41). While important, this review will not delve into detail on them. Mironczuk-Chodakowska et al. (41) provides a detailed review on non-enzymatic, intrinsic antioxidants. One example of non-enzymatic antioxidants is albumin, which is important to grazing ruminant health. Albumin is the major antioxidant in circulating blood, which is continuously exposed to oxidative stress (46).

In ruminants, albumin has been found to be incorporated into colostrum and milk (47). Thus, albumin provides antioxidant defense in several biological fluids, such as blood, colostrum, and milk. In the following section we will go in depth on how a diverse diet providing biochemical diversity in plant secondary compounds can provide extrinsic antioxidant defense.

DIETARY DIVERSITY AND ANTIOXIDANT DEFENSE

Extrinsic (exogenous or dietary antioxidants) defenses against oxidative stress come from food. These antioxidants include vitamins E and C and PSC, such as phenolics, terpenes and terpenoids. When offered an array of forages animals select and consume natural antioxidants at rates below toxic levels of intake (48, 49). Plant secondary compounds, especially phenolic compounds, have been shown to improve antioxidant status and reduce plasma levels of oxidative components. Phenolic and polyphenolic compounds (tannins and flavonoids, from terrestrial plants; phlorotannins, from aquatic plants [seaweeds]) can have free radical scavenging properties. Phenolic compound containing-extracts from the common daisy (*Bellis perennis L.*) showed free-radical scavenging activity of 2,2-Diphenyl-1-picrylhydrazyl *in vitro* (50). This ability was likewise demonstrated with isolated flavonoids from *Opuntia monacantha* (51). Additionally, Chakraborty et al. (52) extracted phlorotannins from three species of red seaweed (Division: *Rhodophyta*) and saw marked reductions in free radicals. This antioxidant activity can have remarkable effects on antioxidant status when applied to plants and animals (53, 54). Kannan et al. (55) reported increased antioxidant enzymes and reduced lipid peroxidation when sheep were treated with a seaweed extract and challenged with transportation stress. Milking goats provided tannins from sulla (*Sulla coronarium L.*) forage, had improved plasma antioxidant capacity (56). Sheep provided plant by-products (tomato pomace and grape skin) had upregulated transcriptional activity to genes that are involved in oxidant defense enzymes (57). When transition dairy cows were provided tannins from chestnut there were lower plasma and milk malondialdehyde (MDA; a marker of lipid peroxidation) and increased antioxidant enzyme activities in plasma and the liver (58). These experiments, and others, highlight the potential of PSC to improve antioxidant status, which would result in a better internal state and improve hedonic well-being of grazing ruminants.

Plant Secondary Compounds as Antioxidants: Potential Modes of Action

Several modes of action exist for PSC, especially phenolic compounds, to exhibit antioxidant activity. One mode would be by providing antioxidant activity directly in bodily fluids and tissues. In order for this to occur, antioxidant PSC would need to be absorbed and incorporated into tissues (59). Evidence for this can be seen by the increased product quality, such as improved shelf life, color stability, flavor, and odor, from animals provided PSC seen by several experiments (59). One experiment provided sheep plant extracts, including rosemary (*Rosemarinus officinalis*), grape (*Vinis vitifera*), citrus (*Citrus paradise*), and marigold [*Calendula officinalis*; (60)]. It was found polyphenolic compounds, including condensed tannins from grapes, are catabolized to monomeric phenolics, become bioavailable, and were present in the blood of the sheep. It was also reported that naringin from the citrus extract was found in the plasma, which is contrary to what occurs in monogastrics (60). Another work reported that the ultraviolet-absorbing compounds in milk result from ingested phenolic compounds from forages [various hays, silages, and fresh pasture; (61)]. Additionally, when supplied tannins from sulla, goat milk was found to have greater phenolic compounds and total antioxidant capacity (56). These experiments indicate that antioxidant PSC such as phenolics can be absorbed from ruminant gastrointestinal tracts and incorporated into milk products thus improving product quality, but also potentially exerting nutraceutical, pharmaceutical, and prophylactic activity.

Plant secondary compounds have also been measured in meat products. When ewes were dosed rosemary (*Rosmarinus officinalis*) extract, their offspring were found to have increased phenolic compounds incorporated in their meat at slaughter (62). This incorporation in the tissue increased the antioxidant capacity of the meat (62). In a similar experiment, Nieto et al. (63) gave pregnant ewes either 0, 10, or 20% of their diet with distilled rosemary leaf and observed delayed lipid oxidation, odor, and flavor spoilage of their lamb's meat due to the additions. In another experiment, rosemary leaf distillate additions to pregnant ewes improved lamb meat quality characteristics (64). These results were corroborated when ewes were provided varying rates of thyme (*Thymus zygis ssp.*) leaves in their diet. Again, the antioxidant additions to their dam improved product quality and shelf life of the lamb meat (65). When lambs received a diet containing quebracho (*Schinopsis lorentzii*) tannins there was a 31.29 and 16.81% increase in total phenols and antioxidant capacity in the muscle compared to the control, respectively. The increased antioxidant status improved meat color stability (54). Similarly, when growing chickens were provided a by-product of the olive oil industry (semi-solid olive cake; "pate"), meat oxidative stability was improved and tyrosol and metabolites of hydroxytyrosol (phenolic compounds) were detected (66). These results support the mode of action for a direct antioxidant activity at the bodily fluids and tissue level by absorbed PSC and this interpretation has also been suggested by Vasta and Luciano (59).

Another potential mode of action for PSC is by providing antioxidant action in the gastrointestinal tract. As discussed above, the gastrointestinal tract is a major source of oxidants. This effect in livestock, to our knowledge, is largely unexplored. However, when sheep had long-term exposure to dietary heavy metals, it was found that there was oxidative damage to the gastrointestinal tract and concluded that lipid peroxidation was one of the mechanisms behind chronic heavy metal poisoning in ruminants (67). In humans, PSC, such as phenolics, have been found to alleviate or prevent gastrointestinal diseases such as ulcers (68). Evidence of antioxidant benefits of PSC in ruminant's gastrointestinal tract is lacking and requires further research; however, as stated above we speculate that the ruminant gastrointestinal tract is a major source of oxidants and postulate that antioxidant PSC would alleviate this production.

Finally, PSC have been found to regulate gene expression to alter antioxidant status. The nuclear factor erythroid 2-related factor 2 (Nrf2) has been identified as the leading transcription factor behind oxidative stress defense (69). Nuclear factor erythroid 2-related factor 2 reduces oxidative stress directly by increasing antioxidant enzyme activity, regenerating oxidized cofactors (e.g., GSSG to GSH), synthesizing these reducing factors (e.g., GSH), and by increasing expression of antioxidant proteins (69). Plant secondary compounds have been shown to activate Nrf2, resulting in increased antioxidant enzymes in farm animals (70). An *in vitro* experiment on bovine mammary epithelial cells showed potential of tea polyphenolics to reduce oxidative stress when challenged by hydrogen peroxide, and that these results were due to upregulation of Nrf2 (71).

Oxidative stress is an important aspect of ruminant management. Reactive oxygen metabolites are both necessary for normal physiological functioning (e.g., redox signaling) but also, when produced at levels that outpace the animal's defense system, can cause negative effects after they damage various biological molecules. The defense system in place for ruminants to handle oxidants are intrinsic antioxidant enzymes and non-enzyme antioxidants, but also extrinsic dietary antioxidants. Plant secondary compounds, which can be commonly found in many forages used in grazing ruminant production systems, provide an interesting opportunity to manage oxidative stress in grazing ruminants as they have several modes of actions. They can remove oxidants in the gastrointestinal tract, after absorption in the small intestine, by being incorporated into milk and tissues, and by regulating gene expression.

Physiological Stress and Hedonic and Eudaimonic Well-Being

Physiological stress is the hormonal response that an organism experiences in response to a stressor, whether abiotic or biotic. Physiological stress manifests itself in the "fight or flight" response in organisms (20). This response is elicited by the release of glucocorticoids (GC). Glucocorticoids, such as cortisol, have been studied as a marker of animal welfare with less cortisol acting as a marker for positive welfare (20). Abiotic stressors include climatic events (e.g., heat stress). Biotic stressors are elicited from the animal's peers, predators and other animal species, animal handling (72), and, more recently suggested, dietary monotony (4, 5, 8, 73). Dantzer and Mormède (20) reviewed the causes of physiological stress and physiological pathway, from stress perception to hormonal responses. In brief, following the experience of a stressor, glucocorticoids (GC) are released following the hormonal cascade from the hypothalamic-pituitary axis (20). In essence GC prepare the animals for the "fight or flight" through several metabolic responses. These include increased gluconeogenesis, reduced glucose uptake by the periphery, suppress insulin, and mobilize energy stores. Additionally, GC can alter behavior and elicit anxiety behavior [e.g., stereotypies; (20)].

Historically, objective assessment of animal welfare has been done by measuring GC in the blood (20). As animal handling to take the blood sample causes a stress response, it has been suggested that fecal cortisol metabolites (74) or hair (75) cortisol levels are more accurate. While cortisol is the most predominant biomarker of welfare, there are several other markers available [see (76) for a recent review]. However, most methods of measuring welfare would only provide insight on the negative state of the animal and it is often assumed that less cortisol provides insight into positive welfare, which may not always be the case (77). This necessitates research into objective markers of positive welfare. Some markers of positive welfare that have been suggested are vocalizations, measurements of neurotransmitters such as endorphins and dopamine, and hormones like oxytocin and serotonin (77, 78). While physiological stress and negative welfare may often be negatively correlated with positive welfare, it is time for the development of standardized methodologies for measuring positive states of animals.

Ethical management of animals has been predominately based upon the "five freedoms." These include the freedom from (1) thirst, hunger, and malnutrition, (2) discomfort and exposure, (3) pain, injury, and disease, (4) fear and distress, and (5) freedom to express normal behavior (79, 80). All of these freedoms relate to hedonic well-being, with the exception of freedom 5. Hedonic well-being is based upon pleasure and comfort seeking (10). More recently, there has been a call in the literature and from the public for animals to have "A Life Worth Living" (81) or "the Good Life" (12). As such, animal welfare concerns are moving away from merely ensuring that animals are provided with the opportunity to perform (by ensuring that they have adequate nutrition, freedom from fear, sickness, and discomfort), to ensuring that they have a life worth living (at least in terms of anthropomorphic understanding of "worth"). This appeals to the eudaimonic theory of well-being. For further readings find (81) and then the invited response (79).

Under eudaimonic theory, well-being is a process and not a state. It stems from the pursuit of a good life through individual choices (10). Much of what is known about eudaimonic well-being comes from philosophy, but recently scientific evidence has been gathered to support this theory using human subjects (82). In grazing animals, more research is required to investigate eudaimonic well-being and we believe that experiments centered around providing choices are particularly needed. Evidence for support of Eudaimonia in livestock has been shown in zoo animals. Giant pandas had lower urinary cortisol when they were provided a choice between two environment enclosures compared to pandas who were only allowed access to the exhibit environment. As the added enclosure area was less enriched, the choice group spent most of their time in the exhibit area, and there were no differences in active time it was concluded that the enhanced animal welfare was derived from the ability of the animals to choose (15). In foraging ruminants, some support for *telos* and eudaimonic well-being may be seen in grazing personalities (8). One example of grazing personalities was described by Bailey et al. (14), who found that there are cattle who prefer to graze in the flat low fields, termed bottom dwellers, and cattle who prefer to climb mountainous areas for grazing, termed hill climbers. It was found that these specific grazing personalities were related to genetic markers (14). This is interesting as *telos* has been described as intrinsic in the genetic coding of animals (12). Thus, we hypothesize that individual animal's personalities, including grazing personalities, provide insight to individual animal's *telos* and thus provide evidence of eudaimonic well-being. Additionally, we speculate that this theory will apply to livestock and that enhanced welfare from dietary diversity both facilitates and is evidence to support this theory, which is discussed further below, even though separating welfare enhancement of dietary diversity between hedonic and eudaimonic well-being is difficult.

LINKING DIETARY DIVERSITY AND PHYSIOLOGICAL STRESS

Choice is a key concept in the eudaimonic theory of human well-being, with the overarching concept being to pursue a life of fulfillment of one's true nature, or *telos*, with choices being important in this pursuit (10). Eudaimonia often stands in contrast to the hedonic theory of well-being, which considers contentedness as the sum of positive and negative affective states, i.e., emotions (10) and which has been a primary focus of studies of animal welfare (13). However, recently several works have explored the effect of choice on livestock welfare. Catanese et al. (83) gave lambs either a choice of different foods contrasting in protein: energy ratios (diversity) or all of those foods provided in a total mixed ration (monotonous). It was found that when animals were allowed to choose, they had lower cortisol, than their counterparts (83). Villalba et al. (73) has also shown similar results in lambs. When lambs were offered a four-way choice between foods which were diverse in nutrient composition or in PSC, there was lower plasma cortisol concentrations compared to lambs who received a monotonous diet of all food options. Manteca et al. (4) and Villalba et al. (5) reviewed dietary choice as an important aspect of animal welfare and related it to animals being able to balance their own nutrients to meet individual requirements through nutritional wisdom, and also to balance intake of PSC so that they can experience their benefits (e.g., reduced gastrointestinal parasites) without experiencing toxicities, which all relate to hedonic well-being. Additionally, hedonic well-being is partly responsible for controlling feeding behavior in ruminants (84). While these are likely true, dietary diversity may also reduce stress merely by providing the animals a choice, if the Eudaimonic theory of well-being can be applied to livestock. Additionally, we postulate that dietary diversity likely enhances both hedonic and eudaimonic well-being (**Figure 1**), as it has been found that these two mental well-being states contribute to welfare in different and overlapping ways (85).

As mentioned previously, dietary choice allows animals to consume PSC at amounts that provide benefits, while staying below the threshold at which negative effects occur. In one experiment, sheep faced either no challenge (received saline injection), an adrenocorticotropic hormone (ACTH) challenge only, or an ACTH challenge plus one of four PSC (containing polyphenols) products (86). Sgorlon et al. (86) examined global mRNA expressions in sheep blood in response to the blood cortisol levels, which resulted from the ACTH challenge. As expected, ACTH treatment caused increased cortisol production after 3 and 51 h. While the sheep that received plant secondary compounds did not experience reductions in cortisol production, it was determined that the PSC altered the molecular signature produced as a result to increased cortisol. Overall it was determined that while ACTH challenge reduced gene expression involved in immune response, when provided PSC products, this effect was attenuated, but the results were dependent upon the product used (86). Thus, PSC may improve the response that animals have to physiological stressful events.

There is a known relationship between diet and the composition of ruminal microorganisms. For example, Tapio et al. (87) in a 4 × 4 factorial, fed dairy cows two levels of forage-to-concentrate (high, 35:65; low, 65:35) with either 0 or 50 g sunflower oil/kg diet dry matter. It was determined that there were taxa abundance changes and microbial interactions that were diet specific. Similar results have been seen in cows fed alfalfa or triticale forages (88). The composition of ruminal microorganisms can likewise influence the ruminal degradability of feeds, but also fermentation end products as specific microorganisms produce different fermentation end products, as they often fill dietary niches (89, 90). As the majority of energy available for ruminants to use for metabolism come from fermentation end products [∼70% in ruminants; (91)] and the ruminal microbiome composition determines the types of fermentation end products produced, several experiments have shown a link between the rumen microbiome composition and feed efficiency traits (92–94). Therefore, the ruminal microbiome is an important aspect of ruminant nutrition and is dependent on diet.

Relatively recently, there has been much work on how microbial fermentation products (e.g., volatile fatty acids) can alter mood, behavior, and subsequently physiological stress, mental well-being, and welfare, through what is termed the microbiota-gut-brain axis. Much of this work has been done with humans. In a review, it was concluded that the gut microbiota in humans can communicate with the central nervous system, subsequently altering mood, cognition, and emotions (95). Likewise, the microbiota-gut-brain axis has been shown to influence behavior (anxiety and social) and memory capacities in non-ruminant livestock (96). Microbial fermentation products are known to alter feeding behavior in ruminants and to provide positive postingestive feedback to the animals, thereby providing positive emotions and influencing the preference for specific foods. For instance, when different flavors were offered to sheep and associated with a low or high addition of exogenous propionate (a glucogenic volatile fatty acid), it was found that at lower additions ruminants developed a dietary preference for the conditioned flavor, whereas at the higher addition the sheep developed aversions to that flavor (97, 98). This relationship makes intuitive sense as the volatile fatty acids provide 70% of the caloric requirements of ruminants (91). In humans and other mammals the microbiota-gut-brain axis has been shown to influence behavior, mood, and emotions, while the only predominant link shown in ruminants is its effect on feeding behavior (96). The availability of information on the microbiome-gut-brain axis and its effect on non-eating related behaviors in ruminants may be lacking because fermentation products represents a much larger contribution to their nutritional requirements than humans or other farm animals. For example, while volatile fatty acids contribute 70% of energy for metabolism in ruminants, it only accounts for ∼10% for humans, 25% for pigs, and 30% for rabbits and horses (91). However, there is still a need to determine how the ruminal and hind-gut microbiome may alter non-eating related behaviors in ruminants.

Dietary diversity would likely influence the ruminal microbiome composition, which in turn may influence the host animal's mood, emotions, and welfare and would influence dietary preference and dry matter intake. A review of the human literature concluded that a diverse diet would supply a wide range of substrates for the microbes to ferment in the gut, which would promote a more diverse microbiome (i.e., microbial species richness). This diverse gut microbiome was suggested to be more adaptable to disruption (99). It is known that diet formulation alters the ruminal microbial species richness. For instance, when grain based diets are fed to ruminants they have a less diverse microbiome compared to forage based diets (100) and differences have also been shown when cows were fed different forages (88). However, to our knowledge, there is no information available for how dietary diversity may influence the ruminal microbiome's species richness. Therefore, there is a need to determine how dietary diversity may influence the microbiome of grazing ruminants. Additionally, while it is known that microbial fermentation products can alter dietary preference and intake behavior in ruminants, there is a lack of knowledge on if the fermentation products could influence other behaviors, and subsequently mental well-being and welfare, in ruminants. However, based on experimentation with other mammals (humans, rats, etc.), there is a strong possibility for dietary diversity to alter the microbiome, mood, and emotions when provided to ruminants.

Grazing ruminant mental well-being and nutrition are closely linked. Hedonic well-being influences voluntary feed intake through changes in opioid, cannabinoid, and the GABA systems, thus providing a reward response and influencing how ruminants like a specific food [(84); **Figure 1**]. By providing dietary diversity, animal well-being may be improved in several ways. One is through improved eudaimonic well-being, by providing the animals with choice, thus allowing control over the animal's environment and the expression of individuality. Another means is through improved hedonic well-being by enhanced internal state by improved nutritional status. Additionally, PSC consumed at an appropriate level, which is allowed by dietary choice, have direct effects on the response that the animal has to physiological stress. Finally, dietary diversity may alter the microbiome-gut-brain axis, which has been shown to alter the mental well-being of other mammals.

NUTRITION AS EFFECTED BY DIETARY DIVERSITY

Ruminants have evolved in ecosystems where dietary choices abound and where they were able to select plants differing in PPC and PSC so that they could consume a balanced diet that met their needs for nutrients, medicine, and prophylactics (9). Dietary diversity and allowing animals to choose from an arrangement of feedstuffs to meet their own requirements is not a new concept (9). As ruminant nutritionists, requirements are typically assessed and food offered to meet those requirements for an average animal. However, if we expect dietary requirements to follow a normal distribution, a small number of animals would be "average" and thus, ∼50% of animals will be fed diets that under supply nutrients and around 50% will over ingest nutrients (1, 2). Therefore, lack of dietary choice may result in individual dietary imbalances. These nutrient imbalances may lead to incidental restriction or augmentation (6). Incidental restriction is a reduction in intake due to negative post-ingestive feedbacks as a result of over consuming specific nutrients and incidental augmentation occurs when animals over ingest nutrients in order to meet their requirements for nutrients that are in lower concentrations in the diet (6). The differences between individual animals are a result of variations in physiological and morphological differences (1) and also due to individual personalities (101). In a grazing context, Parsons et al. (102) found that, overall, sheep prefer to mix their diet and that their dietary preferences change across the day, influenced by sward characteristics and their previous diet. Parsons et al. (102) measured preference by video-recording grazing location (i.e., forage species) and calculating forage intake from previously established intake rates of the respective forages. Individual animals vary greatly with regard to selection of dietary components within and between meals. It's because of these differences of individual animal preference and selectivity that common management goals aim to reduce sorting and selectivity by cattle fed "total-mixed rations" (103). These management goals often involve adding liquids (e.g., molasses or water) to the mixed rations (103). Interestingly several works have shown these management strategies actually encourage feed sorting and reduce dry matter intake and this has been related to the lower dry matter diets having greater temperatures resulting in increased spoilage (104, 105).

The difference in individual animal selection is likely due to individual variation in the internal-state and post-ingestive feedback mechanisms that govern intake. This means that providing animals choice in the dietary constituents, rather than offered as a "total-mixed ration" formulated for the average animal or a non-functional mixed sward (mixed swards planted in a way that inhibit selection) may allow animals to choose from the dietary constituents in order to meet their respective requirements (8). Ruminant producers offering livestock high concentrate diets prefer to feed total mixed rations for ease of management and to reduce risks for negative health problems (e.g., ruminal acidosis and laminitis). However, it has been suggested that by offering choice ruminants can alter eating patterns to account for the later concern (2) and this has been supported by experiments where grain was offered at free choice and pH was measured (106, 107). While there has been much research on feeding total mixed rations in the last 60 years [see (108)], there is surprisingly few experiments which have compared total mixed rations compared to the dietary constituents offered as choice, but many of those that have, found choice to be superior. In an early experiment, reduced dry matter intake, similar performance, and improved feed efficiency were observed when dairy cows were offered forage and grain separately as opposed to being provided a total mixed ration (109). Another experiment conducted in feedlot fed steers provides further evidence for this hypothesis (2). Cattle were offered either a total-mixed ration or the components of the total-mixed ration offered individually. It was found that the diet selected by cattle varied tremendously between animals, but also within animals across days. The cattle offered choice consumed less feed, had similar performance, and lower cost of gain compared to the total mixed ration treatment (2). A separate experiment conducted by the same laboratory with growing sheep found that when lambs were provided choice between three iso-caloric and iso-nitrogenous diets, they had greater dry matter intakes, performance, and feed efficiency, and less cost-of-gain compared to lambs offered only one of the three diets (110). These experiments have been corroborated by other laboratories. When lactating goats were offered choice they consistently consumed less dry matter comparable milk productions compared to their total mixed ration counterparts (111). It is important to note that some experiments have found choice and total-mixed rations to be not significantly different (106, 107) or for total-mixed rations to be superior (112). Likewise, others contend that ruminants possess poor internal wisdom and that they are unable to select diets according to their nutrient requirements (108). These different findings and conclusions may be due to the differences in the dietary options provided. If dietary constituents are not divergent enough in nutritive composition, then animals may not be able to select diets tailored to their specific individual nutrient requirements. Several experiments across multiple species and production settings have shown choice to improve feed efficiencies (either by reducing intake while maintaining intake or by increasing intake and performance) compared to offering a total-mixed ration, which were formulated to be optimal for the average animal. This is clear evidence for the importance of dietary choice as a means for meeting the individual requirements and avoiding incidental restriction or augmentation of intake.

LINKING OXIDATIVE, AND PHYSIOLOGICAL STRESS

In humans, a link between oxidative status (metabolic stress) and physiological stress has been suggested and reviewed, with an apparent vicious cycle where physiological stress increases metabolic stress, which in turn increases physiological stress, etc., resulting in telomere shortening and aging (113–115). This may be especially true in scenarios of chronic stress (115, 116). Aschbacher et al. (116) explored the effect of chronic stress and perceived acute stress and found that there was significant oxidative damage when chronically stressed people experienced a perceived stressor. Chronic stress occurs when there are relatively high levels of glucocorticoids circulation in the blood stream for a prolonged period of time. Chronic stress has been linked to health problems in humans and animals. Several works have reported increased oxidative stress as a possible mode of action behind the cost of chronic stress (117). Orzechowski et al. (118) explored how rat's antioxidant status and oxidative stress changed when challenged with dexamethasone (a synthetic GC; 2-mg/kg of body weight/d). It was found that treatment with dexamethasone decreased blood and muscle glutathione, reduced SOD activity, and increased malondialdehyde [measured by TBARS; (118)]. A meta-analysis by Costantini et al. (117) concluded that GC were significantly associated with oxidative stress and that there were different magnitudes of effects according to tissue, sex, and age. Therefore, physiological stress increases oxidative stress in livestock and other mammals.

There is little direct evidence to link physiological stress and oxidative stress and their subsequent consequence in livestock. The experiments that have explored these relationships generally compared animals before and after a physiologically stressful event. In one experiment, 105 crossbred steers where transported for 19 h and 40 min. This stressful event significantly reduced serum antioxidant capacity and increased malondialdehyde (marker of oxidative stress). It was found that calves with more incidence of bovine respiratory disease also had higher oxidative stress after transportation (119). Other common management practices, which are known to be stressful to animals have been linked to oxidative stress. After sheep were shorn, there were greater circulating malondialdehyde (marker of lipid peroxidation) concentrations than before shearing (120). Finally, malondialdehyde was likewise increased after cattle were dehorned (121). These experiments provide evidence that physiological stress increases oxidative stress in livestock. However, there is less evidence to show that dietary antioxidants can reduce physiological stress. One experiment challenged sheep with injections of ACTH and found that a treatment group provided with supranutritional antioxidants (Vitamin E and Se) had lower circulating cortisol compared to their non-supplemented cohorts (45). Some recent works have shown a positive correlation between isoprostanes, which results from oxidant conversion of arachidonic acid, and cortisol. These experiments explored the effects of a non-steroidal anti-inflammatory drug on reproductive performance of cattle (29, 30). As mentioned previously, isoprostanes result from the peroxidation of arachidonic acid by oxidants and it has been suggested that they are the pathophysiological mediators of oxidative damage (28). A positive correlation between cortisol and isoprostanes provides direct evidence and a potential mode of action for a link between oxidants and physiological stress. However, the relationship between physiological and oxidative stress is an area that requires further investigation in livestock, but there is evidence that improving oxidative status may allow the animal to better recuperate from the stress and reduce subsequent negative effects.

LINKING OXIDATIVE AND NUTRITIONAL STATUS

Metabolic disorders seen in transition dairy cows provides excellent insight into how oxidative stress can be effected by the nutritional status of the animal. Bernabucci et al. (22) followed 24 cows with different body condition scores (BCS) across the transition period (± 30 d at calving). It was concluded in this experiment that oxidative status was related to energy status and that cows with greater weight loss over this period experienced greater oxidative stress (22). This positive relationship between energy demand and negative energy balance and oxidative stress has been shown in several experiments. For example, milk yield is positively correlated to markers of oxidative stress in dairy cows [malondialdehyde, (122) and hydroperoxides (123, 124)]. While most of the experiments focused on nutrition and oxidative stress in dairy cows, the relationships are applicable across ruminant species. For example, when lambs were fed 70 or 80% of their metabolizable energy requirements, they had higher plasma malondialdehyde levels than when lambs were fed 100% of their requirements (125). Level of energy intake is not the only way oxidative status is influenced by nutrition, in fact source of energy can have impacts. When lambs were fed high fat, there was an increase in blood superoxide dismutase levels and glutathione concentrations. This was attributed to increased fatty acid oxidation which would stimulate the production of oxidants (126). Nutritionally related disorders such as subacute ruminal acidosis can also induce oxidative stress. Guo et al. (127) found that when dairy cows were induced with subacute ruminal acidosis, there were lower plasma levels of total antioxidant capacity and higher glutathione peroxidase activity and malondialdehyde concentrations. Nutritional status and oxidative stress are intimately linked. Oxidative stress can be influenced by previous level of nutrition, current energy intake, source of energy, and also nutritional related diseases.

As mentioned above, diet can greatly influence the composition of the ruminal microbiota. Further, we discussed above how microbial fermentation products can influence physiological stress and welfare. As physiological stress and reduced welfare will likely lead to oxidative stress, the effect that dietary diversity may have on the ruminal microbiota, or even the hind-gut microbiota, may provide another mode of action for dietary diversity to reduce physiological and oxidative stress. However, this requires further investigation. Additionally, microorganisms have been directly linked to reductions in oxidative stress. For instance, cattle had less antioxidant activity in their ruminal fluid and in their plasma when they were defaunated (removal of protozoa) compared to their faunated cohorts (39). Also, steers placed in a feedlot had less glutathione peroxidase activity (indicating less oxidative stress) and a greater blood antioxidant level when provided a lactobacillus fermentation product compared to the control steers (128). Thus, dietary diversity may alter mood and behavior, thereby influencing mental well-being and welfare, indirectly by altering the ruminal microbiota composition and also may directly reduce oxidative stress.

CONCLUDING REMARKS

This review has covered oxidative stress, physiological stress, and nutritional status, which are areas of animal science that are important to both producers and consumers. Further, we have provided links between these three areas and have described how dietary diversity links the three. In conclusion, there is evidence to support how dietary biochemical diversity (provided through taxonomical diversity) can reduce oxidative stress directly by providing plant secondary compounds as natural dietary antioxidants and indirectly by reducing physiological stress, which we have reported evidence to influence oxidative status. Additionally, the antioxidant benefits of plant secondary compounds may improve the metabolic response the animal has to physiological stress and therefore improve the response to the perceived stress. Dietary diversity may improve eudaimonic well-being merely by allowing animals to make choices, and thus we postulate that this theory of well-being applies to livestock. Further, diverse diets may alter the microbial-gut-brain axis, which in humans and some non-ruminant farm animals has been shown to alter cognition, mood, emotions, and behavior, as well as dietary preference and eating behavior in ruminants. Finally, dietary choice allows animals to take advantage of differences in plant primary compounds to meet individual animal requirements and thereby improve nutritional status. Improved nutritional status can subsequently have beneficial impacts on oxidative stress by reducing energy store mobilization and physiological stress by improving hedonic well-being. Physiological stress, oxidative stress, and nutritional stress are intimately linked (**Figure 1**) and dietary choice compared to monotony may simultaneously improve all of these items directly and indirectly, resulting in marked improvements in the foraging animal's nutritional status, health, mental well-being, and ultimately their welfare. We conclude that dietary diversity reduces stresses while enhancing hedonic and eudaimonic well-being in ruminant livestock.

AUTHOR CONTRIBUTIONS

MB was responsible for the majority of formulating content and writing the submitted manuscript. PG is MB adviser and assisted in writing, formulating content of manuscript, proofreading, and suggesting edits.

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Pastoral Farming Ethics and Economics–Aligning Grazing Practices and Expectations

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Keywords: animal welfare, body condition, shelter, painful husbandry procedures, values, profitability, community engagement

INTRODUCTION

We all generally value animal welfare–what animals experience, how they perform or whether they are being treated with respect–is important to them and to us. However, animal welfare is contested because humans benefit from compromises to animals and we have different expectations borne of different needs, preferences and prejudices: "some we love, some we hate, some we eat" (1). Also contested is what we think and understand is important to animals, and whether the way animals are farmed is in keeping with their nature. In all, a complex subject, but complexity is a feature of humanity, as noted in the development of early civilisations (2).

Few people see the world as exclusively human and most extend concern to animals, plants and the environment, giving rise to different animal-related values, the "rules" and "expectations" we learn from parents and family life, friends, religion, our trades, and professions, the literature, media, the society we live in and its history. Values, then, are not always shared; reasonable people can disagree; and contested ideas can become difficult to describe, subject to considerable political debate and are unlikely to have simple solutions, at best being managed rather than solved. Our common morality holds that we not harm, that we do good, that we are fair and just, and that we respect people's ability to make their own choices (3). The importance of values to human behavior requires that different values are understood, respected and taken into account, even if they cannot be reconciled. And how humans interact with animals is fertile ground for contested practices and expectations.

The aim of this chapter is to provide a commentary on some of the contested aspects of pastoral farming and how we go about dealing with them. The purpose is to reveal the complexity of the subject and how this gives rise to different views or perspectives informed by different values, and how we engage and evaluate those views.

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THE CHALLENGES OF PASTORAL FARMING

Farming is at the nexus of two worlds, the physical or biological and the social or societal, farming arguably subordinate to both (4). Consequently, the potential challenges can be varied, from dealing with the vagaries of climate to the expectations of people far removed from the land. Most pastoral farmed animals, especially those in extensive environments, have some of the attributes of free-living or wild animals. Although having choice of diet and considerable freedom of movement and behavior, they are under some degree of human management (5–7). For example, social and kin structure may be distorted by culling and grouping, movement may be limited or prevented, food and nutrients are often less varied in composition, parental care of young may be curtailed by weaning, and animals are usually less subject to predation and natural selection but increased artificial selection is likely. Furthermore, changes to pastures, animals and management such as supplementary feeding and artificial breeding are commonly used to minimize ecological constraints and improve animal and farm productivity and efficiency (8, 9). In such a complex physical and social environment, there are potentially many challenges to animal welfare, including, for example, those related to body condition, shelter, exposure to mud, and painful husbandry procedures. These examples are used to highlight some of the different perspectives contributing to animal welfare, perspectives that different people may value differently.

Body Condition

Animals have evolved homeostatic mechanisms to overcome nutritional variation when energy demand exceeds availability, e.g., in winter, during pregnancy, and lactation, or when unable to forage. Body condition score, an accepted measure of energy reserves, generally reflects better access to resources, individuals exhibiting enhanced survival (10–12), growth, reproduction, lactation, and health [e.g., (13, 14)]. However, the relationship is not simple. Dairy cows in higher condition at calving are at greater risk of metabolic disorders (e.g., ketosis, milk fever), while those in lower condition are more likely to have difficulty getting pregnant again. Body condition is, then, an important management tool for optimizing flock or herd health and productivity. For example, body condition can be reflected in ovulation and lambing rates (15), lamb birth and growth rates (16), calf birth weights and earlier returns to breeding (17).

As well as inadequate feeding, loss of condition can reflect underlying disease or parasitism, and is sometimes more prevalent at the end of an economic or productive life. It can also be the result of neglect or failure to care for animals, sometimes the result of people in difficult financial and personal circumstances, such as during droughts, or ill-health, and challenging relationships [see (18)]. Furthermore, maintaining animals in good condition may not just reflect a lack of food, but changing genetics (e.g., selection for production can be at the expense of body condition) and management (e.g., a lack of skills in managing pasture, perhaps exacerbated by farming to generalized recipes rather than having the skills to adapt to novel and changing situations).

Not surprisingly, animal welfare regulatory codes place importance on body condition. Typically, they require that when animals are thin, emaciated or very thin, urgent remedial action is taken to improve condition, or the animal humanely destroyed. Therefore, few farm animals tend to routinely be in such poor condition. However, as has been noted (10, 14) neither the animal welfare decision process, nor the information on which recommendations for optimal body condition are based, are clear. The standards seemingly rely on common sense–emaciated animals represent poor welfare, or poor productivity. However, there are limitations in extrapolating from body condition to animal welfare. For example, at low condition score, the weight of fat in dairy cows may be overestimated (19). Even within a more normal range of scores, body condition may have its limitations. After monitoring milk production, health, and udder and uterine health, Roche et al. (20) concluded that body condition score (at least between 3.5 and 5.5 on a scale of 1–10) "is not a sufficiently sensitive measure to be reflective of cow welfare" in early lactation, despite it appearing optimal for production, reproductive performance and general health. For example, based on measures of liver and immune function, the authors suggested that body condition peripartum and early lactation did not affect cows' abilities mount an inflammatory response.

As well as the understanding of body condition being dominated by measures of animal productivity, further reflection on the relationship with animal welfare reveals a number of other limitations. Firstly, as well as being diagnostic of nutrition, body condition is both a dynamic state and a subjective assessment. Animals at a particular condition score could be maintaining, gaining or loosing condition with different implications for what they feel (e.g., hungry) and are likely to experience (e.g., metabolic diseases), as well as reflect underlying conditions such as ill-health, parasitism, or advanced age, and their seasonal physiology. While it is reasonable to assume that feed restricted animals might be hungry, body condition may not necessarily reflect, or can be taken as a measure of hunger (21). For example, there is natural variation in body condition between individuals and breeds, as well as species. The physiology of some animals may see them lay down fat but then lose their appetite seasonally, e.g., rutting stags. Similarly, an analysis of death rates amongst sheep being exported by sea from Australia suggested that sheep in the fat deposition stage were less able to adjust to feed deprivation (22). It should not be assumed that animals in good condition do not experience hunger, especially when they have been bred to be highly productive. Some sows and breeder meat chickens display extreme examples of what Rauw et al. (23) have suggested is altered, perhaps pathological, hypothalamic mechanisms regulating appetite.

While body condition can be an indicator of hunger, and thus welfare, it is more precisely an indicator of past access to nutritional resources and thus an ability to deal with the constraints of the future environment, be it climate or a need for veterinary care. As such, it serves to illustrate the difficulties in relating how an animal performs with what emotions it may be experiencing, two of the predominant understanding of animal welfare. Nevertheless, body condition is an iceberg indicator, i.e., a key indicator of overall welfare inferring that the animal's care is of high quality and its welfare good (24).

The Provision of Shelter for Pastoral Animals

Pastoral animals experience and usually successfully adapt to a range of climatic factors, whether they are daily and seasonal or extreme and adverse. Adaptation to thermal challenges involves a range of physiological and behavioral systems (**Figure 1**), including shelter seeking behavior, shelter being a resource animals need to ensure their comfort, productivity and survival.

There is a seemingly endless range of possibilities enabling animals to lessen the impacts of adverse weather–contours in the land, hedgerows, trees, gullies, flaxes, vegetation clumps, tussocks, rocks, woolsheds, rushes, etc. The importance of shelter is noted in the internationally recognized "five freedoms."

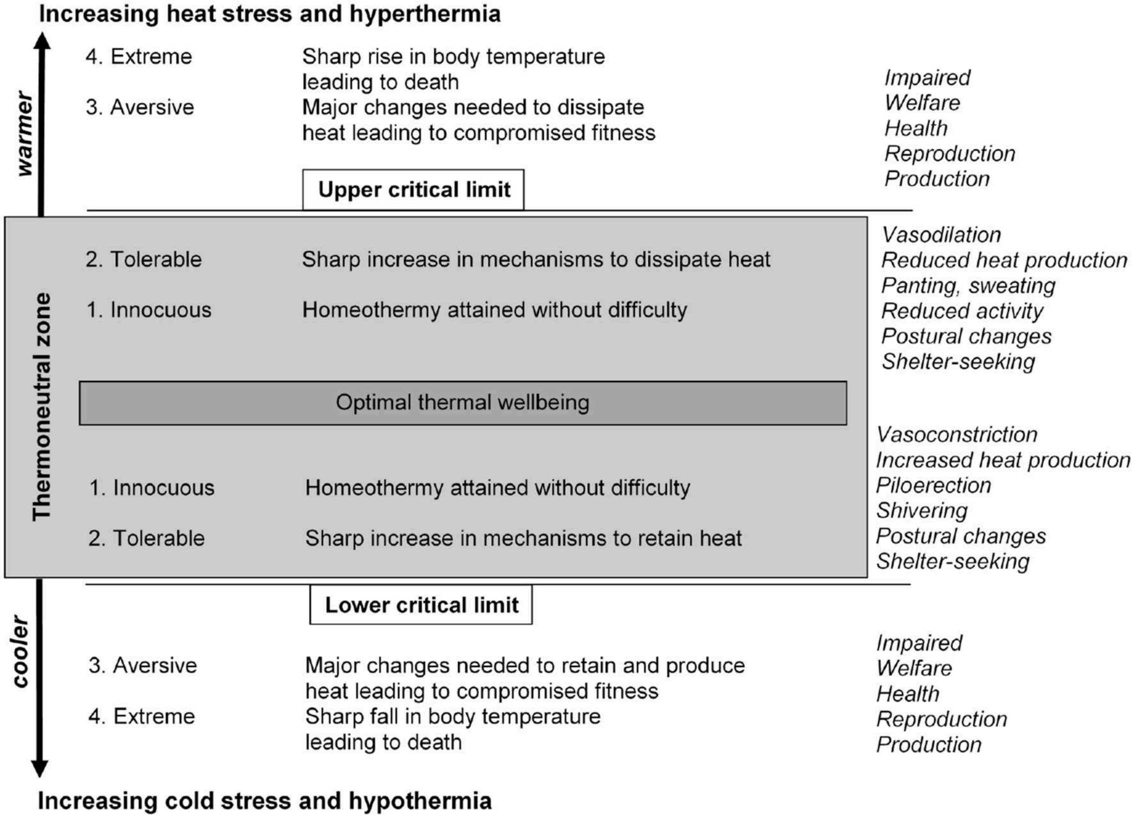

FIGURE 1 | The responses of animals, and the consequences, to thermal challenges evoked by exposure to increasing heat and cold stresses [from (25)].

Freedom from discomfort by providing a suitable environment including shelter and a comfortable resting area (26) addresses, like the provision of food and water, a fundamental need. However, although there are many directives and expectations for the provision of shelter, it remains an example of what Dwyer et al. (27) describe as "stubbornly unchanging." The accumulation of knowledge is sometimes not having a significant impact on the circumstances animals encounter (28).

Given that shelter is important and valued by animals, farmers and the wider community [e.g., (28)], why is it an issue? In a survey (29) barriers to the greater provision of shelter for pastoral animals included resources (time, money, return on investments, etc.), the negative impacts on farm productivity (e.g., the removal of shelterbelts to enable the use large, center-pivot irrigators), inadequate knowledge of means of providing shelter and their success, and the fact that standards are difficult to enforce requiring proof of animal suffering and with a lack of consequences for not providing shelter. Furthermore, many farms appear to provide adequate shelter; others have active plans to provide more shelter; some have other priorities; or are more resistant requiring exposure to the consequences including legal enforcement. In addressing shelter, it may be necessary to acknowledge these factors and aim to achieve a balance between regulation and enforcement and incentives and encouragement.

Exposure to Excessive Mud

Winter brings many challenges, most notably meeting animals' nutritional needs (if well-fed, livestock can usually tolerate variations in the weather). Mud is the inevitable outcome of slow pasture growth in winter, exacerbated by rain, and/or intensive land use, and high stocking densities. It can be uncomfortable, cold and wet. Furthermore, images of miserable-looking animals, deep in mud, has led to public criticism and expectations for improvement (30). Storms, stocking densities, animal preferences, pastures, aspect, soil types, management needs and all the other things that make farming both rewarding and challenging, mean that mud is sometimes inevitable. While feedlots, feeding pads and crop utilization are pragmatic examples of ensuring animals have adequate nutrition over winter, as well as reducing pasture damage and improving feed utilization, they mean animals are concentrated on small hard-surfaced areas. Advances in crop breeding producing markedly increased dry matter per hectare can mean animals live on smaller areas for longer periods, often resulting in mud. On feedlots, energy requirements increase if animals are wet and muddy, especially if not sheltered from the wind, thus mud can also reduce animal performance. Excess energy requirements depend on mud depth, temperature, portion of animal affected, and wind. Liveweight gains can be reduced by 35% in dirt pens

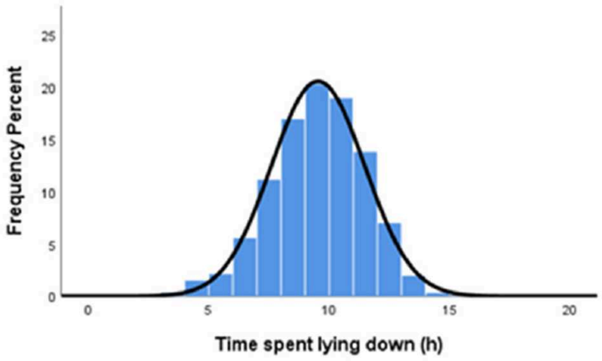

FIGURE 2 | The distribution of lying time (mean 9.5 h) in pasture-farmed dairy cows (1948 cow-days across 10 farms) in large (> 500 cows kept as a single group) herds in Australia (32).

in muddy conditions, and cattle need about 25% more feed to produce the same gains (31).

Excessive or prolonged exposure to mud, especially very wet mud, can potentially impact on animals' needs as follows:

- Proper and sufficient food and water–feed can become contaminated and more difficult to access leading to animals "giving up" and becoming hungry and losing condition, leading to increased risk of metabolic diseases, physical weakness and an inability to stand.
- Adequate shelter–if the site is exposed to adverse weather, especially extremes, with animals unable to seek shelter, animals are prone to discomfort, shivering, hypothermia, and death.
- Opportunity to display normal patterns of behavior–an inability to rest properly because of a preference for dry comfortable resting areas, may result in reduced lying, tiredness, reluctance to move greater distances, inability to move, isolated from mob; possibly increased risk of infection, and typical physiological indicators of stress (more so than moderate feed restriction).
- Protection from, and rapid diagnosis of, any significant injury or disease–mud can increase susceptibility to lameness, mastitis, dermatitis, hypothermia, and metabolic diseases.
- Animals in mud could then, experience discomfort, chilling, skin irritation and pain, weakness, exhaustion, frustration, and depression.

One of the less well-appreciated animal behavioral impacts of mud is on the ability of animals to rest. Dairy cows, for example, may spend, on average, 10–12 h per day lying down although there is much variation between individuals (**Figure 2**). However, lying time is significantly reduced when the animals are exposed to muddy conditions, as little as 3–6 h during the first 24 h. Chen et al. (33) concluded that "muddy conditions, even in the absence of wind or rain, are aversive to cattle and have negative implications for their welfare."

An animal's preferences for lying is dependent on its environment. A dry and comfortable surface, such as pasture, woodchips, or sawdust, is strongly preferred by dairy cows (34). On a wood-chip standoff pad, lying time reduced from an average of 11.6 h a day to 5.6 h over 5 weeks when bedding was not refreshed (35). If faced with choosing between feeding and lying, dairy cows prioritize lying, and depriving animals of the opportunity to lie down, for example when surfaces are wet and dirty, impairs welfare, evident in in altered pituitary-adrenal function indicative of stress (36, 37) and immunosuppression with an increased the risk of infection (33). The importance of lying was seen in tired cows, those from wet, uncomfortable standoff surfaces, preferring to lie down when they might have normally been expected to graze. The altered lying pattern of a herd, more cows lying down sooner, and for longer, on returning to pasture, may be a simple and practical indicator of inadequate resting opportunities on standoff surfaces (35). Interestingly, rumination and resting appear inextricably linked. For example, sheep given finally chopped feed that did not require ruminating became tired and irritable. The addition of hay to their diet enabled rumination and the disappearance of distress and exhaustion (38).

The risks of excessive mud appear to be minimized by providing fresh bedding, environmental buffers (e.g., windbreaks, mounds, shelter) and access to space with dry, comfortable resting areas. Cow cleanliness is becoming an accepted indicator of animal welfare in farm assurance programmes and dirty cows (e.g., flanks, hind limbs, and udders) a measure of an unsuitable environment.

Painful Husbandry Procedures

Fences notwithstanding, one of the features of pastoral animals is that they more likely to be able to behave naturally compared with more confined farm animals. However, and like many of their more intensively farmed counterparts, livestock are subject to painful husbandry procedures in order to enhance animal health and welfare, facilitate husbandry and management, enhance animal products, or reduce the safety risks to humans. Common examples include castration, tail-docking, and disbudding and dehorning. Many of these procedures can cause anxiety, fear, discomfort, pain, or distress associated with mustering, handling and restraint, and acute and chronic pain resulting from the physical interference of sensitive tissues. While many of these impacts have been well-documented and have contributed to greater use of pain relief at the time of the procedure, the possibility of modified behavior resulting from the procedure is less well-known. One example is the practice of tail docking dairy cows to supposedly improve hygiene, in part because of the reduced inability of the animal to swat flies and to communicate its emotional state. However, perhaps the best example of a husbandry procedure designed specifically to thwart behavior, is nose ringing in in grazing pigs. Although not a ruminant, the example is insightful because of the thwarted behavior being the more significant compromise to the animal than other husbandry procedures where the procedure, usually performed without pain relief, is arguably the more significant compromise.

Rooting is the means by which pigs explore and search for, locate and harvest food. The insertion of a ring, clip or wire through either the nasal septum separating the nostrils, or the upper, outer part of the snout, is a common means of preventing pigs from undertaking the behavior, principally to reduce pasture damage, and perhaps lessen soil erosion, nutrient leaching, and internal parasites. Nose rings reduce the time a pig spend rooting (20–30% of waking hours in semi-natural conditions) and result in significantly better grass cover (39).

The "extreme vocalizations" at the time of insertion suggest nose-ringing is painful. It is probably also painful for a period after the procedure since some behaviors take time to return toward normal levels. Typically, pain relief is not provided. However, it is the long-term or chronic effects which are arguably more significant. Firstly, the ring is effective in reducing rooting presumably because it is uncomfortable or painful to root with a nose-ring. Several behaviors are affected with differences in grazing, sniffing, standing inactive, pawing/scraping the ground, and chewing straw, as well as rooting, evident with different rings or clips (40). Secondly, a natural behavior thought to be important to the animal is thwarted, resulting in a degree of suffering although signs of frustration may not always be evident (41). Pigs continue to root in intensive systems even when fed *ad libitum* and housed on wire-mesh or concrete floors, situations where they perhaps have no need for food, and certainly no prospect of digging. This suggests rooting is an important natural or normal behavior, and preventing it is likely to lead to frustration and altered behaviors. Finally, the rooting action is also part of digging wallows, nest building, physical aggression and exploring. Ringing reduces rooting in wallows and in straw, and affects grazing and palpating the ground for nuts, stone-chewing and increases the amount of time spent standing but otherwise inactive. Ringed animals are at a disadvantage when feeding and may need to be kept separately from un-ringed animals to enable the former to obtain their fair share of feed (42).

There have been attempts to provide opportunities to satisfy or divert pigs' urges to root. For example, providing other things to do, or a more satisfying diet; sacrificing rooting areas or provision of root crops; or providing earth- or peat-filled rooting troughs in intensively housed pigs. While the provision of root crops did not appear to prevent rooting in any significant way, the provision of rooting trays resulted in less abnormal behavior, such as belly-nosing or ear and tail biting in intensively housed weaners.

Clearly there are compromises to animals undertaken for human benefit, compromises that different people have different views on. Furthermore, alleviating those compromises inevitably comes at a cost. Typically, we appeal to ethics and economics for some sort of guidance, or to justify compromises are reasonable and necessary, in other words what costs should be borne by the animal or the farmer. The following section is a general introduction from the perspective the author's interest in animal welfare, as well as science and farming.

ETHICS AND ECONOMICS

Any introduction to ethics should emphasize two things–it is complex, and yet it is something we all do. Ethics' complexity is one of its benefits. We and the world we live in, are complex–simplifying it doesn't always work.

Morality has its roots in co-operation between social beings over resources–the land, animals and people, and how we apportion them as well as take responsibility when things go wrong. The terms ethics and morals are often used interchangeably but the former can be understood as the study of the latter, the thinking or "reasoning" behind beliefs of right and wrong actions. There are many different reasons and theories–like different scientific disciplines, e.g., animal behavior, immunology, and reproduction–each providing different and important insights, but also each having their own limitations.

The most well-known theory, consequentialism, is based on whether the likely consequences of an action will have benefits outweighing the harms. For instance, humans have benefited from the milk production of dairy cows, both as a source of food and of industry and commerce. These benefits are taken to outweigh the harms associated with the removal of the calf from the cow at birth, and either their artificial rearing or imminent slaughter. These practices are common to many modern dairy systems. However, relying exclusively on an ethic based on the benefits outweighing the harms is problematic. It does not mean all harms to animals are justified because of the benefits to humans. As both individuals, and as a society, we accept that there are some things we cannot do to animals, no matter what the benefits are. For example, as Rollin (43) asks farmers, would you do anything at all to increase profits and production, such as "torture a cow's eyes with hot needles if it increased milk production?" Rights theories set a limit to actions, regardless of the benefits. This ensures, depending on the circumstances, that there is a limit to animal compromises.

Rights are a social device that makes it easier for people to live with each other by providing a protection or constraint on treatment. Western animal rights theory appears to have evolved from eighteenth century reaction to humans apparently having no obligation to animals or to their treatment. Not surprisingly, the movement resulted in almost complete consensus in the need for the speedy killing of animals when slaughtering, or in eradicating vermin, and in repudiating cruelty to animals (44). This view of rights is reinforced by the idea that animal welfare and animal rights are, despite common perceptions, essentially similar in aims (45)–animals have entitlements or rights to adequate food and shelter etc., which humans have a duty to provide (both for the animals sake and because it makes us better human beings). While the term "animal rights" can, and commonly does, refer to any call for the fair treatment and protection of animals, the more revolutionary rights theories maintain that, because animals are the subjects of a life, they should not be used for farming, in animal research and testing, or even kept as pets (46). While it is understandable, then, that animal rights is often dismissed, especially when associated with revolutionary implications and illegal activities, such a stance does not reflect the complexity and common understanding of the term. On the other hand, common understanding of animal rights may preclude its use amongst some people, even where consideration might result in more equitable use and treatment of animals.

Another ethical theory is pragmatism. It not only considers the consequences of an action, but also emphasizes the legitimate and necessary role that emotions and sympathy play in moral reflection and choice. It is, for example, difficult not to feel something for dairy cows, with whom you've become associated with over many years, being loaded for slaughter at the end of their productive lives. Thus, some ethical theories are impoverished in not realizing the special weight of relationships usually inherent in husbandry and caring (47) and this is most evident in care-based reasoning, especially actions that are informed by an intimate understanding of the circumstances, in the skills of stockmanship and animal care based on a respect for the essence of the animal (48). Individuals commonly draw on tradition and experience and a willingness to learn, personal qualities of empathy and patience, and an understanding of the balance between the expectations of people and the needs of animals in different systems. The attributes of good stockmanship include:

- being able to draw on a lifetime, or intergenerational catalog of practical personal experiences with animals and farming such that actions become second nature, where feel and experience are valued as much as specialized knowledge and measures;
- having personal qualities of patience, empathy and other traits or attitudes considered necessary for working with animals, their impacts having been demonstrated scientifically in modern pig, poultry and dairy cattle farming systems [e.g., (49)];
- an understanding of the constraints and opportunities afforded by the farm environment including the terrain, the climate and the flora and fauna, experience with animals aligned with that of the land and the weather; and
- knowledge of the normal behavior of animals and, being observant, having the ability to recognize and deal with abnormal behaviors.

Much of this knowledge is ineffable and should not be underestimated in the calls for formal training and proof of competency, invaluable as they are in complementing such understanding.

Ethical theories, then, provide different insights for people and one understanding of ethics is that it is the systematic examination of moral issues in the public sphere. The broad view, representing the culmination of a long tradition of moral reflection, as well as expressing the common view of most members of society, is that the use of animals is permissible as long as it is justified and humane (50), respecting the following three principles:

- Harms of a certain degree and kind should not be inflicted on an animal, regardless of any benefits (e.g., mulesing, the surgical removal of skin around breech of lambs to reduce the risk of flystrike, or the use of blunt-force trauma to kill unwanted dairy calves, except in emergencies, are prohibited in New Zealand).
- Any harm (e.g., physical pain, loneliness, degrading use) to an animal must be justified by ensuring the good realistically expected from the harm, outweighs the harm inflicted (e.g., disbudding or dehorning cattle to reduce the risks to human and other animal safety).
- Any harm which is justified, should be minimized as far as is reasonable possible (e.g., undertaking painful husbandry procedures on young animals to reduce the amount of tissue involved, and providing pain relief).

The third of these principles, minimizing the harms, essentially describes good standards of practice, often justified by or drawn from science [e.g., (51)] and good husbandry and encoded in codes of welfare. New Zealand's *Sheep and Beef Cattle*, *Dairy Cattle,* and *Painful Husbandry Procedures* codes of welfare (52–54) describe, for example, stockmanship and animal handling; the provision of food, water and shelter; opportunities for animals to behave normally; addressing health, injuries and disease; and husbandry practices from selection and breeding to animal identification, humane destruction, and minimizing pain and distress.

Although codes of animal welfare have a regulatory role, failure to meet a minimum standard can be used as evidence to support a prosecution whilst equalling or exceeding such a standard can be used as a defense, codes typically have a number of uses and purposes. For example, they also articulate the aspirations of society; raise awareness by drawing attention to issues; give the public an idea of what to expect; and are self-promoting, the standards defining good animal use in a particular industry or country serving to differentiate them from those ascribing to other, especially lesser, standards.

Although there are many ethical insights reflecting common morality and given expression in codes of welfare, arguably one of the more significant is the economic benefits to humans justifying the compromises to animals.

Like animal welfare (55), the term economics has numerous understandings. At one level, it has a business focus, the need to cover expenses and maintain a profit. This is one of the goals of agriculture, along with producing safe and affordable food that is produced fairly and without harming the environment, animals or people. Maintaining profitability is a challenge for any business as decreasing returns and increasing costs squeeze profits. This leads, in many cases, to larger operations benefiting from economies of scale evident in larger farms, flock and herd sizes. And higher animal welfare standards often come at a greater financial cost to the farm system [e.g., see (56–58)]. Profitability is challenging when viewed against the long-term trend of an increasing marketing share of the consumer's spending on food at the expense of the farmers' share (59).

Animal welfare is also an economic concept at another level: the trade-offs we make between our preferences for food and the opportunities for commerce in producing it, vs. our discomfort with whatever animals may experience in the process. Varying social expectations for animal welfare standards mean, for at least some practices, that they may diverge from those able to be provided by commercial farm in animal production–in other words, some improvements come at a cost. Who bears those costs, the methods of placing a value on them, and, ultimately, the behavior of consumers who pay for animal products in the market place, have been extensively described by, for example, McInerney (60, 61) and Norwood and Lusk (62)?

There are many insights of relevance to animal welfare. For example, the marketplace does not reflect the true values of society, only those things exchanged through markets. Nor can it include the views of people not involved in the marketplace–many of those active in advocating for better treatment of animals are, for example, vegans, or vegetarians. Market values can be distorted by subsidies and taxes, the lack of inclusion of externalities, like animal welfare (products from different systems are usually selected on the basis of cost and appearance rather than the impacts of those systems on the animals), and a lack of informed understanding of the differences between different production systems. Finally, consumers value products differently for different reasons and are free to alter their purchasing behavior. Therefore, it is usually necessary for state intervention to ensure efficiency and fairness, for example in setting minimum standards and in redistributing the costs and benefits.

This more involved understanding of economics reveals several interesting aspects about animal welfare. Firstly, food prices vary for all sorts of reasons (seasons, taxes, instore specials, supply) so perhaps the impacts of balancing animal welfare against food security and availability is over simplified. It is important to know the costs of improving animal welfare and how they might be distributed across the food supply chain, as well as the impacts on various consumers. Secondly, the market generally provides little or no reward or incentive to produce to higher animal welfare standards–such standards are increasingly seen as a cost of production, or of market access. As animal welfare is a value largely attributed to farmers providing good husbandry and stockmanship, not consumers, farmers may require economic signals and incentives to reflect the value consumers and society place on animal welfare. Finally, it is important not to overlook the complexity of the food supply system with its many opportunities for people to mistrust, exploit, distort, overlook, or remain wilfully blind to what occurs.

There are, then, many potential economic means of maintaining and enhancing animal welfare. Taxes and subsidies are one option and some countries link subsidies to successful animal welfare inspections. Another method is labeling products from preferred animal welfare-friendly systems enabling motivated consumers to support them. Tradable permits may enable those in more animal welfare-friendly systems to effectively subsidize less humane farming systems. Lastly, quotas can be used to ensure production is limited so that returns cover the expenses of favored systems.

What the above discussion highlights is that animal welfare is not just about the expectations society may have for how animals should be treated by the person in charge of them, but that it is part of a wider and more complex system. A common caricature of much public concern regarding poor animal welfare practices, is that it reflects an excessive focus on profits and uncaring individuals, for example, "farmers are a nasty, greedy, whinging lot." While leaving aside the instances of animal neglect resulting from difficult personal circumstances, relationships and ill-health (18, 63, 64), and the fact that greed may play a part in some cases, such an understanding does not reflect the influence of economic factors. Animal welfare, McInerney (60) argues, is ultimately an economic or socio-political issue, a subset of human welfare. The place and role of economic understanding in animal welfare is yet to be fully realized.

ETHICS, ECONOMICS, AND PASTORAL ANIMAL WELFARE

Maintaining animals in suitable condition, expectations for the provision of shelter and a comfortable resting area free of mud, and the impacts of painful husbandry procedures, highlight the contested nature of pastoral animal welfare. The well-being of animals is compromised for human benefit, often expressed in economics for those involved in farming and its related industries, but also in the supply of safe and affordable food for others. Depending on individual and group perspectives, compromises are sometimes justified, sometimes not. It is society's consensus which ultimately decides when, for example, animals' needs can be legitimately thwarted, or those exposed to excessive mud or hot sun should have access to resources providing greater comfort and well-being. There are balances and limits in the use of animals–generally production is not maximized at the expense of welfare, and welfare is not maximized at the expense of production.

Poor animal welfare raises concerns and expectations amongst farmers, farm industries and the public alike. While, for example, the provision of shelter is part of good farming, there are different understandings of what is good, and different barriers or constraints to providing shelter, including finances, time and resources. Furthermore, there are different expectations of when shelter is required–for the comfort of the animal, that required to maintain its productivity, or that required to survive. Such differences also reflect different understandings of animal welfare–what the animal experiences, how it performs, whether it is natural and even if it is being treated with dignity and respect. Finally, there is the challenge of addressing something best expressed in the view "a cow on a hot day, yeah she's hot, we all get hot. Is that really a problem?" The contested degree of compromise to animals is crucial to determining what compromises animals can be expected to endure, and when assistance or resources should or must be provided, commensurate with public expectations, to assist them to cope.

To the above difficulties, we can further add the insights revealed by using body condition as a management tool, i.e., of the productivity of the herd or flock, and as an indicator of animal welfare, what the individual animal feels or experiences. For example, animals in good body condition traditionally may have been expected to endure muddy conditions. Similarly, the thwarting of animals' behavioral needs, like rooting in pigs, to prevent pasture and soil damage. These examples raise the issue of what needs are important–are some more important than others or, as legislation and the expectations of the five freedoms seem to imply, are all equal and thus must be given equal weight. Can adequate nutrition outweigh the discomfort of mud? And which human needs outweigh animals' needs?

Does protecting the environment outweigh preventing pigs from rooting? One way worthy of further exploration is to perhaps weight animals' needs (65) such those sustaining life (e.g., food), health (e.g., shelter), and comfort (e.g., environmental complexity). This concept is akin to Abraham Maslow's well-known theory of human motivation and has been adapted to animal welfare (66). What it will require, however, is a greater understanding of the importance of social interactions and bonds which lessen individuals' demands for energy expenditure and skills, helping them to survive, reproduce, and care for their offspring, enhancing physical and mental health and thus well-being [see (67)]. The place of social behaviors is yet to be fully integrated into hierarchies of motivation.

Collectively, these aspects highlight the subjective and values-based complexity of animal welfare. Animal welfare, then, has been likened to a "wicked problem" (68)–difficult to describe, complex, changing and subject to inconsistencies and considerable debate. Wicked problems are not easily solved but are, at best, managed and progressed with understanding and compassion. Not surprisingly, there are many directives in guidelines, standards and regulations to provide such resources. Thus, while the resources the animal has access to are in the farmer's hands, expectations are increasingly influenced and determined by the wider community and societal expectations. What is not fully acknowledged is the influence, and indeed role, of that wider community and society, in helping maintain and enhance animal welfare other than in "telling" or "wanting" to "manage a farmer's resources at no cost or risk to themselves" (69).

Though the welfare of an animal is largely dependent on knowledge, beliefs and circumstances of the individuals caring for them, as noted animal welfare standards are determined by society. Consensus requires acknowledging the full spectrum of ethical and economic perspectives that provide opportunities and constraints for individual pastoral farmers. For instance, the recognition that economics is a key driver of farming systems is connected with the view that, at least some consumers see themselves as part of the problem. Society's role in pastoral farming welfare may be addressing the factors beyond the ability of farmers to control, such as financial interest and international exchange rates and consumer preferences, factors which affect the ability of those in charge of animals to care for them. A more sustainable vision of animal welfare borne of understanding the connection between animals and all people, may enable society to fairly balance the demands that livestock have with those of the wider community.

DISCUSSION

Land use has changed from the time of hunter-gatherers and nomadic pastoralists, to extensive and intensive settled pastoralism, and finally industrial or factory farming (70). This has shaped human activities from earliest times (71), enabling many people to live without being directly involved in raising and killing animals for food. These changes have undoubtedly contributed to the different values, preferences and prejudices people have toward the rightful place of animals in society. While the welfare of most animals reflects the care provided by those in charge of them, it is influenced by the wider community, and thus subject to contested scrutiny. The examples described in this chapter illustrate some of the contested issues in animal welfare, tensions borne of animals being compromised for human benefit, whether they are for reasons of financial costs, environmental degradation, or practicality.

Given the different viewpoints and ways of justifying stances, who should help decide society's stance on contested issues? Although there are many perspectives, discussion of three of those: scientists and veterinarians; citizens; and stockpeople, are the subject of this discussion because of the strong beliefs in their place, beliefs that may or may not withstand critical scrutiny. For instance, one perspective is that "there is no doubt that veterinarians are the best equipped and most committed profession to lead the community in animal welfare debate" (72). Such a view ignores the fact that animal welfare is a social construct and not exclusively scientific or veterinary. Furthermore, although veterinarians are well-trained in animal health, they are arguably less familiar with other aspects of animal welfare such as what the animal is experiencing (73), limitations which are now beginning to be more widely addressed in veterinary teaching curricula. Similarly, there are calls for science to guide socially contested issues "with rational application of sound scientific principles." Such calls must be tempered by remembering, for example, that scientific advice need not be sound. For example, as recently as early last century survival rates of children in orphanages were terrible. At the time of the rising recognition of the value of cleanliness in preventing disease, and a desire to make the young field of psychology into a truly objective science, it led to a professional crusade by JB Watson, among others, against the evil of affection ("mother love is a dangerous instrument"). Such advice was given authority because its proponents were objective and scientific experts [see (74)]. In contrast [see (75)], the Scottish philosopher David Hume stated, "reason is the slave of passions and should be." Furthermore, Enrich Fromm held that "reason flows from the blending of rational thought and feeling. If the two functions are torn apart, thinking deteriorates into schizoid intellectual activity and feeling deteriorates into neurotic life-damaging passions." Thus, views based on selected or narrow professional understandings not only have a tendency to measure and value what can easily be assessed rather than what is important to the animal (76), they ignore the value of engaging with all our ways of knowing–ethics, common sense, intuition, imagination, memory, and reason (77).

Another perspective is that advanced by advocacy and activist NGO (non-governmental organizations) interests, often with or through the involvement of popular and social media. Although there are many different motives for an interest in animal welfare (78), including an identity for a minority, a means of expressing prejudiced views and class conflict, to be a part of more general protest movements, or even profit or enlightened self-interest, undoubtedly the main motive is a genuine concern for the well-being of animals. The growth of NGO interests may also reflect persons in charge and regulatory groups not doing their, or society's, job, or at least not engaging in the issue publicly, enabling "the most shrill and dramatic articulations" (79) that tend to demand attention, or those seeking "to privilege the transient urges of the mob over and above social order" (80). This is hardly a sound approach to addressing contested and complex issues. Debates about farming, including animal welfare, have been considered "disappointing intellectually, ethically, and politically." Fraser (81) considers the debate has not resulted in genuine understanding of how farming affects animals, the environment, and the public; the polemic nature of many of the accounts has polarized the debate preventing critical analysis; and the debate has failed to produce shared understanding and consensus. In overcoming these short comings, those in society need to avoid simply aligning themselves with stances and seek knowledgeable analysis of the issues. Such an approach may require the creation of a forum enabling all parties to explore the various aspects of contentious issues (82). There is little time, resource or will to undertake such critical analysis when in the glare of social and public media frenzy.

Finally, we might ask if society, in determining animal welfare standards, is at risk of disregarding the invaluable perspectives of those husbanding animals since it is those persons in charge of animals who are arguably the most important determinants of their welfare. Stockmanship ensures animals have the resources they need to be comfortable, fit and feeling good. Kilgour and Dalton (83) included in the last chapter of their practical guide *Livestock Behavior* that there is the potential for better handling by allowing young animals to learn behaviors, such as lambs learning to eat drought feed whilst they are still with their dams, opportunities generally excluded by management practices such as early weaning and maintaining animals in age and sex cohorts. "Using the old to teach the young" may well apply to stockpeople, professionals and advocates, especially in the current era of narrow specialized expectations.

These different perspectives suggest the issue is not so much who is best placed or qualified to determine animal welfare, but who brings knowledge, and practical experience, whether as a veterinarian, a scientist, a farmer or shepherd, or whatever. It is perhaps more relevant to think of animal welfare in terms of a system, since humans and animals are socially and ecologically interdependent (84). In one depiction (85) at the center of the system are animals. Then there are the persons in charge (e.g., farmers and farm workers); those with oversight of the persons in charge (e.g., animal welfare inspectors and regulatory advisory bodies); and those with an interest in animals (e.g., consumers of food, commerce interests, and animal advocates and activists). Finally, there are citizens, who, while not necessarily having direct vested interests in animals, have a special role in the democratic process. There are many examples of the individuals and groups in society making up the animal welfare system and they can be thought of as actors arranged in concentric bands (**Figure 3**).

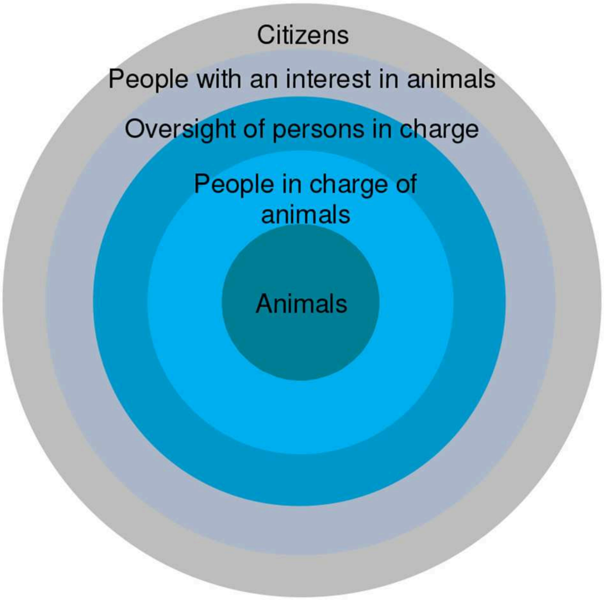

FIGURE 3 | A schematic representation of the animal welfare system [from (85)].

Arranged in this way, the system acknowledges that each group has a role, and thus a responsibility, for animal welfare. Like tourists, individuals within each of the bands see the world from their own perspective in a varied but often limited way. Learning more of the features and expectations of others in different bands may act to change or reinforce our responses, in short having a genuine understanding of each other's interests and roles. It has been suggested that this is undertaken by identifying the issues, providing information and involving people (86, 87). In other words, taking responsibility for complexity by understanding animal welfare as a complex problem, recognizing that there are constraints and opportunities, giving people more autonomy by engaging local institutions, building trust with stakeholders, taking accountability for learning, and broadening dialogues (88, 89). In order to have good welfare, perhaps the most important thing is to give those in charge of animals the confidence, resources and opportunities to develop and deliver what they are best placed to do. Animal welfare is important but not all important–the environment and people, along with the outputs from farming (90) must also be placed within the context of the whole.

Farmers, like many others, are having to respond to a dynamic and complex world and conversations may be better managed as part of a wider debate on environmental management, markets and social expectations. In other words, animal welfare interests must be prepared to couch their preferences within the context of the farming system, and not just the experiences of the animal. It is suggested that society cannot merely tell farmers what to do any more than farmers can expect society to understand farmers' "reality." The future may lie not so much in emphasizing productivity and profitability, but in understanding what animals are experiencing and in building better connections with people to produce more sustainable and equitable farming practices (90). It will be necessary to acknowledge the complexity of the issue borne of different animals, environments and people; and that initiatives may be better managed as part of wider expectations. The future may well-involve society moving from telling or expecting farmers to know how to manage their resources, to encouraging them by providing the confidence, resources and opportunities to provide those resources.

Part of managing expectations may require engagement with the wider community–mediating between the reality of animal needs and the demands of public perception, whether informed or uninformed.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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The Influence of Different Types of Outdoor Access on Dairy Cattle Behavior

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Pasture access for dairy cows is highly valued both by cows and the public at large. When pasture access is not feasible, farmers can provide cows with alternative forms of outdoor access, such as an outdoor bedded pack, that may be easier to implement on some farms. We reviewed the literature on how lying, standing, walking, feeding, social, and estrus behaviors are influenced by pasture and other types of outdoor areas. Pasture allows the expression of grazing and can facilitate the expression of lying, standing, walking, and estrus behaviors. In addition, pasture can decrease the number of negative social interactions between cows, likely because more space per cow is provided than what is normally available indoors. The provision of soft flooring and an open space in outdoor bedded packs appears to provide some benefits for lying, standing, and walking behavior and may also have positive effects on social behavior, especially with larger space allowances. The effects of an outdoor bedded pack on estrus behavior are less well-documented, but the provision of a standing surface that provides better footing than typically available indoors may promote estrus behavior. Alternative outdoor areas assessed to date appear to be less attractive for cows than pasture, perhaps because these areas do not provide the opportunity to graze. We encourage future research to investigate the importance of grazing for dairy cows. The motivation of dairy cows to access alternative outdoor areas should also be investigated. As cow preference for the outdoors depends on many factors, providing cows a choice may be of particular importance.

Keywords: animal welfare, pasture, bedded pack, exercise lot, free range

INTRODUCTION

Pasture access for dairy cows is declining in many parts of the world, even though citizens from different countries view pasture as important [e.g., The Netherlands: (1); Germany: (2); Canada and the US: (3); Brazil: (4)]. Collectively these studies indicate that people value access to natural elements for cows such as fresh air and sunshine and the ability to roam, i.e., elements that extend beyond the provision of pasture *per se*. There is also evidence that cows are highly motivated to access pasture (5). As such, several Nordic European countries have implemented regulations that require farms to provide dairy cows with access to pasture for specified periods of time. Organic standards in many parts of the world also regulate access to pasture, at least for part of the year (6). However, in many parts of the world pasture access is not regulated. When farm size increases, pasture access may also be difficult to implement (7). An alternative to pasture is providing cows access to an outdoor loafing area (i.e., an open area with concrete or other hard flooring) or to an outdoor bedded open pack (i.e., an open area with a soft flooring). Given that these alternative outdoor options generally require less space per cow than pasture and are less subject to damage from cow traffic than pasture, they may be easier to implement on some farms. However, little is known about how alternative outdoor areas influence cow behavior. The aim of this review was to critically assess the scientific literature to understand how key behaviors (lying, standing, walking, feeding, social, and estrus) are influenced by pasture and other types of outdoor area. We also identify gaps in knowledge, especially regarding the use of alternative outdoor areas. Where applicable we draw upon research that investigates cow preference and motivation, as this evidence is especially helpful for drawing inferences regarding the importance of outdoor access to cows (8). We recognize that many aspects of dairy cattle welfare are influenced by pasture and other types of outdoor access, including health and production measures but this is beyond the scope of this review [see (9) for more information]. For the remainder of this review, we will focus on the influence of different types of outdoor access on dairy cattle behavior.

OUTDOOR ACCESS FOR DAIRY CATTLE

In this section, we provide an overview of pasture and alternative types of outdoor access used on dairy farms in those regions where we have been able to find reliable data (Europe, Australia, New Zealand, the United States and Canada). We distinguish between pasture (i.e., an outdoor area with grassland that allows for grazing) and alternative outdoor environments (i.e., any type of outdoor area that has some sort of alternative flooring to grassland, such as concrete or bedding of some sort). Pasture and alternative outdoor areas provide cows with access to the outdoors, but the outdoor environments differ in terms of size and many other features (most notably pasture allows cows the opportunity to graze). This section builds upon the work presented in two recent reviews: one summarized the changes in the global dairy industry affecting dairy cattle health and welfare but did not examine pasture or outdoor access (10) and another focused upon pasture access for dairy cows, but not on alternative types of outdoor access (11).

Europe

Information on pasture access for dairy cows in Europe is not collected in a systematic manner. As such, little is known about which cows are given access to pasture (e.g., young stock, lactating, dry cows, etc.) and duration of access (i.e., days per year and hours per day). In 2019, pasture access in Europe was estimated to range from 95–100% of dairy cows in Ireland, to <25% in Denmark, Poland, and Greece, with most other countries being intermediate [for an overview, please see (12)] (n.b. these figures do not distinguish between farms that provide cows free choice access to pasture from a barn vs. cows housed exclusively outdoors). Data from The Netherlands indicates that in 2018, 71% of the dairy cows aged 2 years and older had access to pasture (13); duration of pasture access was not specified. Regardless, the general trend in the majority of European countries is that the number of farms providing cows with pasture access is declining (12). The exception being some of the nordic countries, such as Sweden, Norway, and Finland, that have implemented regulations requiring farms to provide dairy cows access to pasture for specified periods (12). For example, in Sweden dairy cows must be given pasture access a minimum of 6 h/d, for 60–120 d/y, depending on the region (14). These regulations are based on the assumption that pasture provides cows with an environment in which they can better express natural behaviors such as grazing[1]. Similar to pasture access, there are limited data regarding the percentage of farms in Europe that use alternative outdoor areas. To our knowledge the only available scientific information comes from the 2015 European Food Safety Association (EFSA) report (15) detailing that 3 out of 124 small-scale/non-conventional farms in the convenience sample stated that they used an alternative outdoor area.

Australia and New Zealand

Data collected in 2016 showed that about 99% of Australian dairy farms provided cows pasture access. The large majority (89%) kept cows on pasture year-round; 6% of the farms kept cows on pasture during most of the year but also provided supplementary feed (i.e., partial mixed ration) on an outdoor feed pad; 3% of the farms kept their cows on pasture for less than 9 m/y with a partial mixed ration provided on an outdoor feed pad, the latter two used some type of indoor housing or sheds the rest of the year [(16); personal communication]. Alternatives to pasture are commonly referred to as permanent feed pads with the majority of these using concrete flooring; temporary feed pads also exist and are generally differentiated from the permanent feed pads in that they have either a dirt or rubble (i.e., crushed rock and other materials with a range of particle sizes that can be compressed) base (17).

It is thought that more than 99% of dairy farms in New Zealand provide pasture access during some time or during the full year (DairyNZ, personal communication). Approximately one quarter of farms have an off-paddock system (i.e., an area that cows can be kept on during adverse weather conditions, or to reduce feed wastage) available on the farm. Of the farms using an off-paddock system, 81% do not provide any form of cover. Generally, the lying area comprises at least 80% of the total off-paddock surface area; concrete, gravel, and wood-chips are the most common form of surface material; the remaining area is often a concrete feed pad with feed through [(18); personal communication].

United States and Canada

Pasture-based dairy farming was once the norm in the United States (19), but data from 2013 show that pasture is used as the primary system for fewer than 3% of lactating cows and for 5.0% of dry cows (20). A total of 19.9% and 34.0% of lactating and dry cows, respectively, had some pasture access

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[1] Djurskyddslag SFS 2018:1192. Available online at: http://rkrattsbaser.gov.se/sfst?bet=2018:1192 (accessed May 1, 2019).

(21). Approximately 26% of dairy cows in the US are housed in free-stalls with access to an open/dry lot and ∼17% are housed in open/dry lots with or without access to a barn or shed (8.8 and 8.3%, respectively) (20). Although the majority of US dairy farms are still relatively small (i.e., in 2017, 74% of US dairy farms had <100 cows), 55% of all US dairy cows are housed on farms with >1,000 cows (22). As the percentage of lactating cows that have access to pasture decreases with increasing herd sizes, and an increasing volume of total milk production is produced by larger farms (23), the proportion of US dairy cows that have access to pasture is likely to decline.

The National Dairy Study (2015) conducted in April–May 2015 [for detailed methodology, see (24)] contacted all dairy farms in Canada ($n = 11{,}664$) and obtained information regarding pasture usage. A total of 1,062 producers completed the full questionnaire (9% response rate). Of those farms that responded, 29.1% provided their lactating cows access to pasture with an approximate minimum average of (±SD) 20.5 ± 6.1 w/y. This corresponds to a total of 18.6% of lactating cows, although this number should be interpreted with caution, given that a significant proportion of all participants did not provide the number of lactating cows on their farm. Pasture use differed for lactating and dry cows and also by province (**Figure 1**). A total of 57.3% of farms provided dry cows pasture access, with an approximate minimum average of (±SD) 20.9 ± 9.5 w/y (farms often only provide dry cows access to pasture in spring and summer time, or weather permitting). This corresponds to a total of 49.2% of dry cows, although this number should again be interpreted with caution.

Collectively, the available evidence indicates that pasture use differs by region. Pasture usage is generally expected to decline in Europe and North America, driven by increases in farm size (7, 12). In addition to open lot dairies, some farms provide access to alternative types of outdoor environments such as bedded open packs or exercise lots (i.e., non-bedded areas with concrete or dirt as flooring), although the number of farms doing so appears to be limited outside of the US, Australia, and New Zealand. It is largely unknown what percentage of farms keep their cows outside as opposed to providing cows free choice access to the outdoors and some sort of indoor facility or covered area.

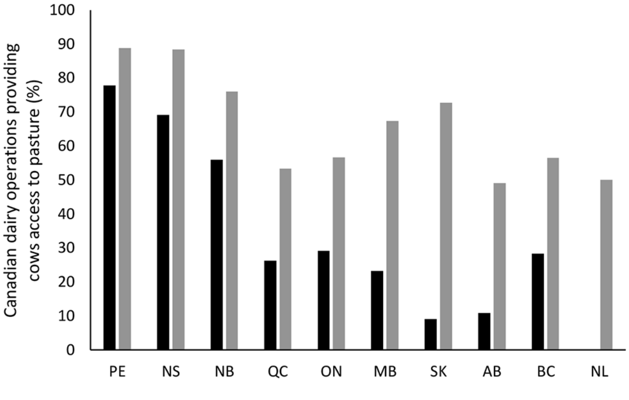

FIGURE 1 | Percentage of operations that allow lactating (black bars) and dry (gray bars) cows access to pasture in Canada, by province (data should be viewed with caution as the survey only had a 9% response rate).

Space requirements for pasture, especially as herd sizes increase, may be one reason why pasture access is not provided. In addition, during some parts of the year pasture access may not be practical, for example during rainy seasons when the soil is subject to damage from cow traffic. This may be a reason why pasture access is more feasible in some parts of the world (e.g., New Zealand, Australia) than others (e.g., Canada and the USA). As will be discussed in the section on feeding behavior, some farmers believe that there are production benefits associated with zero-grazing systems. Farmer characteristics are important predictors of the degree of pasture access provided, as shown in studies with Irish (25) and German farmers (26). Given that the social factors influencing decisions regarding pasture access may differ by country, more research in this area is needed. In addition, most social science work has focussed on pasture access, and information regarding alternative outdoor areas is lacking.

DAIRY COW PREFERENCE AND MOTIVATION FOR OUTDOOR ACCESS

Preference testing requires animals to choose between two or more options (8). The "preferred" option is typically identified as the one that is chosen most often, consumed in the largest quantity, or where the majority of the available time is spent (27). Motivation testing investigates how hard an animal is willing to work to obtain access to a resource (28), i.e., a commodity or the opportunity for the animal to engage in a certain behavior (29). The stronger the motivation to access a resource, the more important that resource is thought to be for the animal (8, 30). Hence, welfare is thought to be more negatively affected if an animal is denied access to a resource for which it is highly motivated (8). Both preference and motivation tests may be affected by the animals' familiarity with the resource and can be influenced by the context in which the experiment is conducted (e.g., weather, time of day, etc.). These and other factors [for a comprehensive overview see (8)] should be taken into account when designing these types of experiments.

Several studies have shown that dairy cattle have a partial preference for pasture access (5, 31–35), with cows choosing to spend from 8% (34) to 72% (35) of their available time on pasture. Experience plays a role in dairy cattle preference for pasture. The cows used in the study of Charlton et al. (34) were reared indoors, potentially explaining why they only spent 8% of their time on pasture. Preference for pasture is influenced by environmental conditions, with high temperature-humidity index (THI) (31), and rainfall (31, 33, 34) decreasing the time spent outside. Cows prefer to spend time on pasture at night rather than during the day (31–33), possibly to avoid high solar radiation during the day (36). Several motivation tests have shown the importance of pasture access for cows, especially at night (5, 33). The quality of the indoor environment may also influence the value of outdoor access for dairy cattle. In a study by Falk et al. (37), cow preference for pasture was not influenced by the number of lying stalls available indoors (24, 16, 8, or 0 stalls per group of 24 cows), showing that even when overstocked cows preferred to be indoors for much of the day and on pasture at night. More research on how the indoor environment influences preference to be outdoors is needed. For example, the provision of an indoor open pack may influence the preference for pasture or an alternative outdoor area.

Despite numerous studies on the importance of pasture access for dairy cattle, little is known about what aspects of pasture are important to dairy cattle. For example, it is not known whether this preference for the outdoors is driven by a desire for more space, cooler air, softer surfaces, grass to graze, or some combination of these and other factors. When free-stall housed, mid-lactation dairy cows could choose between a large pasture or a smaller (i.e., 12 m^2/cow) outdoor sand pack during the night in late summer, they spent around 90% of their time on pasture and only 1% on the sand pack (38). This preference could have been driven by the larger space that was available on the pasture compared to the sand pack, the ability to graze on pasture, or other factors. Similarly, Kismul et al. (39), showed that early-to-mid lactation cows with access to a small exercise pasture (0.2 ha grass-covered paddock with little herbage and provided *ad libitum* grass silage indoors) spent 44% of their time outside, compared to 81% for cows provided access to a production pasture (larger pasture with ample herbage and restricted access to grass silage indoors) (both groups were given 8.5 h/d outdoor access). Jørgensen et al. (40) showed that cows provided access to an exercise paddock (0.74 ha in size in a small forest) spent less time outdoors than did cows provided access to pasture (a total 2.8 ha in size that was used for strip grazing). These two latter studies were based on a single group of cows in each treatment and thus should be interpreted with caution given the lack of replication. When preference of free-stall housed cows for access to an outdoor pack was tested in summer and winter, cows spent 25% of the time outside in summer and only 2% in winter (41). Cows especially spent time outside during summer nights (50.0 ± 8.4%) rather than during summer days (3.3 ± 1.3 %) and generally avoided adverse weather (i.e., snow, strong wind, and/or low air temperatures) during the winter months. Haskell et al. (42) investigated the use of an outdoor concrete loafing area by free-stall housed cows when given the option during the day. These authors reported that the cows spent about 14% of their time outside on the concrete loafing area during the day in spring and summer, with the majority of this time being when the weather was sunny; cows rarely went outside in the rain. Except for cows given access to the production pasture in the study of Kismul et al. (39), feed was provided indoors in all studies such that cows could fulfill 100% of their nutritional needs without the need for grazing. Given that cow preference for pasture and alternative outdoor areas is affected by many factors, providing cows a choice to access the outdoors may be of particular importance. In addition, providing animals controllability over their environment likely enhances their welfare (43).

In the following section, we will outline how various dairy cattle behaviors (i.e., lying, standing, walking, feeding, social, and estrus behaviors) are influenced by different types of outdoor access, and how providing choice to access the outdoors can affect behavior.

THE INFLUENCE OF DIFFERENT TYPES OF OUTDOOR ACCESS ON SPONTANEOUS DAIRY CATTLE BEHAVIOR

Lying, Standing, and Walking Behavior

Lying is a highly motivated behavior in dairy cows, with cows prioritizing lying over feeding after a period of deprivation of both behaviors (44). Heifers appear motivated to lie down for 12 to 13 h/d when housed in a tie stall (45); cows trained to push open a weighted gate to access an open deep-bedded lying area worked to maintain a lying time of 13 h/d (46).

Generally, cows housed on pasture have lower lying times compared to when housed indoors. For example, cows kept on pasture lay down for 10.9 vs. 12.3 h/d when housed in a free-stall barn (47). Other studies reported average daily lying times between 7.5 and 9.5 h/d for cows housed on pasture (48–50). Cows in free-stall barns typically lie down for 10–12 h/d (51–55), though large variation in average lying times exist between farms [between 9.5 and 12.9 h/d was reported by (52); between 8.7 and 13.2 h/d was reported by (55)]. Despite having lower lying times when housed on pasture, cows given the choice between pasture, and a free-stall barn generally chose to lie on pasture rather than indoors [e.g., (31, 56)], except during summer days when cows generally stay indoors [e.g., (31, 37)]. Cows are able to engage in a broader range of lying positions when housed on pasture, including lying flat on the side (57); the ability to adopt these positions may help explain cow preference for lying on pasture compared to the more restrictive lying environment of free-stalls. The surface type may also influence preference: when cows had a choice between an outdoor wood-chip area and pasture, they spent most of their lying time on the grass (58).

The lower daily lying times on pasture may be a consequence of time spent grazing, but to our knowledge no studies have attempted to disentangle whether indoor-housed cows provided access to pasture prefer to graze or to lie for long periods of time. Typical grazing times are difficult to estimate, given that these depend on several factors, including herbage height (59, 60), herbage allowance (61), and concentrate supplementation (62). Feral cattle spend from 6.8 to 13.0 h/d grazing [reviewed (63)], and Holstein–Friesian cows appear to spend about 9.2 h/d grazing (64, 65). Given these estimates, grazing time does not appear to be affected by lying time, but future research should investigate this. Higher lying times indoors may also be a consequence of boredom; an alternative explanation for the longer lying times reported in free-stalls compared to pasture is that cows are seeking refuge from the concrete standing surfaces elsewhere in the barn; soft, dry standing surfaces are rarely available indoors (66, 67). Lying stalls were designed to provide cows with a place for lying and not for standing. The ability of the cow to use the lying stall for standing is affected by the positioning of the neck-rail, with more aggressive positions (closer to the curb) increasing two foot standing (perching) in the stall (68). Hence, perching may be a result of cows looking for a soft place to stand, especially when the placement of the neck rail prevents cows from standing with all four feet in the stall (68). When housed in pens with rubber flooring in front of the feed bunk, cows spent less time perching, and standing fully in the free-stalls and less time lying down in the free-stalls, and more time standing idle at the feed bunk (66, 69). Boyle et al. (70), however, found no difference in lying time between cows housed in free-stall pens with concrete or rubber flooring, but found that cows housed on concrete stood more in the free-stalls compared to cows in pens with rubber flooring. In the latter case, cows stood more on the rubber flooring at the feed face, again suggesting that cows seek refuge from standing on hard surfaces. Taken together, these studies indicate that standing on a soft surface is important for dairy cattle.

These results may also help explain the partial preference of free-stall housed cows for outdoor bedded packs. Fregonesi et al. (71) showed that cows preferred to spend time both lying and standing fully in an indoor open bedded pack compared to free-stalls, potentially because of the less restrictive environment of the open pack. A study by Smid et al. (38) provided free-stall housed cows free access to an outdoor open sand pack or pasture during the night. Although the amount of time spent in each location differed, the proportion of time cows spent lying down outside was similar when given free access to a sand pack (55%) or pasture (52%), indicating that cows may find the outdoor pack comfortable for standing. Another study provided cows free access to an outdoor wood chip pack in summer and winter and found that in summer, 54% of the time that cows were outside was spent lying down. This again shows that cows preferred to stand on the outdoor pack for a significant amount of time. In winter cows spent little time outside and of the time spent outdoors only about 5% was spent lying down (41).

Cows generally walk more on pasture than in a free-stall barn [e.g., (72, 73)], likely because of the need to move while grazing. Exercise has been suggested to be positive for dairy cattle welfare (73), although the higher energy expenditure of cattle on pasture compared to zero-grazing systems may pose challenges (74). Pasture systems are often associated with lower body condition scores in dairy cattle [e.g., (75, 76)] emphasizing the need for good pasture management. The increased opportunities for exercise in outdoor areas compared to the generally more restrictive indoor housing environments may also provide benefits for animal welfare. This may be especially true in bedded packs as cows prefer to walk on softer materials such as rubber than on concrete flooring (67), potentially because they are more prone to falling and slipping on concrete (77).

Feeding Behavior

Dairy cattle are able to utilize high roughage diets, but to maintain milk production and minimize body condition loss (75) many dairy cattle are fed more energy dense diets [often provided as a mixture of roughage and grain products, or as a total mixed ration [TMR; (78, 79)]. Ration formulation varies based on the nutritional demands of cows [see (80)]. On average, milk production increases when the diet is supplemented with grain (81, 82), and the perceived production benefits of feeding a mixed ration is one reason why cows are no longer kept on pasture (21).

It is important to distinguish between choice and forced outdoor systems. When cows were provided a choice between free-stall housing and pasture, they maintained much of their TMR intake, and increased their feeding rate as compared to when they were confined in the free-stall barn (31). A similar result was reported by Smid et al. (41) who provided cows access to an outdoor wood-chip pack and found that cows showed a small decline in their feeding time in summer, but no decrease in feeding time in winter, compared to when confined in the free-stall barn. When cows were provided access to pasture or an outdoor sand pack during the night, they had lower feeding times than when kept indoors day and night. However, regardless of the option to go outside during the night, cows maintained their feeding times indoors during the day (38). Overall, these studies indicate that, when provided a choice to access pasture or an alternative outdoor area, cows maintain much of their TMR intake. Cows can also maintain their intake (and milk production) when kept on pasture at night and indoors during the day, relative to cows kept permanently indoors (83).

An important difference between pasture and alternative outdoor areas is that only the former allows grazing. Cows given access to an alternative outdoor area are generally provided access to a TMR or partial mixed ration (PMR), feed sources that do not allow them to engage in typical "grazing" behavior. It is likely that cows are motivated to graze, but little work has addressed the importance of grazing for cow welfare. The inability to engage in natural feeding behaviors is associated with the development of stereotypic and other abnormal behaviors in many animal species [e.g., pigs: (84), giraffe: (85), chimpanzee: (86); horses: (87)]. Stereotypic behaviors often resemble the behavior that is thwarted (84). When grazing, cattle roll their tongue around the grass to ingest it; this behavior resembles tongue playing or tongue rolling [i.e., "twisting and twirling with the tongue, either inside or outside the open mouth," (88)], one of the most common stereotypies in cattle. As described by Beauchemin (89), cows fed a TMR use their lips to ingest feed, as opposed to their tongue to ingest long-stemmed forage or when grazing grass (90). In experimental settings, oral stereotypies in cattle were never observed on pasture (90–92), but were present in loose housing (91). Thus, the method of feed ingestion may be as important for animals as the goal itself (i.e., ingesting feed). Interestingly, in mountain breeds such as Brown Swiss and Simmental, tongue rolling is more prevalent compared to other cow breeds (93). The reason for this is unknown. Jerseys also seem to be especially orally motivated, showing a higher frequency of cross-suckling than Danish Red or Holstein–Friesian calves (94). The lower prevalence of tongue rolling in other breeds does not necessarily indicate that they are less motivated to graze or to obtain roughage.

Prior experience may play an important role in determining the preference for pasture (11). Naïve heifers grouped with cows that had experience with grazing had a lower latency to graze compared to groups consisting of only naïve heifers. Differences in grazing behavior between the treatments were found only for the first hour after pasture introduction, however (95). Heifers that had experience with pasture spent less time grazing, but more time ruminating, compared to heifers with no experience with pasture. This potentially indicates more efficient grazing behavior of the former (96), grazing itself may not be the only factor influencing the preference for pasture access. As pasture provides cattle with roughage, grazing is confounded with roughage consumption. Research in this area is again limited, but the development and frequency of stereotypies has been linked with feeding low amounts of roughage (97). Calves appear to prefer long over chopped hay (98), and work on beef cows found that they were highly motivated to obtain roughage, especially when kept on a low-roughage diet (99). Collectively, these results indicate that access to roughage and the manipulation of feed are important to cattle, as also suggested in a review (100) on the importance of straw for dairy cattle.

The time of day that cows spend feeding indoors is mainly determined by the time of fresh feed delivery (101). On pasture, cows feed mainly during the day, with intense grazing bouts at dawn and dusk (102–104). Cows housed on pasture often show synchronized feeding (105) and lying behavior (73, 106), which is thought to be positive for their welfare, perhaps especially so for more subordinate cows (107).

Given that milk production per cow has more than doubled in the last 40 years (108), selection for milk yield may cause high producing dairy cows to be especially motivated to consume a high energy ration like that which is normally provided indoors. Given the selection for high milk production and the correspondingly high energy requirements, it has been questioned if certain dairy genotypes are suitable to be housed exclusively on pasture (109). We encourage research to disentangle the importance of grazing, roughage and energy provision for dairy cattle. Given that cows are unable to perform grazing behavior in alternative outdoor systems, understanding the importance of grazing for dairy cattle welfare will also provide insight into the acceptability of providing cows these alternatives. Studies have investigated the effect of outdoor access on feeding behavior, but these studies have not reported effects on drinking behavior. Given that the stocking rate for drinking places is typically higher than that for feeding places (110), and competition around the drinker may be expected, future work on drinking behavior is required.

Social Behavior

Social behavior includes positive and negative (i.e., agonistic) interactions. Positive interactions in cows have not been studied extensively but there is some evidence that allogrooming (i.e., social licking) is important (111, 112). In contrast, agonistic interactions between cows have been well-studied and consist of multiple forms of aggressive behavior, such as displacements, pushes and head butts (88, 113, 114). Housing is thought to play an important role in the frequency and display of these interactions (115).

In free-stall housing, competition for resources such as feeding and lying areas may pose challenges. It is well-known that increased stocking density leads to increased competition for access to the feeding area in free-stall housed dairy cattle [e.g., (116, 117)]. When given a choice, cows prefer to have greater inter-cow distances than what is normally available in indoor systems (118, 119). Tresoldi et al. (115) investigated social behavior in dairy heifers housed in either a free-stall barn or kept on pasture. When housed in free-stalls, heifers exhibited a 4-fold increase in the number of social interactions (allogrooming as well as agonistic interactions) compared to when housed on pasture, but the ratio of positive to negative interactions was the same in the two environments. Less space was available indoors than on pasture, leading the authors to suggest that the higher number of social interactions observed indoors was a consequence of a higher stocking density.

Similar observations have been made for other types of outdoor areas. Heifers on an outdoor wood-chip pack, provided an individual space allowance of 8 m^2 on the pack and 6 m^2 on the concrete feeding area, showed increased frequency of play behavior, including social play, compared to heifers housed inside a free-stall pen that provided 5.3 m^2/heifer (120). These heifers also had a higher frequency of allogrooming, but showed no difference in the frequency of agonistic interactions. Schütz et al. (121) reported that a minimum of 6 m^2 of space allowance per cow was needed on an off-wintering rubber pad during an 18 h stand off period to maintain daily lying times similar to that observed when cows were housed on pasture. When cows were provided less space (3 or 4.5 m^2/cow), the reduction in both lying time and lying bout duration and frequency was thought to be due to increased agonistic behavior. Free-stall housed cows given access to an outdoor open wood-chip pack spent more time outside during the night with increasing outdoor space allowance (range of space allowances tested: 4–16 m^2) (122). Interestingly, outdoor space allowance did not influence the number of displacements from a lying position that cows were engaged in on the outdoor pack. However, as the authors argue, this latter finding may be a consequence of cows having the opportunity to avoid agonistic interactions by moving indoors, particularly when outdoor space per cow declines. Another study (42) reported that, compared to high-ranking cows, low-ranking, free-stall housed cows used an outdoor concrete loafing area more during the pre-feeding and feeding period, suggesting that the use of the outdoors may in part be affected by social rank. There is also some preliminary evidence suggesting that cows housed on an out-wintering woodchip pad showed a higher synchrony in lying and feeding behavior than free-stall housed cows (123). These authors argued that increased synchrony may be a positive indicator of welfare, but the work should be viewed with caution given that there were only two replications per treatment.

As an increase in space allowance generally results in reduced interactions between cows, it follows that providing cows with an additional outdoor space will result in a decline in social interactions. However, there has been little experimental work looking at how much space cows require on an alternative outdoor area. The Canadian Dairy Cattle Code of Practice (124) states that resting areas in bedded-pack pens must provide 11 m^2 per mature cow, but no justification is provided for this number. New research is required to investigate the space requirements of individual cows when provided different forms of outdoor access. Studies should include social rank when investigating cow preference, as social rank may play an important role in the preference of dairy cows for certain environments. In addition, the effect of the choice to go outdoors on social interactions should be investigated, especially on outdoor areas other than pasture that typically provide less space per cow.

Estrus Behavior

The estrus cycle in dairy cows is, on average, 21 days in length (125), with estrus behavior expressed between 2 and 24 h (126). Estrus behavior in dairy cows can be divided into primary (i.e., standing to be mounted) and secondary signs (i.e., anogenital sniffing, chin resting, successful, and unsuccessful mounts) (127).

Free-stall systems where cows are for the most part continuously housed on concrete flooring (representing the vast majority of US dairy operations; (21) pose a challenge for estrus expression. Cows housed in free-stall barns with concrete flooring have fewer standing estrus events (128) and a lower frequency of standing to be mounted compared to cows housed on pasture (129). Similar results were found comparing concrete with other types of flooring; for example, cows had a lower duration of estrus as well as a lower frequency of mounting and standing to be mounted when kept on concrete compared to dirt flooring (130). The effects of rubber flooring in loose housing systems are variable; cows housed on rubber mats showed a higher frequency of mounting than when housed on concrete (77), but no beneficial effects on estrus behavior of rubber over concrete flooring were found by Boyle et al. (70). Differences in rubber quality may explain this difference (131, 132).

Vailes and Britt (133) suggested that cows may feel unsure of their footing on concrete and are therefore less inclined to perform estrus behaviors on this flooring. Concrete flooring has been linked with more slipping during mounting compared to pasture (128) or rubber flooring (77). In the latter study, 19 out of 23 mounts on a concrete floor were accompanied with collapsing or slipping. Little information is available regarding the effect of alternative outdoor areas on estrus behavior. Cows housed in a covered straw yard had more successful mounting attempts compared to cows housed in a free-stall (134), possibly because the straw flooring provided them with better footing. Indeed, when given a choice between concrete and dirt, cows in estrus spent more time on dirt than on concrete and preferred to mount other cows that were in estrus on dirt rather than on concrete (133). However, the latter study was conducted with individual cows that were given 30 min to interact with two tied cows, one on concrete and one on dirt; to our knowledge no research has examined preferences for different types of flooring during estrus in dairy cows housed under commercial conditions.

Concrete flooring can also increase the risk of lameness in dairy cows [e.g., (47, 135, 136)]. Lame cows may be less inclined to engage in estrus behaviors, especially if the flooring contributes to their pain. Lame cows have lower behavioral estrus expression than non-lame cows (137). In addition, falling and slipping when mounting can increase the risk of trauma and lameness.

Based on these results, housing systems with softer, high traction flooring such as pasture, dirt, or deep bedded packs may facilitate the expression of estrus behavior in dairy cows. Access to an outdoor area with better footing than is provided by concrete may be especially beneficial to cows in estrus.

CONCLUDING REMARKS

Pasture can provide cows with an open area and a soft surface that allows the expression of grazing and facilitates the expression of lying, standing, walking, and estrus behaviors. In addition, keeping cows on pasture decreases negative social interactions between cows, potentially because cows on pasture engage in fewer encounters compared to when housed indoors. Alternatives to pasture include outdoor loafing areas (often with concrete flooring) or outdoor open bedded packs. Given challenges with concrete or other hard flooring in terms of lying, standing, and walking behavior, bedded packs may be more suitable than concrete loafing areas. Access to an outdoor open bedded pack can facilitate lying, standing and walking behavior and may also have positive effects on social behavior. The benefits of an outdoor bedded pack on estrus behavior warrants more research, but the available evidence shows that outdoor bedded packs can provide better footing than is available indoors, minimizing slips which can be beneficial for estrus behavior. Alternative outdoor areas assessed to date appear to be less attractive than pasture, perhaps because these areas do not provide the opportunity to graze. We encourage future research to investigate the importance of grazing for dairy cattle welfare. The motivation of dairy cows to access alternative outdoor areas should also be investigated. Given that cow preference for the outdoors depends on many internal and external factors, providing cows a choice between well-managed indoor and outdoor areas may be of particular importance.

AUTHOR CONTRIBUTIONS

All authors contributed equally to conceptualization. A-MS performed the literature search and wrote the initial version of the review. DW and MK provided critical revisions.

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Effects of Housing System on Dairy Heifer Replacement Cost From Birth to Calving: Evaluating Costs of Confinement, Dry-Lot, and Pasture-Based Systems and Their Impact on Total Rearing Investment

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Replacement heifer rearing is critical for the future of dairy operations, to improve genetic merit and maintain herd size. A myriad of options exist on how to manage, feed, and ultimately raise replacement heifers. Pasture is perceived to offer optimal welfare and an economical housing system for replacement animals, but confinement systems are gaining popularity. This study investigates the costs associated with replacement heifer management decisions from birth to calving, considering the factors of housing systems, labor, feed, and health. The objective of this study was to develop an economic model to determine the cost of raising a replacement heifer managed in confinement, dry-lot, and pasture-based scenarios post-weaning. We accounted for variation in feed, labor, and health inputs and quantified the impact of these individual management decisions. An economic simulation with 10,000 iterations were completed for each situation using @Risk and PrecisionTree add-ons (Palisade Corporation, Ithaca, NY) where health incidence, commodity prices, and management variables were made stochastic. Published literature or sample farm data created parameters used in Pert distributions. Costs and biological responses were reflective of published surveys, literature, and market conditions. Management decision inputs had 3 main factors: housing type, ration composition, and labor utilization. Housing systems were calculated separately for confinement, dry-lot, and pasture scenarios. The mean total cost (min, max) to raise a replacement heifer from birth to calving, assuming the same pre-weaning strategy of group housing with an automatic calf feeder, was found to be $1,919.02 ($1,777.25, $2,100.57), $1,593.57 ($1,490.30, $1,737.26), and $1,335.84 ($1,266.69, $1,423.94) for confinement, dry-lot, and pasture, respectively. Total housing cost per replacement heifer was $423.05, $117.96, and $207.96 for confinement, dry-lot, and pasture management systems, respectively. When compared to total cost, housing contributed 21% for confinement, 7% for dry-lot, and 15% for pasture. Upon analysis of all scenarios, utilizing pasture to raise heifers resulted in a lower overall cost when compared to confinement housing options. Percentage breakdowns of feed, labor, housing, and fixed and variable costs provided more information on efficiency rather than total cost, which makes each situation different in relation to on-farm cost. This cost analysis is critical to assisting farms in making decisions in the utilization of their resources for replacement dairy heifers.

Keywords: stochastic model approach, dairy economics, dairy calf, young stock, dairy management, on-farm decision tools

INTRODUCTION

Access to pasture is generally assumed to improve welfare for dairy cattle [reviewed by (1)]. However, dairy cattle in many parts of the world are housed on zero grazing or continuous housing systems, especially North America (2, 3). Many of these cows have already entered the milking herd, where confinement housing is used as a tool for more intense management. Pasture is still an important part of the housing system in many of these intensive farms, where pasture is still commonly used in spring and summer to feed heifers and animals with low energy demands, for reasons of lower feed and labor cost.

Replacement heifers are the second largest annual operating expense on the farm, behind only feed cost (4). The cost of raising a replacement heifer is increasing and plays an important role in dairy enterprise economics (5, 6). Heifer raising cost is directly related to feed, housing, and labor demand, which can all contribute to increased cost. In the Netherlands, the difference in actual and perceived cost of heifer retention averaged $898.19 (7). The difference in cost is accounted for in the operation, but it is normally misallocated to another area of dairy expenses. Therefore, determining the true on-farm cost of raising replacement heifers is the first step in better managing these costs.

The decisions that producers make regarding housing options can impact total cost, the development of heifers, and labor utilization. In 2014, the most common housing types for weaned heifers were (1) group housing in a barn and (2) open, dry-lot areas with a barn or shed shelter. While these two housing systems represent over half (54.6%) of all heifers in the United States, over 10 different housing management styles were represented (8). Housing of replacement heifers accounted for 17% of the total cost to raise a weaned heifer in a report from Wisconsin, USA (9). Inputs contributing to housing costs include barn payments, electricity, bedding, and maintenance costs. A potential cost-saving and animal welfare-friendly option would be to raise heifers on pasture. Pasture is utilized by 13.1% of producers for weaned heifers (8). Dairy operations in the Eastern region of the USA are utilizing pasture more than those in the West (10). The adjustment period appears to be quick regardless of whether heifers are kept on pasture for the entire period as a heifer or a select time frame; heifers in the milking herd that were housed previously in confinement for at least a year acclimated to pasture within 3 days.

Analyzing replacement heifer raising costs can uncover additional information about resources utilized on the farm and can assist in evaluating the efficiency of an operation. Feed costs are the primary expense, accounting for 60–73% of all expenses during the rearing period (5, 6). In a 2013 survey of Pennsylvania producers, labor utilization (the second largest contributor to cost) was a clear distinction between efficient and inefficient farms. Farms labeled as efficient were allocating on average $140 in labor resources for each replacement heifer (6). Additionally, biological management decisions can influence the total cost of raising a replacement heifer. For example, raising replacement heifers to be bred to calves at 24 vs. 25 months has the potential to save considerable costs for the dairy enterprise (4). Decreasing cull rates of the milking herd has a direct influence on the cost of the entire heifer raising enterprise, by lowering the required number of heifers to be raised [(4, 11)].

There are multiple options for how to raise replacement heifers on farm, with each decision presenting a unique cost. Many current investment decisions made on dairy operations are based on tradition or intuition, providing an opportunity for more objective methods of investment analysis (12). While there are many factors that influence decisions about dairy heifer raising, including tradition, animal welfare, and environmental concerns, herein we focus on economic efficiency as a primary decision point in heifer raising. The objective of this study was to develop an economic model to determine the cost of raising a replacement heifer managed in confinement, dry-lot, and pasture-based scenarios post-weaning. Furthermore, we account for additional variation in feed, labor, and health inputs and quantify the impact of these individual variables on the total cost.

MATERIALS AND METHODS

A heifer cost simulation model was created in Excel 2013 (Microsoft, Redmond, WA, USA) utilizing @RISK add-ons (Palisade Corporation, Ithaca, NY) at the University of Kentucky Dairy Science program. This model serves as the extension to a pre-weaning model described in Hawkins et al. (13). Briefly, the pre-weaning period is an intensive time for raising replacement heifers and total costs for changes ranged from $258.56 to $582.98 (13). Because of the variation in cost during this time period in this analysis, all heifer calves are assumed to follow the growth and cost patterns seen from heifers raised on an automatic calf feeder in group housing, fed milk replacer, and allotted 8 L of milk per day. The total cost found (±SD) was $352.40 ± $16.70 per calf for the pre-weaning period. This accounts for variation in diarrhea and respiratory illness, mortality rate, and weaning age.

Replacement heifer costs were separated into age groups (3–6, 7–10, 11–14, and 15 months to 60 days pre-calving) representing common biological and management changes, such as weaning or reaching puberty, or a change of housing (such as housing heifers on pasture after breeding). Each age group was developed in a new layer within the model to be calculated separately and summed at the end. This opens the possibility of changes within each age category in further model development without changing the rest of the calculations. Management decision options were required for 3 main factors: housing type, ration composition, and labor utilization. The cost associated with each decision was calculated by day; therefore, within each month group, a producer could allocate how many days heifers were utilizing specific resources. This structure allows for more flexibility to account for differences from one farm to the next. Housing could be one of three options: confinement, dry-lot, or pasture. Rations were utilizing corn silage or pasture supplemented with grain. A visual representation of post-weaning management decision pathways for housing, feed, and labor are outlined in **Figure 1**. Based on previous decisions, only one possible option may be available. For example, if pasture is used within the heifer rearing system, then the only labor option would be time required to care for a heifer on pasture.

Breeding and health-related costs were calculated separately. Health costs per age group were combined with the corresponding month totals, while totals for breeding were incorporated into the final overall cost calculation. All calculated total costs per age group and management style were presented in an overview spreadsheet.

Variables related to health incidence, commodity prices, and on-farm management variables were made stochastic with @RISK simulation. Pert distributions were utilized with parameters set from published literature or sample farm data. A convenience sample of 12 dairy farms located in the states of Ohio and Indiana provided annual financial data to aid in the creation of assumptions. **Table 1** outlines the key assumptions made by authors for the calculation of the cost of replacement heifers from weaning to calving.

Housing

Housing costs were calculated separately for three potential options: confinement, dry-lot, and pasture. For the confinement housing scenario, a barn cost per replacement heifer was calculated. The required square meter of barn space was calculated based on the age group and number of animals from the input page. Square meter requirements per replacement heifer began at 2.8 m^2 at 3–6 months and increased 0.93 m^2 with each age group (16). The total required m^2 was multiplied by the construction cost per m^2 to calculate the barn value. Barn payments were calculated, including interest and depreciation, then broken down by total number of heifers utilizing the barn. Dry-lot and pasture housing scenarios both incorporated land value as the base of housing cost. Pasture, as a housing system, was calculated separately than the nutritional content gained by using pasture as a feedstuff. Average per acre rental rate in Kentucky was used as the assumption to value the land (15). Annual pasture maintained per acre was assumed at $31.50, accounting for seed, equipment, upkeep, and labor. Based on the University of Massachusetts recommendation, 0.5 acres is required per 227 kg of animal and was used to determine the number of replacement heifer per acre. Daily pasture price per animal was calculated using Equation (1). For dry-lot housing, 55.7 m^2 was required per replacement heifer and used to calculate required spacing. Additionally, dry-lot housing included the calculated investment of 3.71 m^2 shade per replacement heifer, valued at $0.13 per m^2 (17). All housing options accounted for

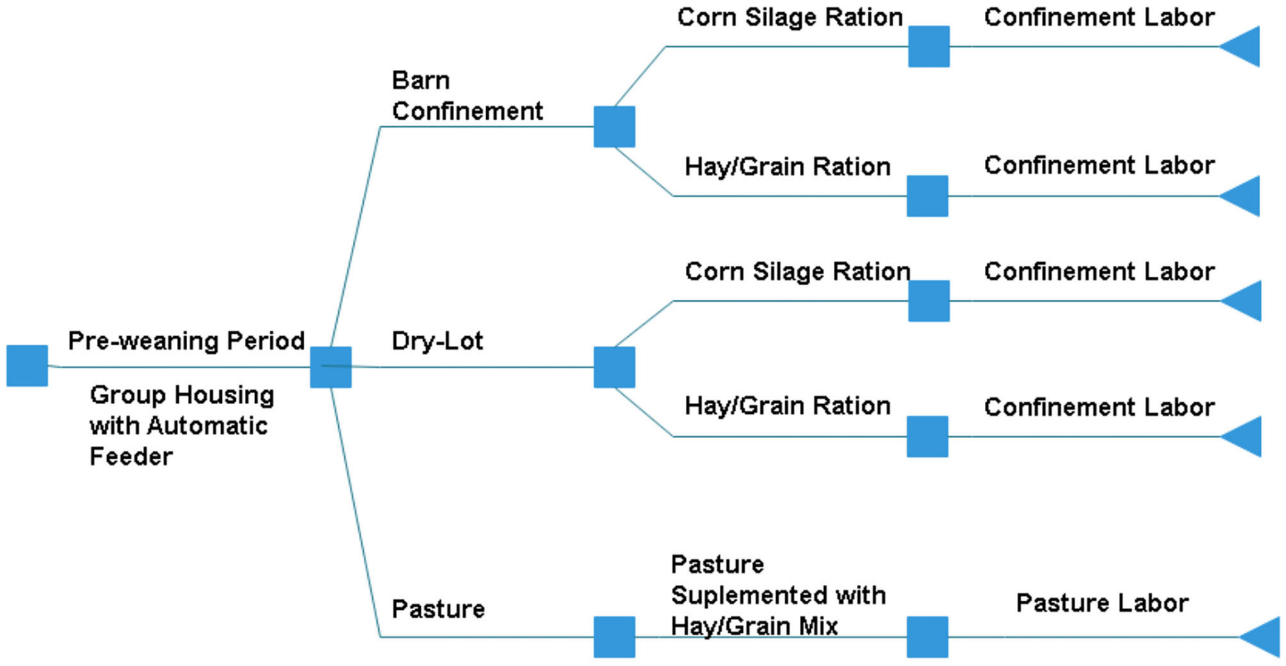


FIGURE 1 | Possible management decision options for producers to raise heifers post-weaning in the model.

water consumption with water valued at $0.75 per cubic meter of water.

$$[(\text{Annual Rental Rate per Acre} + \text{Annual Pasture Maintenance per Acre}) / 365 \text{ days}] / \text{Number of Animals per Acre} \quad (1)$$

Feed

Feed costs were calculated following the nutritional requirement of Holstein dairy heifers in each stage of growth following the NRC (18). Heifer requirements are shown in **Table 2**. Options for diet formulation included two diet types: R1, comprised of silage, forage, corn, soybean meal, and distillers grain, or R2, which included the utilization of pasture into the diet while supplemented with forage and corn. All rations included a mineral premix and assumed heifers would consume 2.2% of their body weight on dry matter basis. Feed cost was calculated as the average of USDA agriculture commodity market reports from January 2014 to November 2018. Feed cost and rations were both inputs into the model. Therefore, in the available economic model, the user can alter the model to be reflective of their farm or unique conditions.

The three commodities outlined in **Table 2** were made stochastic by assuming a 15% increase or decrease to create a minimum and maximum price. Distribution of the commodity prices is shown in **Table 3** for corn, corn silage, and soybeans as a result of the stochastic simulation model. Most values used for feed cost calculations were within 2 standard deviations from the mean. The mean remained the same average value set from USDA published market reports. Shrink of forage and concentrates was accounted for in the daily cost of the feed using Equation (2). An assumption of shrink was made at 10% for silage and forage feedstuff, and 3% for concentrates, based on communications with forage specialists.

TABLE 1 | Key assumptions presented in the model to determine the cost to raise a replacement dairy heifer from weaning to calving.

Variable	Value	Source
Number of heifers raised annually	1,000	
Hourly employee labor	$14.00	Based on Adcock et al. (14)
Hourly management labor	$22.00	Based on Adcock et al. (14)
Interest rate	7%	
Construction per M^2 frame	$13.00	(9)
Weaning age	65 days	(9)
Value of newborn calf	$100.00	Based on USDA market reports
Whole milk value (cwt)	$15.00	Based on USDA (8)
Milk replacer value (22.7 kg)	$65.00	Based on average market price
Manure management ($/head/month)	$0.90	(9)
Pasture rental rate (improved pasture)	$40.00	(15)

Values were found in published literature, extension surveys, and USDA market reports.

$$\text{Total Daily Cost of Individual Feedstuff}/(1 - -\% \text{ shrink}) \quad (2)$$

The projected body weight of replacement heifers in each month group was based on a weaning weight of 88 kg and 0.8 kg average daily gain of heifers post-weaning, following results found in Hawkins et al. (13).

Labor

Required labor hours varied from confinement housing to a pasture-based system. Published surveys of producer-reported time required per heifer were used in the calculation of labor cost. Equation (3) explains how the total labor hours (TLH) were used to determine how many hours of labor are required per replacement heifer.

$$\text{Labor Required per replacement heifer} = TLH / \text{Total Number of days the replacement heifer was in the rearing program} \quad (3)$$

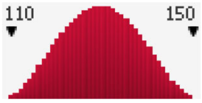
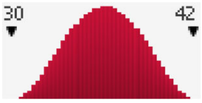
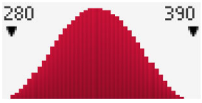
To determine the labor cost (LC) within each age group, the total number of days within each month period is multiplied by labor

TABLE 2 | Projected weight and nutritional requirements for dairy heifers.

Age group (months)	Projected Wt.* (kg)	DMI (kg/d)	ME (Mcal/d)	CP %
3–6	148	4.2	9.6	15.9
7–10	245	6.2	14.1	13.1
11–14	340	7.9	18.2	11.7
15–calving	544	12.2	27.5	13.3

Diets were balanced for NRC provided weight requirements which most closely matched projected weights. 150, 250, and 350 kg, respectively.

TABLE 3 | The distribution, mean, SD, minimum, and maximum of commodity prices per ton used to calculate feed cost of dairy heifers post-calving.

	Distribution	Mean	SD	Minimum	Maximum
Corn		$130.00	$7.37	$111.35	$148.91
Corn Silage		$36.26	$2.06	$30.96	$41.54
Soybean Meal		$333.00	$18.88	$284.80	$381.85

Values were developed using the @RISK. Assumed commodity prices were based on USDA monthly reports from January 2014 to November 2018 for corn and soybeans. Corn silage was valued based on corn commodity price.

requirement (LR). The resulting variable is the total number of hours of labor required per heifer within each age group (TLR). Equation (4) represents the final step in calculating the cost of labor per heifer (19). Hourly cost associated with more than one employee working on heifers at a time was calculated into the cost.

$$\text{LC} = \text{TLR} * \text{Number of Employees} * \text{Employee Hourly Wage} \quad (4)$$

Pasture-based scenarios followed the same labor calculations outlined above. An assumption was made based on lack of published literature for TLH required per heifer in a pasture-based scenario. 1:02 min was assumed for labor required per heifer; this was broken down from the 3 h of labor requirements per day to care for 175 heifers. The model allows for labor to be provided hours per replacement heifer or total labor hours per day and then divided to get a per replacement heifer cost.

Health

An external sheet is included in the model to calculate health costs by age group. A standard vaccine protocol was used as the assumed costs. Health related expenses for pre-weaned calves were included in the assumed pre-weaned replacement heifer cost used in all scenarios. **Table 4** outlines the vaccines and treatments provided to each age group and subsequently included in the overall cost. Labor requirement for working replacement heifers to provide these injections and treatments through working facilities was accounted for by an additional \$0.20 per dose (20). The sum of these expenses resulted in a health cost per age group.

Breeding

Variation of synch protocols, visual heat detection, or a combination of both was incorporated to account for difference preferences in breeding protocols. After six possible breeding cycles, 7% percent of heifers were assumed to be culled because of unsuccessful breeding. In this situation, Equation (5) was used to determine the additional cost incurred by the remaining heifers on the operation. This accounts for the cost of raising heifers that did not complete the heifer-raising program.

$$[\text{Value of Newborn Heifer} + (\text{Total Cost at 13 months} \\ -\text{Springer Heifer Value})^{*}\%\text{Culled}]/\text{Remaining Heifers} \quad (5)$$

Heat detection and conception rate were used to determine the number of heifers culled because of breeding performance. In the model, 176 heifers were in the age group to be bred and considered "at risk." The number inseminated was a function of how many heifers "at risk" were detected to be in heat. The number of pregnant heifers was a result of inseminated heifers multiplied by the conception rate. The difference between "at risk" and pregnant heifers were considered open. This open population would become the "at risk" heifers in the following cycles. Our model allowed for a heifer to complete 6 cycles before she was culled. Services per pregnancy were the sum of all inseminations, divided by the total number of pregnancies. The number of heifers within each group was dependent on how many heifers were culled in the breeding tab.

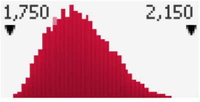
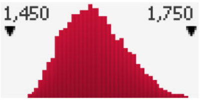
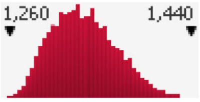
RESULTS AND DISCUSSION

The mean total cost (min, max) for a producer to raise a replacement heifer from birth to calving, assuming the same pre-weaning strategy of group housing with an automatic calf feeder, was found to be \$1,919.02 (\$1,777.25, \$2,100.57), \$1,593.57 (\$1,490.30, \$1,737.26), and \$1,335.84 (\$1,266.69, \$1,423.94) for confinement, dry-lots, and pasture management systems, respectively (**Table 5**). These averages follow the trend of previously published literature, resulting in average values within 1 standard deviation of presented averages (6, 9, 21, 22). The contributions of feed, labor, housing, and fixed and variable

TABLE 4 | Outline of the health protocol followed by the authors to create health-related expenses for each age group of heifers.

Health description	**Age group (months)**			
	3–6	**7–10**	**11–14**	**15–Calving**
Dewormer	X	X	X	X
Fly treatment	X	X	X	X
Respiratory vaccine	X			X
Leptospirosis vaccine	X	X	X	
7-way vaccine	X	X	X	X
E. coli vaccine				X
Brucellosis vaccine	X			
Staphylococcus aureus vaccine				X
Vitamin A&D				X
Total cost	\$11.60	\$6.03	\$6.37	\$8.10

TABLE 5 | Three main housing scenarios were evaluated incorporating the variation represented through stochastic variables.

	Distribution	**Mean**	**SD**	**Minimum**	**Maximum**
Confinement		\$1,910.02	\$58.78	\$1,777.25	\$2,100.57
Dry-Lot		\$1,593.57	\$44.09	\$1,490.30	\$1,737.26
Pasture		\$1,335.84	\$28.78	\$1,266.69	\$1,423.94

The distribution of total cost, mean, SD, minimum, and maximum is shown for each of the housing types selected.

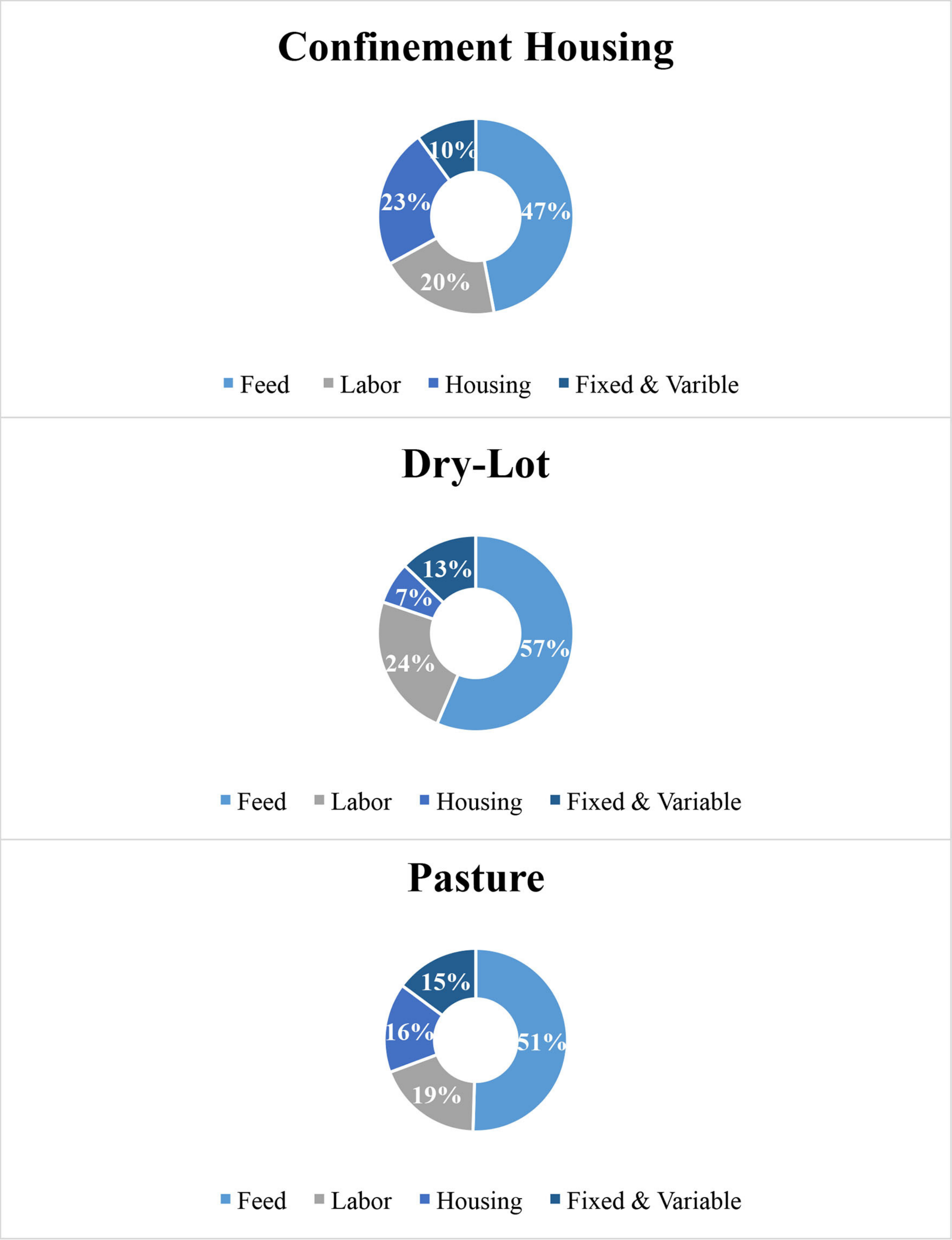


FIGURE 2 | Percentage breakdown of the contribution of housing, feed, labor, and fixed and variable costs in the total replacement heifer rearing period for confinement, dry-lots, and pasture.

costs toward this total cost are reported in **Figure 2**. The two largest contributing variables to the total cost were feed and labor expenses in all management situations, always representing at least 60% of the total cost.

Housing

Total housing cost per replacement heifer was $423.05, $117.96, and $207.96 for confinement, dry-lot, and pasture management systems. When compared to total cost, housing contributed 21%

for confinement, 7% for dry-lot, and 15% for pasture. When the sum of variables reported in published surveys is calculated to match the variables presented in our housing group, the average producer-reported housing costs $280. This represented 18% of total allocated cost in an industry-wide report from Wisconsin, USA (9). Most published surveys do not distinguish between housing management system, which may explain the largest cost represented in confinement. Housing cost was the highest for confinement housing because of the additional cost of barn infrastructure. The monthly barn payment per replacement heifer, accounting for interest and depreciation, was $4.81. This model assumes the payment of the barn; therefore, calculated costs may be higher than seen of cash expenditure expenses at the farm. The main contributor for the pasture-based scenario was the value of the land that the replacement heifers were occupying and the associated opportunity cost. With current assumptions, replacement heifers were costing producers $0.06 per day or $1.80 per month for the land as a housing system, excluding additional value of land as a feed source. Because of the nature of dry-lot housing, more heifers could occupy the same acre in comparison to pasture, reducing the land cost per replacement heifer.

Feed

Feed cost is dependent on input for price per ton and allotment of feed. Total feed cost, under current assumptions, was $932.14, $932.14, and $702.17 for confinement, dry-lot, and pasture management systems, respectively. Confinement and dry-lot scenarios have the same feed cost because both situations are reliant on delivered feed, including a silage ration. As a percentage of the total cost, feed cost contributed 47, 57, and 51% for confinement, dry-lot, and pasture scenarios, respectively. Feed cost is consistently the largest expense on farm in published replacement heifer raising cost, ranging from 51% to over 70% (6, 21). Percentage of feed cost is higher for dry-lots and pastures, partly due to the lower total cost and reduced emphasis on infrastructure found in the housing cost of confinement. This relationship is important when analyzing replacement heifer costs on farm, because we can assume that when comparing percentages of the total cost, confinement will have a lower total percentage of cost in comparison to a pasture setting.

Labor

Labor was broken down by paid hourly employees and hourly management employees, but labor is reported as the sum of these two expenses. The mean labor expense for confinement, dry-lot, and pasture was $932.14, $932.14, $702.17, respectively. As observed in feed cost, the labor for confinement and dry-lot scenarios are considered the same due to similar time and skill requirements. Labor accounted for 20%, 24%, and 19% of the total cost in confinement, dry-lot, and pasture housing scenarios, respectively. Labor accounted for on average 18.2% of the total cost of Wisconsin dairy producers, just below our calculated percentages (23).

A perceived challenge with this input is determining the time strictly used for caring for replacement heifers. This is particularly important on farms where labor is not hired specifically for the post-weaning replacement heifer period. For example, laborers may split time between feeding and care of replacement heifers and the milking herd, making it difficult to develop a true assumption for the relationship of hourly paid employees and management requirements. We have assumed 10% of the hourly labor was equivalent to the management labor required for replacement heifers. In some situations, management may have varied from this assumption.

Breeding

Heat detection varied based on management decisions and set reproductive performance. Cost to sync one replacement heifer, utilizing CIDR technology for breeding, was an investment of $19.60 per heifer. Incorporating visual observation into the breeding protocol added an additional cost of $4.68 per replacement heifer. Therefore, heat detection programs utilizing both visual observation and a sync program totaled $24.28 per replacement heifer.

The assumed base reproductive performance was a 65% heat detection rate and a 55% conception rate. Following the herd model of 1,000 heifers annually, 84 replacement heifers would be in the initial “at risk” group of pregnancy. Under our base assumptions after 6 cycles, 7% of the replacement heifers (or 6 heifers) would be culled for reproductive reasons. The cost accrued before breeding for confinement, dry-lot, and pasture management decisions was $1,197.85, $1,063.32, and $927.77, respectively. When distributed over the remaining heifers, there was an additional cost of $8.38, $6.65, $5.13 per replacement heifer for confinement, dry-lot and pasture housing systems, respectively.

Total cost for breeding with a sync protocol and visual heat detection, accounting for additional expenses due to reproductive culls, was $66.95 per replacement heifer. This accounted for 3.4, 4.2, and 5.0% of costs in confinement, dry-lot, and pasture-based management scenarios, respectively. If only visual heat detection was utilized, the percentage of the total cost decreased to 2.2, 2.6, and 3.2% of each management scenario.

Total Cost

Total replacement heifer raising cost ranged from $1,266 to $2,100 per head. The lowest cost was a result of pasture management decisions, with total cost increasing as infrastructure requirements increased. This model assumed a constant average daily gain across management scenarios, and thus, age at first calving was also consistent. However, many reports of average daily gain of heifers in pasture-based scenarios may be below other housing systems, which could increase the rearing period and increase the presented total costs.

When analyzing replacement heifer cost as an enterprise on the dairy operation on an annual basis, the number of replacement heifers raised can have a large impact on total cost. When the current assumption of the number of replacement heifers raised on farm was reduced by 5% (e.g., 500 heifers annually reduced to 475 replacement heifers), the cost per replacement heifer increased by $85.54, $67.75, $61.89 per heifer for confinement, dry-lot, and pasture management scenarios, respectively. Despite this increase in cost per heifer, the total annual investment in replacement heifers decreased by $7,109, $5,873, and $1,078 annually for each of the respective management scenarios. These results are more variable than the conclusions made by Tozer and Heinrichs (4), who valued a 1% decrease in cull rate of the milking herd which had the potential to decrease overall replacement heifer costs by $1,000–1,500. In addition, our results follow a similar trend found in Mohd Nor et al. (11) where a 5% decrease in cull rate had the potential to decrease replacement heifer costs by $6,500 annually. While heifer raising is often considered a separate enterprise from the dairy herd, management decisions have a large influence on the entire operation.

This study highlights the influences that each factor can have in the different scenarios studied and how it impacts the total rearing cost of replacement dairy heifers. Further studies should investigate the on-farm true cost and the use of economic models for decision-making on-farm.

CONCLUSIONS

Utilizing pasture to raise heifers resulted in a lower overall cost when compared to confinement and dry-lot housing options. Percentage breakdowns of feed, labor, housing, and fixed and variable costs provided more information on efficiency rather than total cost. The model and results presented are dependent on the inputs and assumptions made by the authors. Actual costs calculated may result in higher or lower totals when individual farms utilize the program; nonetheless, the authors determined the model to be highly effective in calculating the cost of raising heifers on an individual farm. This cost analysis is critical to assisting farms in making decisions in the allocation of their resources to raise or purchase replacement dairy heifers. However, a myriad of factors in addition to cost influence decisions around dairy heifer replacement raising on farms, such as tradition, animal welfare, and environmental concerns; these factors in decision-making should be further explored.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AH and JC: conceptualization and writing—original draft preparation. AH, DA-P, KB, and JC: methodology and writing—review and editing. AH, KB, and JC: formal analysis. AH: investigation. JC: resources and project administration. AH and KB: visualization. KB and JC: supervision. All authors contributed to the article and approved the submitted version.

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Timing and Duration of Observation Periods of Foraging Behavior in Natural Grasslands

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The goals of this study were to evaluate the length of time grazing which should be monitored over a 24-h period to predict the grazing behavior of beef heifers within a season and determine the patterns of foraging activity over 24 h. A database was constructed between 2010 and 2012 for beef heifers managed under rotational grazing in a natural grassland. Grazing, rumination, and other activity times were assessed visually during 24 h on 15 occasions. Data were classified according to climatic seasons, generating 12 replicates in summer, 18 in spring, 24 in autumn, and 36 in winter. Treatments were the evaluation of four distinct periods: from sunrise to sunset (DAY-SUN), daylight duration from dawn to nightfall (DAYLIGHT), DAYLIGHT plus 2 h (DAYLIGHT+2), DAYLIGHT to midnight (DAYLIGHT to 0), and the entire 24 h period (CONTROL). Differences for grazing, rumination, and other activities were found in all seasons for the evaluation periods. Sampling sufficiency was reached only with the DAYLIGHT to 0 and CONTROL for all four climatic seasons. The DAYLIGHT to 0 treatment covered 75% of a 24-h period and 95% of the mean foraging time took place during this time interval. Considering grazing distribution during a day, in the warm seasons, the major grazing period during mornings occurred earlier than in the cool seasons, and in cool seasons, the grazing peak was observed during the afternoon. Visual observations from dawn until midnight represented the total grazing time and natural behavior of heifers and could be used to represent grazing activities for the entire day.

Keywords: grazing behavior, grazing distribution, foraging activities, monitoring behavior, grazing patterns

INTRODUCTION

Grazing behavior evaluations can be an important issue when establishing management goals because the behavior of animals on pastures provides clues with which to determine if pasture management decisions are suitable (1) and whether animals are expressing their natural behavior, which is a good indication of animal welfare in pasture-based production systems. Furthermore, the behavior of animals in controlled situations, such as grazing trials, can provide insights into the production data collected in those situations.

The southern Brazil Campos grasslands (2) are the major foraging systems for beef cattle reared in this region, and the same is valid for other nearby South American countries, such as Argentina, Uruguay, and part of Paraguay. Thus, developing management tools for these grazing systems can provide productivity gains, as well as ensure the welfare of animals. Grazing systems in these natural grasslands are considered an important marketing advantage for these countries, and monitoring ingestive behavior could attest to the adequate state of animal comfort. Management systems that do not take into account whether animals can attain daily forage intake to meet their nutritional requirements may be inefficient.

Among behavior variables, time spent grazing, and ruminating is the main measured indicative and key variables used as indicators of management efficiency and animal welfare. Other relevant variables of management efficiency could be estimates of dry matter (DM) intake and forage quality that relates to forage on offer and sward structure (3), but these are not animal behavior traits. For example, ruminants commonly have grazing times between 450 and 600 min/day in temperate pastures and rarely forage < 360 min/day, with times that may exceed 760 min/day on subtropical and tropical pastures (4, 5). In the southern Brazil Campos grasslands (6), without limitations to inhibit potential intake (e.g., sward height or herbage mass), the time spent grazing commonly ranges between 500 and 650 min/day (7–9), regardless of the grazing method used (10). This variation in grazing time indicates potentially diverse situations and challenges that animals can face, even in situations with abundant forage allowance, attesting to the complex interaction of animals with plants and sward structure. On subtropical natural grasslands, in a wide range of forage allowances, Trindade et al. (11) indicated that both lower and higher levels could limit forage intake, due to limited bite volume and mass at allowances lower than 8 kg of DM/100 kg of liveweight and to limited bite selection above 16 kg of DM/100 kg of liveweight. These bite variations are linked also to grazing time along the day, indicating a "standard range" of 500–600 min/day. However, observed grazing times outside of "standard range" are indicative that something is wrong with animal management, which could even decrease welfare by forcing unusual behavior on animals, such as grazing during hot periods of the day. Notwithstanding, all these protocols of forage allowance and sward structure could be far from the possibility of farmers to follow at paddock level and depend on shade availability and water quality and availability. However, observing grazing times at key periods of the day could give clues to adequate grazing management.

Although foraging behavior studies have already been defined as important evaluations, the extent of the evaluations (e.g., during the daylight periods or 24 h periods) that best represent animal behavior remains uncertain. Both protocols were found in the international literature (daylight × 24 h). This is due to three main factors: (i) availability and costs of skilled observers; (ii) circadian behavior rhythms associated with daylight, especially in temperate climates; and (iii) the need for artificial light during the night.

The 24-h visual evaluations may currently be less feasible, in part, because they require a large number of trained people. Furthermore, artificial light during dark periods may affect the natural behavior of animals (12), especially if the animals are not very tame or not used to being under artificial light. Thus, many research groups are searching for accurate automatic recording methods of behavior (13). Nevertheless, continuous 24 h assessments are the most accurate for evaluations, and methods need to be calibrated for automatic recording and evaluating a longer and fixed period, regardless of the climatic season (1,440 min/day). On the other hand, evaluations performed only during daylight periods, regardless of the time interval, have been justified based on the pattern of ruminants' diurnal foraging behavior, especially for the main meals during the day, and have lower labor requirements (14, 15).

The problem lies in the autumn and winter assessments. In these climatic seasons, restriction to daylight evaluations could be seriously biased because of the reduced day length (photoperiod) and, more importantly, because during this period the quantity and quality of the forage are substantially different from that during other periods of the year, especially in natural grasslands. Moreover, daylight observations do not consider natural animal behavior. Usually, animals tend to graze at night (16), mainly in tropical and subtropical conditions. Preference for grazing at night could occur because of more comfortable air temperatures during this period (12), although nighttime grazing activity has a shorter duration compared with that during daylight. However, these grazing events could represent as much as 35% of the total grazing time over 24 h in hot weather or during long nights (17–19). Furthermore, during these foraging events at night, (19) demonstrated that animals have a heavy bite mass (19). This part of the day needs to be considered in assessments that consider animal welfare and evaluate the efficiency of the management system.

Thus, considering the importance of the daylight period on behavior and the interaction of the daylight period with temperatures in subtropical environments, the objectives of this work were to evaluate for how long foraging should be monitored over a 24-h period to predict foraging behavior of beef heifers within a season and determine the patterns of foraging activity over 24 h.

MATERIALS AND METHODS

Local, Climate, Experimental Area, Area Management, and Behavior Assessments

The experimental area is located in the southern part of Brazil, Rio Grande do Sul state, with the center of the experimental area at ∼29°43′30′′S, 53°45′33′′W. This area belongs to the Federal University of Santa Maria (UFSM). The local climate is classified as subtropical humid, with a mean ambient annual temperature of 19.2°C and a mean annual rainfall of 1,770 mm at 95 m above sea level (20). During the trial, the mean maximum temperature was 22.7°C and the mean minimum temperature was 17.1°C, the mean precipitation was of 130.6 mm per month, November is the wettest month (294.9 mm), and October is the driest (53.6 mm).

The experimental area was 22.5 ha, which was divided into six rectangular paddocks of 3.5 ha each. Each of these six areas was then subdivided into seven smaller sub-paddocks and managed with a rotational grazing method. The criteria that defined the rest period of sub-paddocks was the thermal sum accumulated (degree Celsius per day; degree day, DD) for the duration of leaf elongation of two functional groups of grasses (21) (*as described below*) that compose the swards of Campos grasslands.

To define the rest intervals of the rotational grazing method (original trial treatments), mean phyllocron (time in DD for complete leaf elongation) of functional groups A/B and C/D (375 or 750 DD) was multiplied by the number of expanding leaves per tiller, generating the rest periods of each sub-paddock. The number of expanding leaves of grasses in the functional groups is intrinsic to the genetic traits of plants and defines the time of rest intervals (21). Following this logic, over 3 years, three paddocks were managed using a rest interval of the accumulated thermal sum of 375 DD, and the other three paddocks were managed using 750 DD of the accumulated thermal sum. Therefore, the occupation period was defined by dividing rest intervals (in thermal sum) of each treatment by the number of sub-paddocks, less one (sub-paddock under occupation), resulting in the time, in degree Celsius, of occupation of each sub-paddock. The accumulated thermal sum to manage the paddocks generated a varying number of days for occupying the sub-paddocks, according to ambient temperature and weather seasons.

The 375-DD rest interval was based on the accumulated temperature for elongation of 2.5 leaves per tiller of grasses of functional groups A and B [e.g., *Coelorhachis selloana* and *Paspalum notatum*; (22)]. The 750-DD rest interval was based on the accumulated temperature for elongation of 1.5 leaves per tiller of functional groups C and D [e.g., *Aristida laevis* and *Saccharum trinii*; (23)]. Those species had an important contribution to sward composition of the area and, consequently, on available herbage mass.

Over 3 years, when measuring the rest interval effects, a total of 15 experimental evaluations of beef heifer grazing behavior were conducted. All assessments were performed with visual observations during 24 consecutive hours. In each year, a variable number between 24 and 36 beef heifers (at least four heifers per paddock) was evaluated with variable body weights (177–215 kg) and age (12–24 months) (**Table 1**). Variations in weight and age were within the range for heifers rearing to breed at 2 years of age.

During the grass growing seasons over the 3 years (springs, summers, and autumns), heifers were only supplemented with mineral salt and had access *ad libitum* to freshwater. During the first winter (2010), heifers were supplemented with mineral protein salt *ad libitum* (24). During the second winter (2011), heifers were supplemented with grounded corn at a proportion of 0.5% of body weight (BW). During the third winter (2012), the heifers were supplemented with 0.5% of BW with wheat bran (85%) and glycerol (15%). In all winter seasons, except for the first when mineral protein salt had its intake limited by NaCl concentration, the intake was not higher than 200 g per animal. In the second and third winter, supplement did not exceed 0.5% of BW and was available only from 09:30 to 10:30 h, when grazing activity is lower. The stocking rate adjustments were made each 28 days using 4.5% of the herbage allowance (4.5 kg MS per 100 kg of BW) considering 70% of grass leaf blades creating part of the sward mass.

TABLE 1 | Mean initial body weight, age, and number of beef heifers monitored during a study to quantify the daily foraging activities.

	2010[a]	2011[b]	2012[b]
Body weight	215	177	185
Age	18	12	12
Monitored animals	24	36	24
Breed type	Angus	Angus; Charolais × Nelore	Angus
Date of behavior evaluations[c]	June 11 August 15 September 30 December 17	January 20 April 09 June 04 July 19 September 03 November 18	January 16 March 24 May 26 July 07 September 12

Body weight is expressed as kg BW. Age is expressed as months. "Monitored animals" is the number of animals assessed in each evaluation year. Breed type represents the breed of animals assessed each year. Date of behavior evaluations represents the dates when the assessments were performed.

[a] *From September to December.*

[b] *In 2011 and 2012, heifers started with 12 months remaining in paddocks until they reached 24 months.*

[c] *June, July, and August represent winter; September represents spring; December and January represent summer and March; April and May represent autumn.*

The experimental area was arranged in a randomized block design with the two rest periods as the treatments (375 and 750 DD), with three area replicates (six paddocks with sub-paddocks, three for each rest period), using rotational grazing management. The blocking criterion was the relief. Details regarding management can be found in Barbieri et al. (10).

Before this 3-year experimental period and 15 behavior assessments, behavior variables were tested through an analysis of variance model (using $P \leq 0.05$ as the significance level). Because there were no differences between grazing and rumination time between 375 and 750 DD treatments, all data were used to form a larger database. Then, these databases were combined by year, generating four evaluations in the first year (4×6 paddocks $= 24$ replicates), six evaluations over the second year ($6 \times 6 = 36$ replicates), and five evaluations in the third year ($5 \times 6 = 30$ replicates). To this new analysis, the year was used as a block in the statistical model to remove possible climatic differences among the years. Next, data were clustered by season, regardless of year (blocked), which generated 12 replicates in summer, 24 replicates in autumn, 36 replicates in winter, and 18 replicates in spring. Each replicate evaluated at least four heifers. Even after clustering the data (years and climatic seasons), grazing and rumination time did not present significant differences between the 375- and 750-DD treatments, making it possible to pool all data to perform the timing and sample sufficiency analysis. The original data and animal performance can be found in Soares (25).

Natural Grassland Characterization

The pasture that composes the Campos grasslands (6) presents a well-defined double layer canopy structure. In this case, the lower strata were composed of short-grass species, such as *Axonopus affinis* and *Paspalum notatum*, mostly with a prostrate growth pattern and were the major grasses in the above-cited A/B functional groups. These species were highly preferred by free-ranging cattle. In the upper strata were grass species with a tussock-like growth habit, such as *Andropogon lateralis* and *Aristida laevis*, the major grasses in the above-cited C/D functional groups (26). Moreover, the experimental area was mostly composed of C_4 metabolic cycle grasses (above 75% of herbage mass). During the cool seasons, sward production was dramatically reduced concomitant with the decrease in its nutritional value.

The herbaceous vegetation of the area consisted (mean contribution for green herbage mass) primarily of *Andropogon lateralis* (±37%), *Aristida laevis* (±14%), *Saccharum trinii* (±6%), *Shorgastrum nutans* (±6%), *Paspalum plicatulum* (±3%), *Axonopus affinis* (±6%), *Paspalum notatum* (±9%); species within the Umbelliferae family, including *Eringium horridum* (±3%); and ±16% representing other plant families, including woody plants (each with negligible amounts; <1%). Furthermore, 117 species, representing 33 grass genera, have been documented in this experimental area (27). Species classified as A and B functional groups (*Andropogon lateralis*, *Axonopus affinis*, *and Paspalum notatum*) comprised 52% of the mean green herbage mass and those as C and D groups (*Aristida laevis*, *Saccharum trinii*, *Shorgastrum nutans*, *and Paspalum plicatulum*) comprised 29% of the herbage mass. These species and group contributions varied throughout the year, mostly because of the variations in environmental temperatures over time (seasons). See Cruz et al. (21) for details on functional groups. The quantity of senescent plant material also changed across seasons, being lower in the spring (±20% of total herbage mass) and greater in the winter season (±55% of total herbage mass). All these values (species contribution and botanical composition) were sampled using the BOTANAL method, as described by Tothill et al. (28).

Over the 3 years, herbage mass (HM) was measured, each 28–32 days, using a visual standard comparison, calibrated with a double sampling technique (29), with 20 visual samplings and six samples cut at ground level, using 0.25 m^2 quadrats. All regression equations derived from visual assessments were above 0.7 determination coefficient (R^2). In each evaluation of HM, sward height was measured with a sward stick at the same points as HM evaluations. We did not consider the tall tussock grasses in sward height measurements.

Grazing Behavior

All 15 grazing behavior evaluations began on the second day of occupation of the sub-paddocks, regardless of whether the management was 375 or 750 DD (dates in **Table 1**). The mean time of occupation of the sub-paddocks was 4 days (range from 2 to 5 days in spring and summer) and 7 days (range from 5 to 10 days in autumn and winter). In all assessments, the experimental unit was the sub-paddock and the variables explored were the mean values of at least four tested animals.

Previously for each assessment, heifers were exposed to night observations with flashlights to acclimate them to this type of light and they were habituated to close handling by people using daily supplementation on grassland. Thus, flashlights and close observations appeared to have minimal effects on the behavior of the animals. In each evaluation, trained evaluators were placed at ground level in "easy-to-view" locations for heifer behavior recording. Four trained evaluators were used for each sub-paddock, taking turns every 2 h.

Total grazing, rumination, and other activity times were visually recorded, every 10 min over 24 consecutive hours, and the results were expressed in minutes per day. Considering that sub-paddocks were 0.5 ha, and animals expressed their behavior in groups, each observer was able to handle a single paddock observation. The recording frequency was chosen based on previous data reported by Gary et al. (30) and Mezzalira et al. (31). Grazing was defined as including time spent searching, selecting, and gathering (eating) forage, similar to that previously described by Hodgson (18). Rumination time was defined as the cessation of grazing and the beginning of jaw movements. Time of other activities was considered the time when animals were not foraging or ruminating and could be idle, engaging in social activities, drinking water, or eating supplements (32).

Treatments for Timing, Sampling Sufficiency Evaluation, and Natural Behavior

As previously described, an analysis of variance showed no differences among treatments (375 and 750 DD rest intervals). Thus, data were recombined in five treatments regardless of the rest intervals. New treatments consisted of the comparison between timing and duration of observation periods to test the sufficiency of sampling duration for grazing time analysis and to determine the behavior of heifers in the natural environment. Again, each paddock area was used as a replicate in each season, generating 12 replicates in summer, 24 in autumn, 36 in winter, and 18 in spring. Differences among seasons were not compared because of the differences in day length among seasons and differences in green biomass availability and herbage quality.

Validation of the timing and duration of observation periods in each season accounted for grazing, rumination, and other activity times observed over uninterrupted periods of 24 h. Treatments consisted of the evaluation of four distinct periods having different lengths based on the following selected intervals: *sun duration*—during the day from sunrise to sunset (DAY-SUN); *daylight duration*—from dawn to dusk (DAYLIGHT); *DAYLIGHT plus 2 h* (DAYLIGHT+2); *DAYLIGHT to midnight* (DAYLIGHT to 0); and the *entire 24 h* (CONTROL) (details in **Figure 1**). All data were compiled by fractioning the CONTROL dataset.

To obtain the times of sunrise and sunset, historic data were used (mean of 30 years) registered by the National Institute of Meteorology (INMET) station, located 3 km from the experimental area. The mean time of sunrise and sunset was

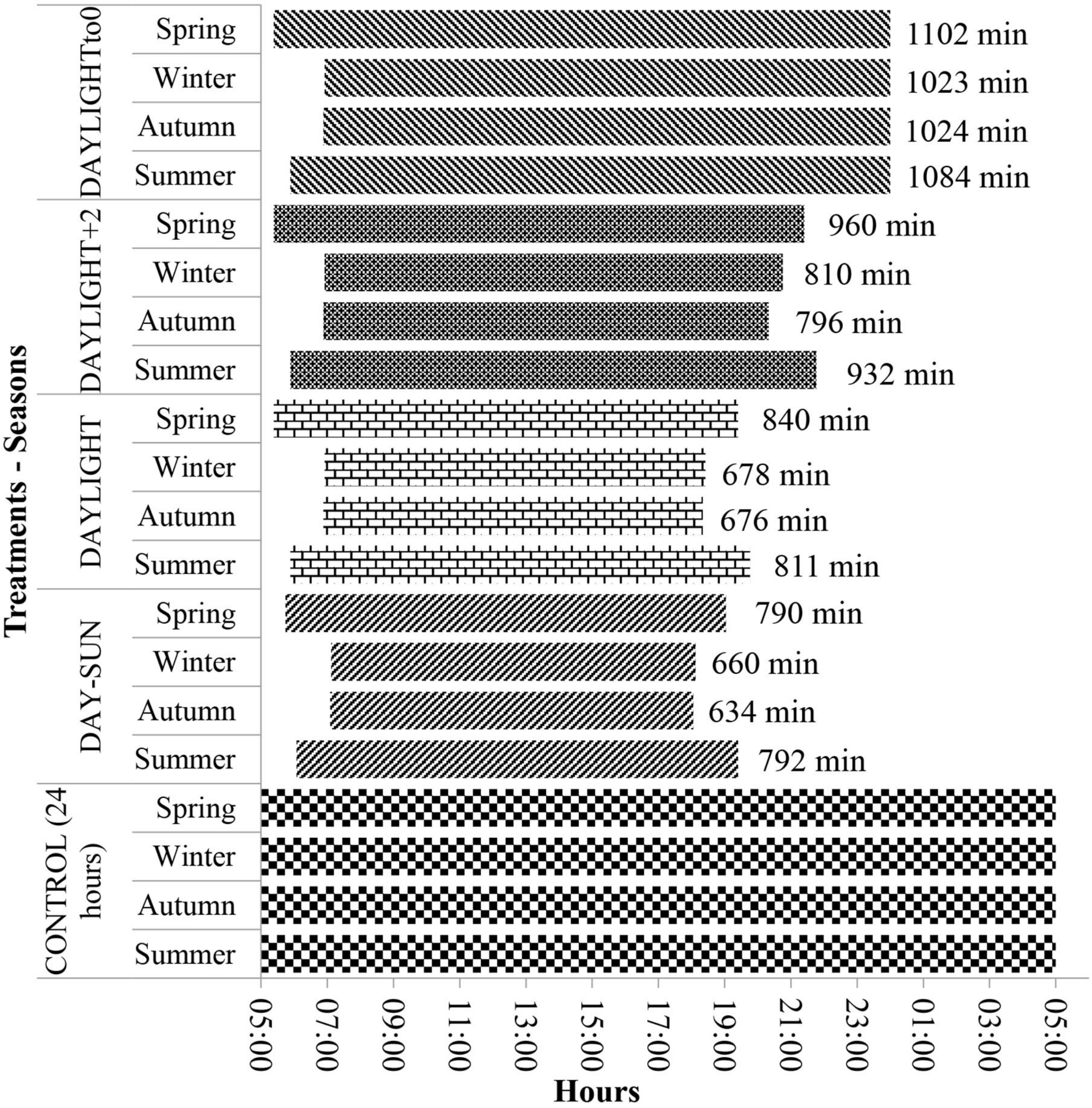

FIGURE 1 | Graphical timeline representation of the timing and duration of evaluations of grazing behavior in 24 h (control; 1,440 min assessment) and the tested periods of time (treatments): DAY-SUN (sunrise to sunset), DAYLIGHT (down to nightfall), DAYLIGHT+2 (down to nightfall plus 2 h after dark), and MIDNIGHT to 0 (down until midnight).

calculated for each season. Using these times, the beginning and the end of the DAY-SUN treatment were identified (**Table 2**). This information also was used to obtain the *dawn* and *dusk* durations [sun position 6° above (sunrise) and −6° below (sunset) the horizon (33)], and this time was added to the DAY-SUN treatment. The mean values of dawn and dusk (in minutes, mean of each season) were added to the mean sunrise and sunset hours for each season to determine the start and the end of behavior evaluations that defined the DAYLIGHT treatment (see **Table 2**).

In addition to these observation periods, to determine if animal behavior was being represented, other evaluation periods were observed. In the DAYLIGHT+2 treatment, animal evaluations were considered from dawn until 2 h after dusk ended. For the DAYLIGHT to 0 treatment, behavior was compiled between dawn and midnight (00:00; midnight). For DAYLIGHT+2 and DAYLIGHT to 0 treatments, the end of the evaluations was considered a fixed period of time. The evaluation period (time), even within the same treatments (except in the CONTROL treatment), was different between climatic seasons, and this occurred because of photoperiod changes among climatic seasons (**Figure 1**), influencing the time the assessments began.

TABLE 2 | Mean hour of dawn, sunrise, sunset, and dusk among the four climatic seasons during a study to quantify the daily foraging activities of beef heifers.

Event	Climatic seasons			
	Summer	**Autumn**	**Winter**	**Spring**
Dawn	05:53	06:53	06:55	05:23
Sunrise	06:04	07:05	07:07	05:44
Sunset	19:24	18:03	18:07	19:02
Dusk	19:46	18:20	18:25	19:24

See the text for the definitions of dawn, sunrise, sunset, and dusk. Mean hours of the events are an average of the last 30 years, provided by the National Institute of Meteorology (INMET).

Behavior Data Analysis

The statistical analyses used was a block design model where each year was considered the block. Each paddock was considered a replicate (mean of animals inside the paddocks) and there were six replicates (paddock number in the experimental area) in each trial (15 trials for 3 years). In spring, data were analyzed with 18 replicates, in summer with 12, in autumn with 24, and in winter with 36 replicates. Results are presented separately by season because of the differences in day length among seasons.

The analysis of grazing time (minute per hour during 24 h) was performed using the mean values of grazing time from all replicates in the database. For this analysis, data were separated by climatic seasons, and using mean values of all replicates in each climatic season, the grazing time (minute per hour) was calculated for each hour of the day. From this, the grazing time in each hour between climatic seasons was compared.

Initially, data were submitted to a Bartlett test followed by a Shapiro–Wilk test to determine the homogeneity of variances and normality of residuals, respectively. After confirming this, the data were submitted to an analysis of variance and F-test. Mean comparison analyses were conducted using PROC MIXED (Tukey test) in SAS 9.2 software, including the model effects of blocks (years) and treatments (evaluation periods). The criteria for sampling sufficiency of the duration of observation periods were defined as occurring when comparisons between the CONTROL and treatments were similar. For all statistical tests, significance was defined as $P < 0.05$.

RESULTS

The main objective of this article was to evaluate the extent of observations of grazing behavior over a 24-h period to accurately represent this behavior, so we will discuss our data considering at the same time feasibility and representativeness to predict foraging behavior mediated by pasture management practices.

Sward Characteristics

Mean herbage mass maintained during the experimental years was 3,871 kg DM/ha, ranging from 3,017 to 4,242 kg DM/ha. Furthermore, mean sward height, without tussock species, was 20 ± 3.9 cm, ranging from 17.3 ± 3.3 to 22.5 ± 4.1 cm. Sward characteristics were similar among the paddocks and typical of this grassland formation.

Timing and Duration of Observation Periods

There were differences ($P < 0.05$) for grazing, rumination, and other activity times among all seasons and treatments within 24 h (**Table 3**). There were differences in grazing time even among lower observation period treatments (DAY-SUN vs. DAYLIGHT) in summer and winter seasons. Furthermore, these treatments presented lower ($P < 0.05$) foraging times than did the time observed in the CONTROL. In summer, grazing time measured in the DAY-SUN treatment represented only 82.7% of the total time spent grazing over 24 h and this treatment evaluated 56.9% of day length. In DAYLIGHT, grazing time observed represented 88.1% of the total grazing time and the DAYLIGHT evaluated 60.4% of day length.

In winter, the DAY-SUN treatment represented only 47.2% of day length and covered 74.9% of the grazing time observed in the CONTROL. In the DAYLIGHT treatment, 79.7% of the foraging time of the CONTROL was represented and 50% of the day length was observed. Rumination and other activity times were similar between DAY-SUN and DAYLIGHT treatments among all seasons. However, rumination time was lower in these treatments relative to that of the CONTROL.

In the DAYLIGHT+2 treatment, grazing time differences were observed in the spring, autumn, and winter when compared with other evaluation periods. In this treatment, grazing time was greater than the time spent grazing in the two treatments that evaluated grazing time only during the day clarity period (DAY-SUN and DAYLIGHT) and lower than the grazing time observed in DAYLIGHT to 0 and CONTROL treatments. In spring, DAYLIGHT+2 evaluated 68.1% of the day length and grazing time represented 88% of the CONTROL. During autumn, this treatment evaluated 57.5% of the day length and 81.5% of the grazing time in the CONTROL. During winter, DAYLIGHT+2 represented 84.1% of the CONTROL grazing time, evaluating 58.4% of the day length. Regardless of the season, the DAYLIGHT+2 treatment reached the grazing time representativeness of time grazing by the heifers observed over 24 h. In general, rumination and other activity times increased with the increase in the evaluated period during all seasons.

Grazing time only began to be represented when a large portion of the night was added to the evaluation period. Sampling sufficiency of the duration of the observation period was achieved when the evaluations were undertaken until midnight (DAYLIGHT to 0 treatment) in the four seasons (treatment DAYLIGHT to 0 vs. CONTROL; summer $P = 0.485$; spring $P = 0.278$; autumn $P = 0.212$; winter $P = 0.196$).

Using this evaluation period, during summer, 77.1% of the day length with 97.8% of the CONTROL grazing time was used. In spring, the evaluation period had 76.4% of day length and achieved 91.5% of the foraging time observed during the CONTROL period. During winter, grazing time in the DAYLIGHT to 0 represented 91.5% of grazing activity of the CONTROL, evaluating 72.9% of the day length. In autumn,

TABLE 3 | Grazing, rumination, and other activity times of beef heifers in a natural grassland managed under rotational grazing among the four climatic seasons.

	Evaluation periods (treatments)					STD*	*P*-value
Min/day	**CONTROL**	**SUN-DAY**	**DAYLIGHT**	**DAYLIGHT+2**	**DAYLIGHT to 0**		
Summer							
Grazing	648^{a}	536^{c}	571^{b}	597^{b}	634^{a}	11.2	0.001
Rumination	517^{a}	191^{d}	196^{d}	267^{c}	321^{b}	13.8	0.001
Other act.	275^{a}	93^{c}	103^{c}	126^{bc}	155^{b}	13.8	0.001
Spring							
Grazing	692^{a}	549^{c}	575^{c}	609^{b}	633^{a}	14.7	0.001
Rumination	473^{a}	164^{d}	176^{d}	230^{c}	311^{b}	10.2	0.001
Other act.	275^{a}	97^{c}	109^{c}	$141^{b,c}$	156^{b}	12.4	0.001
Autumn							
Grazing	637^{a}	449^{c}	475^{c}	521^{b}	602^{a}	12.9	0.001
Rumination	469^{a}	114^{d}	122^{d}	176^{c}	270^{b}	9.2	0.001
Other act.	334^{a}	107^{c}	113^{c}	133^{c}	178^{b}	10.4	0.001
Winter							
Grazing	597^{a}	447^{d}	476^{c}	502^{b}	566^{a}	7.7	0.001
Rumination	437^{a}	91^{d}	95^{d}	167^{c}	303^{b}	8.1	0.001
Other act.	406^{a}	142^{d}	$149^{c,d}$	171^{c}	191^{b}	9.1	0.001

See text for definition of the different treatments (Control, SUN-DAY, DAYLIGHT, DAYLIGHT+2, and DAYLIGHT to 0). Grazing, rumination, and other activities (Other act.) are expressed in minutes. Different lowercase letters in a line differ by Tukey test at 5%.
**standard mean deviation.*

grazing time in the DAYLIGHT to 0 represented 94.8% of the activity of the CONTROL, evaluating 73.6% of the day length.

Considering the time spent in rumination and other activities, even with the increase in the observation periods, differences were observed when compared with that of the CONTROL ($P < 0.05$). Overall, in summer and spring, rumination time during periods of natural clarity (day) represented only 37.6% of rumination time compared with the 24-h period (CONTROL). The remaining rumination time (62.4%) was observed during dark periods (night). In the cool seasons (autumn and winter), 23.9% of rumination time was observed during light periods and 76.1% overnight. Furthermore, the remaining activities followed the same pattern; during summer and spring, 31.1% of other activities occurred during the day (natural light), whereas the remaining time (68.9%) was observed during darkness. In the cool seasons (mean of autumn and winter), 43% of other activities were distributed in the day and 56.9% during darkness.

Diurnal Foraging Patterns

Grazing time distribution over 24 h presented some similarities among seasons, mainly when comparing among warm seasons (summer and spring) and cooler seasons (autumn and winter) (**Figure 2**).

During warm seasons, the first intense grazing cycle (or peak) occurred earlier than in cooler seasons, at ∼04:00 in the morning. At 05:00, grazing activity was more intense during warm seasons than during cool seasons ($P < 0.05$). In cooler seasons, the first grazing peak started at ∼06:00 h. The difference ($P < 0.05$) in grazing intensity (time spent grazing per hour) between warm and cool seasons was observed until 08:00. In all seasons, after this intense activity, grazing activity was reduced until 10:00 h (more details in the Supplementary File; **Table 4**).

During late morning, at ∼11:00, a second intense peak of grazing activity occurred in the summer and it was different from that of other seasons ($P < 0.05$). In this same time of the day (late morning and early afternoon), cool seasons and spring had low intensity and more constant grazing distribution. Regardless of the season, during late afternoon, and at the beginning of the night (16:00–20:00 h), a second intense grazing peak was observed. This peak in grazing activity started earlier in the cool season as compared with that of warm seasons. In winter and autumn, this intense grazing activity started at ∼15:00–16:00 h. This grazing peak had, approximately, a duration of 3, 1 h less than the duration of the grazing peak observed during the warm season. In summer and spring, the intense foraging activity occurred between 17:00 and 21:00 h.

After this grazing peak in the late afternoon, grazing activity was reduced during the early evening. In cool seasons, this grazing activity reduction ranged from 19:00 to 22:00 h. During spring, this reduction was shorter and ranged from 21:00 to 22:00 h, and in the summer, grazing activity was evident from 21:00 to 0:00 h. Furthermore, in spring, autumn, and winter, heifers had another short grazing peak during the night (between 23:00 and 01:00 h). Only during summer did heifers present low foraging activity during the night.

DISCUSSION

During the 3 years when grazing behavior was evaluated, herbage mass and sward height did not present values in the range of sward structure considered limiting for beef heifer intake on

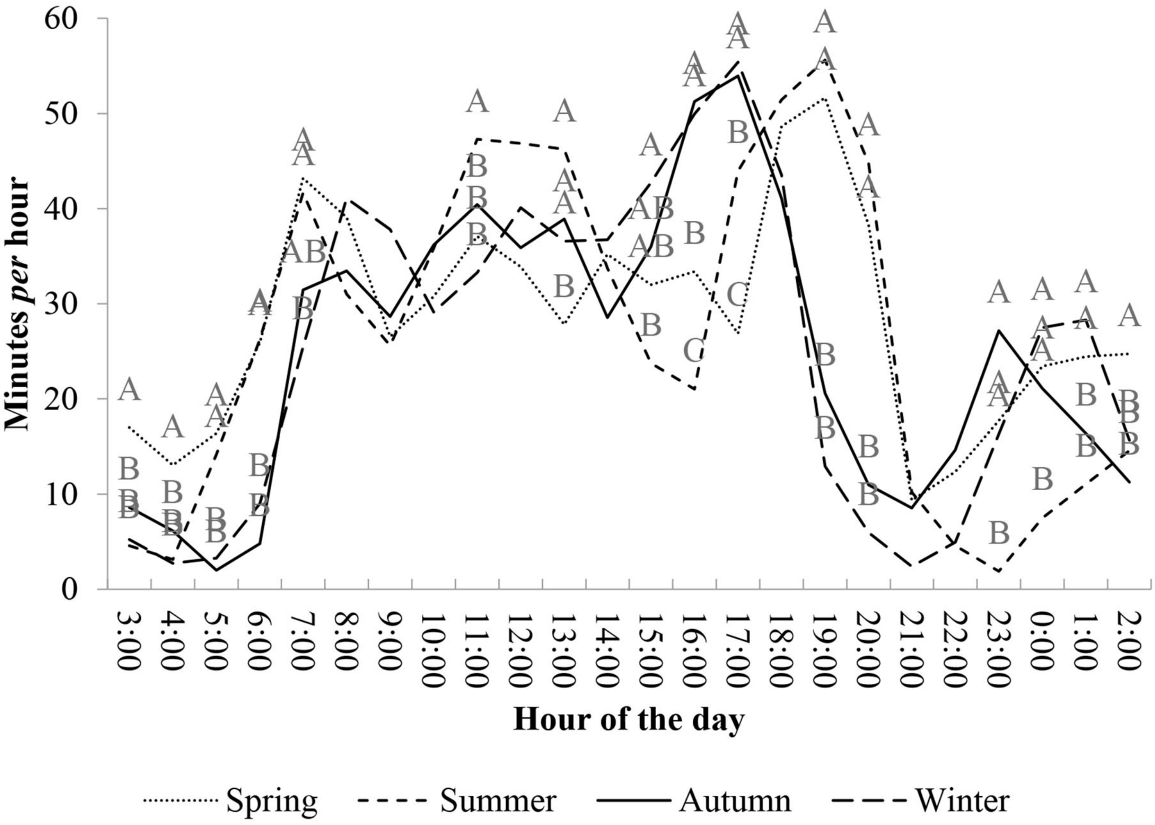

FIGURE 2 | Mean foraging time (minutes per hour) of beef heifers, over 24 h, managed in natural grassland under rotational grazing method among the four climatic seasons over the years of 2010–2012 (*different capital letters in a column differ by Tukey test at 5%).

natural grasslands (1). Thus, the similarities allowed us to assume that the grazing behavior of beef heifers in this study was not influenced by these factors.

Recently, animal behavior evaluations (or trials) have focused on the observation periods during daylight observations, regardless of the climatic season or pasture type (8, 11, 34, 35). This observation period was based on the major grazing events featured by the animals by their natural behavior, mostly observed in temperate climate conditions. In such situations, the weather is characterized by milder environmental temperatures during the daylight period and cold environmental temperatures during the night (14). Thus, because of thermal comfort, grazing activity occurs predominantly during daylight hours (15) and major grazing events occur near sunrise and sunset, with the latter having greater intensity and longer duration (36).

However, in tropical and subtropical conditions, the temperature distribution is different over a 24-h period, especially in different seasons, and as seen in our results, to maintain animal welfare, animals distribute their grazing activities differently, having more dispersed foraging activities over 24 h as compared with those in temperate climates. In support of this, in subtropical and tropical climates, animals can conduct a significant portion of grazing during non-daylight hours together with rumination and resting (12, 19, 37).

Another important feature related to diurnal ingestive preferences of animals in temperate climates is the high nutritional quality of C_3 grass species, a typical trait of the pastures in temperate climates that easily supports animal nutritional demands over the day when the sward is high or herbage mass allows high bite masses. Because of this, night foraging activity is usually characterized as occurring in shorter intervals and less intense bouts. Overall, night foraging represents a small percentage of daily foraging time and contribute minimally to daily herbage intake in temperate climates (16, 38). However, in subtropical natural grasslands, as in our experiment and as observed by Trindade et al. (11), sometimes the nutrient concentrations of the pasture are poor, and consequently, animals have to spend more time during the day to attend to their energetic requirements, even with no limiting intake factors based on sward conditions (high or mass).

Thus, evaluations of grazing time, which consider only day length (DAY-SUN and DAYLIGHT), are incomplete in the representation of grazing time and natural behavior of heifers over 24 h (CONTROL). In these situations, grazing time was significantly lower than grazing time normally presented by the animals. Even when 2 h was added past sunset (DAYLIGHT+2), the time spent grazing was significantly lower than grazing time measured over 24 h. This definitively suggests that there is

TABLE 4 | Hourly mean foraging distribution of beef heifers over 24 h foraging behavior assessments in a natural grassland managed under rotational grazing.

Timetable		Time foraging (min/h)				Standard error
		Autumn	Winter	Spring	Summer	
00:00	01:00	21.1^{a}	27.5^{a}	23.4^{a}	1.8^{b}	3.45
01:00	02:00	16.4^{b}	28.3^{a}	2.4^{a}	7.5^{c}	2.97
02:00	03:00	11.3^{b}	16.7^{b}	24.7^{a}	11.0^{b}	2.76
03:00	04:00	8.6^{b}	5.2^{b}	16.9^{a}	14.6^{a}	2.7
04:00	05:00	6.1^{b}	2.7^{b}	13.1^{a}	4.6^{b}	2.3
05:00	06:00	2.0^{b}	3.3^{b}	16.4^{a}	3.1^{b}	3.21
06:00	07:00	4.8^{b}	9.0^{b}	25.9^{a}	14.2^{a,b}	4.13
07:00	08:00	31.5^{b}	25.4^{b}	43.1^{a}	26.3^{b}	4.4
08:00	09:00	33.5	41.1	39.1	41.6	3.47
09:00	10:00	28.6	37.8	26.6	31.1	3.64
10:00	11:00	36.2	29.1	30.7	25.6	4.11
11:00	12:00	40.4	33.3	37.1	35.8	3.38
12:00	13:00	35.9	40.1	33.9	47.4	3.61
13:00	14:00	38.9^{a}	36.6^{a}	27.8^{b}	46.8^{a}	2.48
14:00	15:00	28.5^{b}	36.7^{a,b}	35.2^{a,b}	46.3^{a}	2.41
15:00	16:00	36.0	42.7	31.9	33.7	2.84
16:00	17:00	51.2^{a}	49.9^{a}	33.4^{b}	23.7^{b}	3.33
17:00	18:00	53.9^{a}	55.4^{a}	26.9^{b}	21.1^{b}	4.47
18:00	19:00	41.1	43.6	48.6	43.9	3.02
19:00	20:00	20.6^{b}	12.9^{b}	51.7^{a}	51.4^{a}	4.95
20:00	21:00	10.9^{c}	5.9^{c}	38.3^{b}	55.6^{a,b}	5.43
21:00	22:00	8.5^{b}	2.4^{c}	9.4^{b}	44.8^{a}	4.19
22:00	23:00	14.6	4.9	12.3	10.2	2.43
23:00	00:00	27.2^{a}	16.3^{a}	17.3^{a}	4.6^{b}	3.84

Time foraging is expressed in minutes for each daily hour. Different lowercase letters in a line differ by Tukey test at 5%.

significant nighttime foraging (**Figure 2**). Champion et al. (14) and Gregorini et al. (12, 19) suggest that both sheep and cows may have significant meals at night. In temperate climates, ruminants have approximately three major grazing events per day: at sunrise, around 12:00, and sunset (36). However, this pattern is flexible and affected by external environmental conditions, especially environmental temperatures. According to Gregorini et al. (12), an adaptation could be an increase in the length of grazing events and a decrease in the number of meals during short days, or ruminants could increase meal numbers, including times at night to allocate these meals.

To faithfully represent the natural behavior of heifers, it is possible to confirm that the time extent of grazing behavior assessments that should be evaluated needs to include part of the night. In our case, despite being 35 min less total grazing time than the CONTROL, evaluations using all day and until midnight showed no differences ($P > 0.05$) when compared with the natural behavior of heifers. To achieve this representativeness, we evaluated 75% of the entire day.

Additionally, our data support that in a tropical climate situation, sunlight (including dawn and dusk) has a strong influence on animal activity (39), even in warm environments. Furthermore, another important fact is that grazing events, which occur after sunset, should not be underestimated (38). Nevertheless, trials assuming that grazing time observed only between sunrise and sunset (natural light) represents an accurate estimate of the grazing time are underestimating the real time that animals spend foraging. This bias can be magnified when this incomplete information is used to estimate/calculate other behavior variables (e.g., bite mass) causing serious misunderstandings, especially in trials were the bite mass is estimated by the division of daily animal intake by the daily bite number, which, in turn, is estimated by multiplying the bite rate by total grazing time.

Of course, to determine the "size" of the evaluation period that must be performed, baseline experiments need to be conducted. In our case, it was possible to reduce the total evaluation period by 25% with no effects in grazing representativeness [all seasons mean: 94% of the total grazing time observed in the CONTROL treatment ($P > 0.05$)]. This protocol reduces possible overestimations of other dependent variables of grazing time and allows the comparison among trials conducted in similar conditions, mostly by representing the actual grazing time of animals.

Grazing activity occurs mainly during daylight and the influence of day length changes the foraging patterns of animals (**Figure 2**). Moreover, the different grazing peaks during different seasons demonstrate the ability of animals to adapt their ingestive activity to variations in daylight, reserving most rumination and rest activities for periods of darkness to maintain their welfare. Furthermore, there are other factors to determine this pattern, such as the difficulty of food selection during dark periods (15), defense mechanisms (40), and hormonal factors (41).

The extent of grazing taking place during daylight in summer and spring (higher temperatures) compared with that in autumn and winter (lower temperatures) was not highly variable, even though the peaks in this behavior occurred during different periods of the day. In summer and spring, grazing begins earlier in the day compared with that of autumn and winter seasons. Consequently, grazing peaks during the morning are more intense in warm seasons than cool ones. This probably occurs because of the longer photoperiod, which encourages the animals to begin foraging earlier (12, 19), and thus, it reduces the need for foraging during the hottest period of the day (late morning/early afternoon). After the first meal (morning grazing peak), animals decrease the time spent grazing, probably because of rumen filling (3).

Another important practical information indicated by our data is related to the use of feeding supplements in production systems. When the use of supplementary sources of feeding is necessary, supplements should be offered to the animals between the grazing peaks. In our subtropical environment, this means offering it from 08:30 to 09:30 (spring–summer) and from 09:30 to 10:30 (autumn–winter) (**Figure 2**). Thus, using this information, it is possible to reduce herbage substitution by the supplement, and this was already used in that particular environment (42, 43). Furthermore, when energetic supplements are used, this management schedule allows better use of herbage nitrogen (44).

Grazing peaks earlier in the afternoon during autumn and winter compared with that of summer and spring, which may be a consequence of the interaction of photoperiod and environmental temperatures. The first is related to the light period when animals can distribute themselves for better grazing activity (15), avoiding the high-temperature periods of the day. Secondly, animals start grazing when temperatures are milder (late afternoon). In seasons with high environmental temperatures, this grazing peak [mainly in summer (12, 19)] is slightly longer than in other seasons.

The longer duration and later start of the afternoon grazing peak probably influences the later onset of grazing during the nighttime period during summer. Only during summer did animals not obtain a meal during the night between 22:00 and 01:00 h. Grazing events over the night are also necessary for the animals to maintain their metabolic heat production (by rumen fermentation) during cool seasons (45). Furthermore, our data of nighttime behavior observations contradicted the assumption that heifers do not forage for significant periods at night (16, 37, 38). Therefore, if one of the experimental goals is to measure the length of foraging events and represent natural grazing activity, it is necessary to accurately evaluate periods of nighttime grazing, especially under subtropical and tropical climate conditions.

CONCLUSION

Visual observations beginning at dawn until midnight represented the total foraging time in a subtropical natural grassland. This period could be used to represent grazing activities performed during 24 h, as well to evaluate the natural behavior of heifers, and this would be useful for the calibration of automatic recording devices.

Diurnal evaluations of grazing behavior of beef heifers do not represent the necessary time to represent grazing activities in natural grasslands in subtropical and tropical conditions.

Beef heifers managed in natural grasslands have a diurnal pattern of grazing. However, there are significant grazing events in dark periods, and there are also significant changes between seasons in the times that animals perform these events. Farmers could use this daytime pattern to establish key periods of the day for observations of grazing behavior, such as the end of the morning grazing peak or the beginning of the evening peak.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal ethics committee—Federal University of Santa Maria.

AUTHOR CONTRIBUTIONS

FJ, ES, and LO compiled and organized the data, ran the statistical analyses, wrote the article, and assisted in the behavioral evaluations. BK, PC, and LM were responsible for the trials wherein data were collected, as well work conducted on the behavior assessments. FQ revised the manuscript. All authors contributed to the article and approved the submitted version.

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Beef Cattle on Pasture Have Better Performance When Supplied With Water Trough Than Pond

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The behavior and performance of steers on pasture regarding water availability in troughs or in ponds were compared. Eight paddocks were randomly allocated to one treatment: POND (∼30 m of diameter) or TROUGH (water trough, 120 cm diameter and 60 cm high and 500 L capacity). Eight groups of six beef steers were randomly assigned to one of the paddocks. The first 10 days were considered for animal habituation. Animals were individually weighed (days 0, 30, 60, and 90). Beginning in the day after each weighing on days 30 (Month 1), 60 (Month 2), and 90 (Month 3), behavior and animal distribution in the paddock were recorded by direct visual observation in three periods of 4 consecutive days. Water temperature and fecal and herbage DM were also recorded in these periods. Water intake was measured during 16 random days in the troughs. Data were analyzed using Generalized Linear Mixed Models, with treatment and period as fixed effects. TROUGH steers gained more weight (0.44 vs. 0.34 kg/day/animal; $P \leq 0.007$) during the experiment and were heavier than the others at the end of the study ($P \leq 0.05$). POND steers spent more time drinking water, but TROUGH steers increased the number of drinking events throughout the study ($P \leq 0.05$), suggesting an adaptation for the new type of water source. Both treatments increased grazing time throughout the study, but not ruminating time ($P \leq 0.05$). Walking time differed between treatments in all periods of behavior observation ($P \leq 0.05$). Events of animal licking and ingesting salt of POND steers reduced throughout the study ($P \leq 0.05$). The number of drinking events of TROUGH steers increased throughout the study, and drinking events were longer for POND steers than TROUGH steers ($P \leq 0.05$). TROUGH steers spent more time on pasture on Month 2 ($P \leq 0.05$). Period collection did not affect the water intake of TROUGH treatment ($P > 0.05$). This study demonstrates that water available in troughs rather than ponds for steers on pasture has positive effects on their weight gain and affects cattle behavior.

Keywords: water intake, average daily gain, beef cattle welfare, extensive pasture, behavior

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INTRODUCTION

Water supply and presentation for bovines have been subjects of increasing interest as they affect cattle drinking behavior and preference (1–4). Water quality also affects grazing behavior and cattle performance (5). The lack of appropriate water supply may lead to water restriction, with detrimental effects to water consumption and animal welfare (6–8) as well as animal performance (5, 9, 10).

Dry matter ingestion and cattle performance are closely related to water consumption. Restricted water intake reduces feed intake (11, 12) and may result in lower weight gain. The need of water for appropriate growth, reproduction, digestion, excretion, and all body processes and metabolism is well-known (13). Water required by cattle is achieved from the sum of that intake from plants and other feeds, plus that consumed as free water (13), with the later being usually the major part of the water consumed. Water is perhaps the cheapest nutrient to offer in most production systems, and certainly the most important, affecting directly all body functions. Nevertheless, allocation of water to cattle is mostly underestimated, especially in situations where beef cattle are raised on pasture.

To assure adequate water ingestion for beef cattle in pasture-based systems, a maximum of 250-m walking distance from the water source is recommended (14). However, this recommendation depends on the paddocks size and their arrangements as water source location and water availability that can influence the distance that animals travel in search for water and the number of visits to the water trough. If water is outside of small paddocks, e.g., in the corridor, even a 150-m distance will affect the number of visits, water consumption, and access of subordinate animals (7). A water source located inside the paddock has positive effects on animal performance (15).

The use of water troughs decreases the energetic demand of animals to find a water source (16) and is a useful management strategy to improve the distribution of animals in pastures in order to preserve natural water sources (17), as off-stream watering generally shifts cattle drinking from river to water (18). However, careful placement is required to improve the likelihood that cattle will find and use these water sources and thus decrease their dependence and use of permanent streams and associated riparian areas (19).

Furthermore, it provides availability of water in adequate quantity and quality to the animals. Any low manure contamination in the water from the pond can affect water intake. Dairy cattle can detect low levels (as 0.005% in the water) of manure contamination in their drinking water, avoiding drinking it whenever is possible (20). Clean water available in a trough instead of pond water pumped to a trough or direct access into the pond resulted in an increase of 23% in yearling heifer performance (9). Among different water troughs, cattle may have preferences. They prefer and drink more water from a round plastic than from a squared concrete trough (4). Likewise, they prefer and drink more water from larger than from smaller troughs (2) and deeper and wider to shallow water troughs (3).

There is a growing concern regarding the environmental impact of cattle accessing natural water sources (21, 22). In fact, when having the choice beef cattle would prefer to drink in a water trough than in a natural stream (1). In that study, however, cattle performance was not evaluated. Perhaps the scarcity of information on water source and beef performance is one of the reasons why the vast majority of cattle on pasture drink water from streams, rivers, lakes, or ponds. Farmers usually consider enough having any natural source of water for beef cattle and are not aware of any effect on cattle performance, drinking behavior, or welfare, regarding the source of water. This is largely the reality of cattle on pasture in all countries. With the aim of bringing information on that issue, this experiment was designed to compare the behavior and the performance of beef steers reared in a continuous grazing system, regarding water availability in troughs or in ponds.

MATERIALS AND METHODS

The experiment was carried out in a private farm (Cacupé Farm) in the municipality of São Gabriel, the South of Brazil, at the geographic location of 30°20′S and 54°19′W, with an average altitude of 124 m. It was carried out from January to April of 2005, when air temperature ranged from 17.1 to 31.1°C, and total rainfall was 165 mm along the 4 months. Before the beginning of the experimental period, all animals were kept under a continuous grazing system (200 ha) with *ad libitum* mineral salt and water from natural ponds. The pasture was mostly composed by native species, as Uruguayan rice grass (*Piptochaetium montevidensis*), Spanish clover (*Desmodium incanum*), strongback (*Desmodium adscendens*), rescue grass (*Bromus catharticus*), cane grass (*Eragrostis plana*), Australian jointvetch (*Aeschynomene falcata*), and beard grass (*Andropogon bicornis*).

Study Description

The area of the experiment (32 ha) was equally divided into eight paddocks, with similar pasture botanical composition (as described), natural shade (trees), and mineral salt mix offered *ad libitum*. The eight paddocks were randomly allocated to one of the treatments: POND treatment, four paddocks had water available in a pond of ∼30 m of diameter. TROUGH treatment, four paddocks had water available in a round water trough made of polythene (120 cm diameter and 60 cm high and 500 L capacity; Tigre®, Joinville, SC, Brazil). The water from one of the ponds was pumped to a 2,000-L reservoir and then distributed by gravity to the water troughs. A floating ball controlled the water level of the troughs.

A total of 48 beef steers, crossbred of Nelore and Hereford, with average age of 15 months and weighting 189.1 ± 35.35 kg were used. Animals were blocked by body weight and randomly allocated to one of the eight groups of six. Then, the groups were randomly assigned to one of the eight paddocks. All animals were identified by ear tags and coat color and were individually marked with numbers on their sides with black livestock markers (Raidex®, Dettingen; Erms, Germany). Animals from both treatments had no experience with the water trough before the study.

Measurements

Weight Gain and Dry Matter Intake

Animals were individually weighed (individual scale CAUDURO 40100−1,500 kg, Cachoeira do Sul; Brazil) located next to the paddocks at the beginning of the study (Day 0) and on days 30, 60, and 90, always at 9:00 h, after 3 h of fasting. The average daily gain (ADG) was determined by the difference between weights on Day 30, Day 60, and Day 90 divided by the number of days between each measurement (i.e., 30).

Behavior and Distribution

Animal behavior was directly recorded in three periods of 4 consecutive days (named Month 1, Month 2, and Month 3), starting in the day after each weighing on days 30, 60, and 90. In the first 2 days of each period, two groups of each treatment were observed simultaneously from 6:00 to 12:30 h on day 1 and from 12:30 to 19:00 h on day 2. On days 3 and 4, the same procedure was made with the remaining two groups of each treatment. That is, each group was observed for 13 h per period. Four observers watched different groups simultaneously. The observers were trained before the study to ensure interobserver reliability (23), and they were balanced across groups and treatments, in order that every observer recorded equally both treatments. The four observers were the same throughout the entire study.

Behaviors were recorded every 10 min using the instantaneous scan sampling technique (23, 24). The behaviors observed were grazing (animal with the mouth below or at the level of the forage or grabbing forage, may be stationary or moving forward), ruminating (animal chewing with lateral jaw movements with the head at the same level or above its body, lying or standing), walking (animal moving, with the head above the superior level of the forage), and other (any other behavior not described above, such as mineral salt and water ingestion and interacting with other animals), according to the definitions adopted by the Laboratory of Applied Ethology and Animal Welfare (LETA) of the Federal University of Santa Catarina (7).

All events of animal licking and ingesting salt were recorded by continuous observation. The number and duration of drinking bouts were also recorded. All events of drinking (i.e., drinking bout) were defined as the beginning to the end of submerging lips in water with perceivable swallowing movements at the throat.

Location of the animals in the paddock (shade, pasture, or water source) was recorded every 20 min. The animal was considered in the shade when the head and most of its body was covered by the shade; at the water source, when standing or lying at <5 m from the water source.

Water Intake, Water Temperature, and Climatic Parameters

Daily water intake was measured during 16 random days of the experiment (on days 32, 39, 40, 45, 51, 52, 60, 61, 65, 66, 67, 68, 79, 85, 86, and 87). Only water intake from TROUGH treatment was recorded. During these days, the volume of water required to fill the trough in 24 h was measured using a flow meter (Tecnobrás®, Brazil; precision of 0.01 L) attached to the water inlet.

During the behavior observation days, the temperature of water from ponds and troughs was measured every 2 h from 7:00 to 19:00 h, using floating thermometers (Dolphin®, Guangdong, China) submerged at ∼4 cm under water surface. If an animal was drinking water at the same moment of water temperature measurement, the observer waited until the animal gets a distance from the water source. Daily climatic parameters were obtained from the Meteorological Station of *Fundação Estadual de Pesquisa Agropecuária* (FEPAGRO) in the city of São Gabriel.

Dry Matter of Fecal and Herbage Sampling

The collection of fecal samples was carried out between 7 am and 9 am of the following day after the behavior observations, with the animals in their respective paddocks. The sample was collected close to the soil, immediately after defecation, disregarding the bottom and top parts. Fecal samples were placed in identified sterile plastic bags and were stored at −18°C. To obtain the DM content, the samples were placed in an aluminum tray, weighed and oven dried at 100°C for 48 h, and then weighed again.

Right after the collection of fecal sampling, pasture sampling was carried out. Five herbage samples of 0.25 m^2 were randomly collected from each paddock (25). The samples were cut close to the ground and immediately placed in identified sterile plastic bags and stored at −18°C. To obtain DM contents of herbage, samples were placed in identified paper bags and dried in forced circulation oven at 60°C for 48 h (26).

Statistical Analysis

Descriptive statistics were calculated using Microsoft® Excel® for Windows. Data from water intake of TROUGH treatment measured during 16 random days were grouped into two periods, where Months 1–2 covered data collected from days 32 to 59 and Months 2–3 covered data collected from days 60 to 87. The total amount of water drank during 24 h in each paddock was divided by 6 to achieve an average of water consumption/animal/day. ADG, final body weight, water temperature, water intake, and DM of fecal and herbage data were analyzed using Generalized Linear Mixed Models (Proc Glimmix) of Statistical Analysis Software (SAS) 9.3. Models included treatment and period as fixed effect. The interaction between treatment and period was removed from the models as it was not significant ($P > 0.10$). In the models for ADG, final body weight, and DM of fecal, animal was used as the experimental unit. In models for water temperature, water intake, and DM of herbage, paddock was used as the experimental unit. For water intake and DM of herbage, gamma was included as the type of distribution.

The frequency of grazing, ruminating, walking, and other behavior; the frequency of position of the animal in the paddock (shade, pasture, or water source); the frequency of events drinking water and licking and ingesting mineral salt; and the duration of drinking bouts were also analyzed using Generalized Linear Mixed Models (Proc Glimmix) of SAS. Models included treatment and period as fixed effect. With exception of ruminating behavior and the duration of drinking bouts, interactions between treatment and period were included in the models as they were significant ($P \leq 0.05$). Animal within paddock was used as the experimental unit, and gamma was

TABLE 1 | Effect of treatment and period on ADG, water temperature, and DM of fecal and herbage sampling (LSM ± SEM).

	Treatment			Period				Statistics		
	POND	**TROUGH**	**s.e.m.**	**Month 1**	**Month 2**	**Month 3**	**s.e.m.**	**Treatment**	**Period**	**Treatment × period**
ADG* (kg/day)	0.34y	0.44x	0.037	0.47a	0.50a	0.21b	0.036	≤0.007	≤0.001	NS
Mean water temperature (°C)	28.7	29.3	0.46	29.7	28.1	29.2	0.57	NS	NS	NS
DM of fecal (%)	17.6x	16.1y	0.26	16.4	17.0	17.1	0.30	≤0.0001	NS	NS
DM of herbage (%)	45.5	47.7	1.40	42.6b	46.3b	51.3a	1.72	NS	≤0.01	NS

NS, non-significant; ADG, average daily gain; DM, dry matter; s.e.m, standard error of means. Different letters "a, b" represent significant differences (P ≤ 0.05) between periods of observation. "x, y" represent significant differences (P ≤ 0.05) between treatments.

included as the type of distribution. Results are reported as least square means (LSM) with the associated standard error of means (SEM). Statistical differences are reported when $P \leq 0.05$, and tendencies were reported when $0.05 < P \leq 0.10$.

RESULTS

Weight Gain and Dry Matter Intake

TROUGH steers had higher (29%) ADG than POND steers ($P = 0.007$; **Table 1**). ADG was lower on Month 3 than Month 1 and Month 2 ($P \leq 0.0001$; **Table 1**) in both treatments. Despite that the initial body weights were similar in all groups (189.1 ± 35.35 Kg; $P > 0.05$), TROUGH steers (228.2 ± 1.50 kg) were heavier than POND steers (219.4 ± 1.50; $P \leq 0.0001$) at the end of the study.

Behavior and Distribution

Treatment did not affect the ruminating time ($P > 0.05$), but it was lower on Months 2 (18.4 ± 0.87) and 3 (20.0 ± 0.94) than on Month 1 (27.2 ± 1.28; $P \leq 0.05$). For grazing, walking, and other behavior, there were interactions between treatment and period of observation ($P \leq 0.05$; **Figure 1**). On Month 2, TROUGH steers spent more time grazing than POND steers ($P \leq 0.05$); in contrast, POND steers spent more time grazing than TROUGH steers on Month 3 ($P \leq 0.05$). Both treatments increased grazing time throughout the study ($P \leq 0.05$). Walking time differed between treatments in all periods of behavior observation ($P \leq 0.05$). Within treatment, walking time was higher on Months 1 and 2 for POND steers, while it was lower on Month 3 ($P \leq 0.05$). Other behaviors, which included any other behavior not previously mentioned, such as mineral salt and water ingestion and interaction between animals, differed between treatments in Month 2 ($P \leq 0.05$). Within treatments, POND steers spent more time performing other behaviors on Month 2, while TROUGH steers spent more time in other behaviors on Month 1 compared to the other periods of behavior observation ($P \leq 0.05$).

There were interactions between treatment and period of behavior observation on the number of events of animal licking and ingesting salt and number of drinking bouts (**Figure 2**). The number of events of animal licking and ingesting salt of POND steers was higher on Month 1 than Months 2 and 3 ($P \leq 0.05$), while it remained constant for TROUGH steers ($P > 0.05$). In Month 3, animals in the TROUGH treatment had a higher number of drinking bouts than in the POND treatment ($P \leq 0.05$). The number of drinking bouts of TROUGH steers increased throughout the study ($P \leq 0.05$), while it remained constant for POND steers ($P > 0.05$). There was no interaction between treatment and period of behavior observation on the duration of drinking bouts ($P > 0.05$). However, drinking bouts were longer in POND steers (59.2 ± 3.32) than TROUGH steers (43.5 ± 3.19; $P \leq 0.001$) and both treatments had longer drinking events in Month 1 (65.9 ± 4.49) than Month 2 (47.6 ± 3.73) and Month 3 (40.5 ± 3.45; $P \leq 0.001$).

The effect of treatment and period on animal distribution on paddock is presented in **Figure 3**. POND steers spent a similar time on shade during the three periods of behavior observation, which was similar for time spent on pasture and near to water source ($P > 0.05$). In contrast, TROUGH steers spent more time on pasture on Month 2, which was balanced with less time on shade and water source in this respective month ($P \leq 0.05$).

Water Intake, Water Temperature, and Climatic Parameters

Period of data collection did not affect the water intake of TROUGH treatment ($P > 0.05$). During the recording days, TROUGH steers drank 12.5 ± 0.87 L/animal/day ($P > 0.05$).

Averages of minimum and maximum air temperature were calculated for the months of January (min 19.6 ± 2.5°C; max 31.1 ± 2.6°C), February (17.5 ± 1.6°C; max 29.8 ± 2.1°C), March (17.1 ± 3.5°C; max 28.3 ± 4.3°C), and April (17.7 ± 2.4°C; max 29.3 ± 2.1°C). Total rainfall during the 4 months was 165 mm, distributed in 21 days (January: 22.4 mm/5 rain days; February: 33.5 mm/4 rain days; March: 29.5 mm/4 rain days; April: 80.2 mm/8 rain days).

Minimum water temperature was not affected by treatment; however, it was lower on Month 2 than on Month 1 and Month 3 ($P \leq 0.001$, **Table 1**). In contrast, period did not affect the maximum water temperature, although it was higher on the TROUGH than on the POND treatment ($P \leq 0.05$; **Table 1**). Average water temperature was not affected by treatment or by period (29.0 ± 0.49°C; $P > 0.05$; **Table 1**).

Dry Matter of Fecal and Herbage Sampling

TROUGH steers presented lower fecal DM than POND steers ($P \leq 0.0001$; **Table 1**), without effect of period or interaction on fecal DM ($P > 0.05$). In contrast, herbage DM was not affected by treatment ($P > 0.05$) but by period, being higher on Month 3 than on Months 1 and 2 in both treatments ($P \leq 0.01$; **Table 1**).

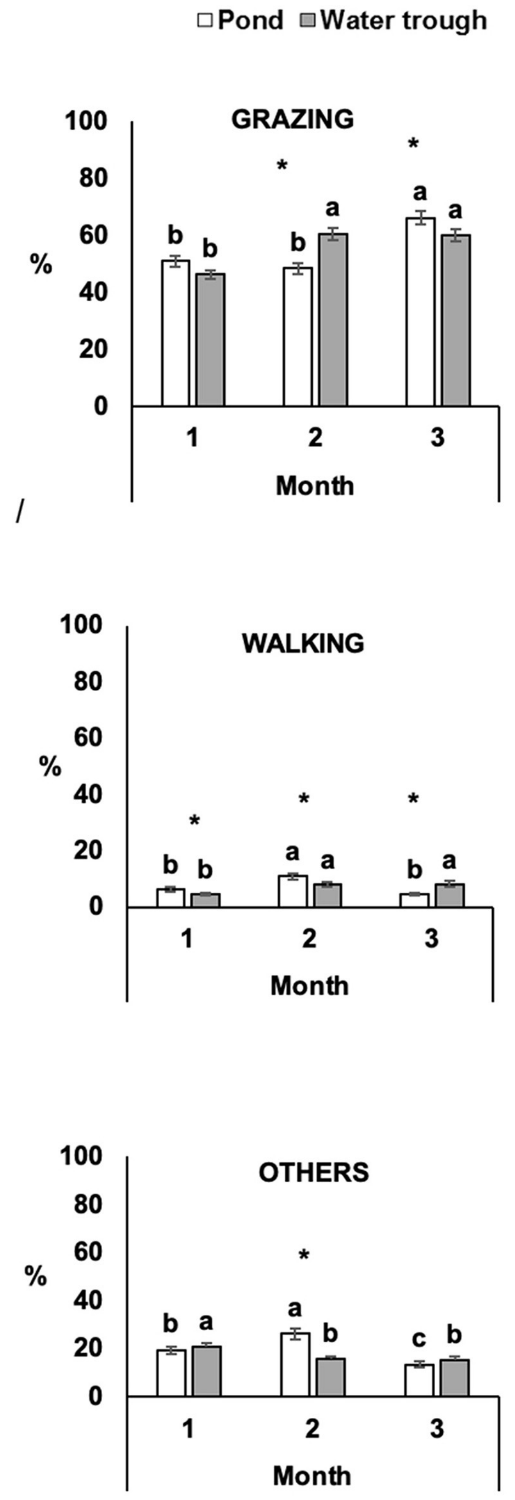

FIGURE 1 | Effect of treatment and period on steer behavior (least square means ± s.e.m.). Grazing, walking, and other behaviors are expressed in least square means ± s.e.m. of the percentage of animals performing each behavior on the three periods of observation. Different letters "a, b, c" represent significant differences ($P \leq 0.05$) between periods of observation within treatment. "*" represents significant differences ($P \leq 0.05$) between treatments within the period of observation.

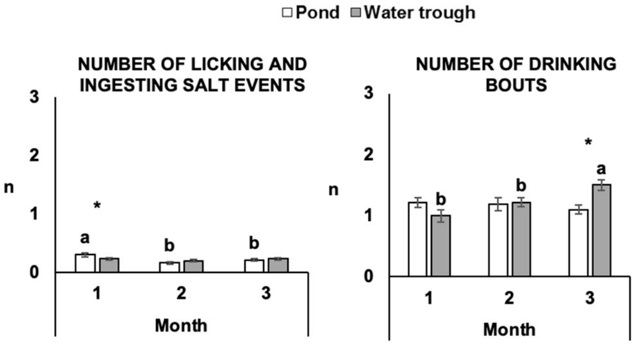

FIGURE 2 | Effect of treatment and period on the number of events of animal licking and ingesting salt and number and duration of drinking bouts. The number of events of animal licking and ingesting salt and drinking bouts is expressed in least square means of the number of events per animal per period of observation. The duration of drinking bouts is expressed in least square means of the duration (s) of events per animal per treatment or period of observation. Different letters "a, b" represent significant differences ($P \leq 0.05$) between periods of observation within treatment. "*" represents significant differences ($P \leq 0.05$) between treatments within the period of observation.

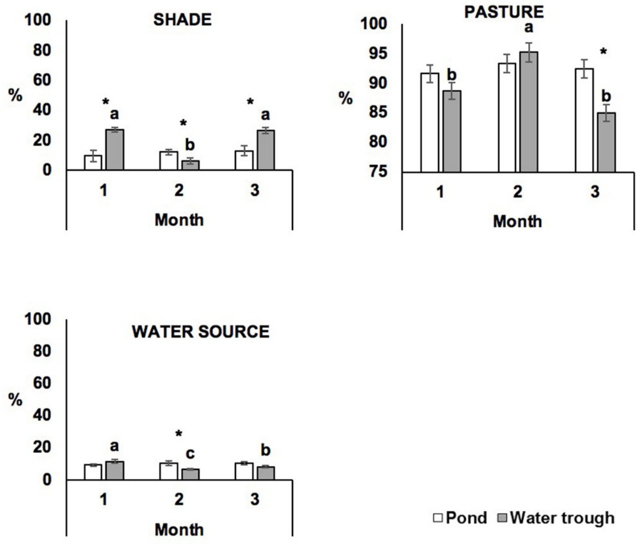

FIGURE 3 | Effect of treatment and period on steer distribution (least square means ± s.e.m.) on the paddock. Shade, grassland, and water source are expressed in least square means ± s.e.m. of the percentage of animals positioned on the three periods of observation. Different letters "a, b, c" represent significant differences ($P \leq 0.05$) between periods of observation within treatment. "*" represents significant differences ($P \leq 0.05$) between treatments within the period of observation.

DISCUSSION

The findings of this study showed that water supplied in a trough rather than in a pond has positive effects on animal performance and behavior. Although having the same body weight at the beginning of the study, TROUGH steers had an ADG 29% higher than POND during the experiment and were 4% heavier at the end of the study. Our findings confirm the previous study showing that cattle drinking clean water delivered to a trough gained 23% more weight than cattle drinking from a dugout (9). Similarly, yearling heifers gained 23% more weight when drinking clean water delivered to a trough than those drinking directly from a pond and 20% more than those drinking pond water pumped to a trough (5). Cows and calves with off-stream water also gained more weight than no-off stream animals (27). Brew et al. (28) reported that water intake of beef cattle is positively correlated with feed intake and ADG, which are in line with our findings as steers from both treatment reduced ADG and drinking time throughout the study.

Despite that grazing time was longer on POND treatment on Month 3, the difference of final body weight between treatments may be due to the fact that TROUGH steers increased their grazing time from Month 2, while POND steers only showed an increase on Month 3 compared to the beginning of the study. A previous study also reported an effect of water source on grazing time: cattle with access to clean water also spent more time grazing and less time resting than animals receiving pond water pumped to a trough and those with direct access into the pond (5). In our study, a systematic approach to ensuring similar pasture availability and consumption was not carried out, so it was not possible to affirm that nutrition did not influence weight gain.

DM intake is the most important factor for water intake in bovines (29), followed by milk production, sodium intake, and high temperatures (6). Earlier studies reported a positive correlation between water intake and DM intake (6, 29–31) and salt ingestion (6, 32). Animals under water deprivation have a reduction in food consumption and an increase in urine concentration (33). In contrast, cows accelerate drinking water intake to excrete a large amount of potassium and nitrogen into urine in excess of their needs (34). Despite that DM of herbage mass increased throughout the study in both treatments, the water intake from the TROUGH steers was not affected by the period of the study. Paddocks were set up on the same pasture, and the herbage content of both treatments was equal, as shown in **Table 1**; therefore, the higher fecal DM of POND steers could indicate that this group had a lower water intake than TROUGH steers. Moreover, fecal DM did not follow the increase in DM of herbage between periods, suggesting an effect of treatments on the moisture content of the feces.

The duration of drinking bouts was longer in steers with access to POND than in TROUGH. Besides that, the number of drinking bouts increased on steers with access to TROUGH in the following periods, being higher than POND in the last period of behavior observation. It has been shown, in a number of experiments reported in a systematic review, that an increased frequency of drinking water resulted in increased weight gain in beef cattle and milk production in dairy cows (35), as was found in this experiment. The increase in the number of drinking bouts of steers with access to TROUGH in this study could be due to animal adaptation to the new type of water source. In the Bagshaw et al. (36) study, beef cattle in the grazing system also increased the use of trough over time.

Water intake by cattle can be affected by other factors including weather conditions, water quality, and height of the water trough. Increased THI (temperature–humidity index) resulted in cows drinking more water, spending more time at the drinker, making more visits to the drinker, and competing more at the drinker (37). Among other characteristics, cattle can discriminate for and select water based on organic solid contents (38) and they reduce water intake due to suspended particulate matter that can influence its appearance, odor, taste, and physical and chemical properties (39). Also, they have an aversion to drink water containing feces (5). In the case of the present study, animals could enter in POND water. Therefore, it was prone to manure contamination from both erosion of the soil adjacent and direct defecation and urination into the water by drinking animals (40). However, despite that suspended particulate matter could be higher on POND treatment, the chemical composition and microbiological quality of water from both treatments did not differ, as water of the troughs was pumped from the pond.

While in POND treatment water was offered at the ground level, in TROUGH it was 60 cm high. In the study of Machado Filho et al. (2), the cows drank more water from a larger and higher trough, also 60 cm high, when they had access for 24 h than on the smaller version (30 cm height). Likewise, beef heifers preferred and drank more from a 60-cm-height round plastic trough than from a 50-cm-height squared concrete trough (4), and dairy cows took more sips, spent more time drinking, and drank more water from higher (60-cm) and larger troughs than small ones (30-cm-height) (3, 41).

The current study was conducted during the hottest months of the summer, under high temperatures, and the average of maximum air temperatures was above 28°C. Temperature and humidity have a direct relationship with cattle water consumption (32, 37, 42). Month 2 of behavior observation was the month with the lowest minimum and maximum air temperatures compared to the other 2 months. Apparently, differences in weather conditions among months did not change POND steers' spatial distribution. However, in Month 2 TROUGH steers spent less time near the shade or water source, therefore spending more time on pasture. In fact, TROUGH steers grazed longer in Month 2, therefore spending more time on pasture. Conversely, in Month 3 POND steers spent more time on pasture and less time in shade than TROUGH steers, and they also grazed longer in this period. These results are unlikely to be explained by the weather conditions but might be related to water intake, once dry matter intake is closely related to water (13).

The availability of shade is essential for grazing animals, and their absence can reduce their well-being (43) and modify their behavior; that is, cows can spend more time around the water source when the shade is unavailable or insufficient (44). In addition, the quantity of forage in the field is likely to alter cattle behavior around water sources (36). In the present study, it was not possible to control the distance between the shade and the water sources (pond or trough), but this could explain the difference between treatments on their spatial distribution throughout this study. The location of the trough seems to be one of the critical factors affecting drinking behavior by cattle (7, 36).

Mean water temperature did not differ between TROUGH and POND treatments. Therefore, the differences found in this study are unlikely to be due to water temperature. Previous studies have reported that beef cattle drink more warm than cold water (45). This finding was later confirmed on dairy calves (46), lactating dairy cows (47), sheep (48), and goats (49).

CONCLUSION

This study demonstrates that water available in trough rather than ponds has positive effects on steer performance and affects beef cattle behavior. Steers supplied with water on trough gained more weight and were heavier than the other group of animals at the end of the study. In general, POND steers spent more time drinking water but TROUGH steers increased the number of drinking events throughout the study, suggesting an adaptation for the new type of water source.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because at the time of fieldwork, Brazil didn't have any regulation requiring previous evaluation from Ethics Committee for non-invasive studies. Even though the activities of the experiment followed conduct respectful to the welfare of the animals involved according to Brazilian Federal Law No. 11.794 of October 8, 2008, later approved, which regulates use of animals in research. Animals were individually weighed following the routine of the farm, and the only direct intervention was marking animals using nontoxic markers for identification in the field. The other activities during the experiment were restricted to visual observation. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

LP and GB conceived, planned, designed the experiment, and wrote the manuscript. GB and DT made the data collection and fieldwork. DT performed statistical analysis and contributed to the manuscript. LP, GB, and DT interpreted data. All the authors approved the final version of the manuscript.

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Preventive Hoof Trimming and Animal-Based Welfare Measures Influence the Time to First Lameness Event and Hoof Lesion Prevalence in Dairy Cows

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Background: The objectives of this study were to, (1) investigate the impact of the Dutch five-step hoof trimming (HT) technique on time to lameness and hoof lesion prevalence in grazing (GR) and non-grazing (NGR) dairy cows, and (2) determine the association between potential benefits of HT and animal-based welfare measures during lactation**.** A total of 520 non-lame cows without hoof lesions from 5 dairy farms (GR = 2, NGR = 3) were enrolled at early (within 30 days in milk; DIM) and late lactation (above 200 DIM), and randomly allocated to either trimmed (HGR or HNGR) or control groups (CON-GR and CON-NGR). Locomotion scores, body condition, hock condition, leg hygiene, and hoof health were assessed at monthly intervals until the following 270 days in milk. The data were analyzed using Kaplan-Meier survival analysis, multivariable Cox, and logistic regression models. The overall incidence rate of lameness was 36.2 cases/100 cows/month, with corresponding rates of 27.4, 31.9, 48.4, and 45.8 cases/100 cows/month in HGR, HNGR, CON-GR, and CON-NGR, respectively. Time to first lameness event was significantly higher in HGR (mean ± S.E; 8.12 ± 0.15) compared to CON-GR (7.36 ± 0.26), and in HNGR (8.05 ± 0.16) compared to CON-NGR (7.39 ± 0.23). The prevalence of hoof lesions in the enrolled cows was 36.9%, with a higher occurrence in CON-GR (48.8%) than HGR (23.2%), and in CON-NGR (52.6%) compared to HNGR (32.2%). The majority of hoof lesions were non-infectious in grazing (HGR vs. CON-GR; 21.3 vs. 33.3%) and non-grazing herds (HNGR vs. CON-NGR; 25.0 vs. 40.4%). The risk of lameness was higher in underconditioned cows (Hazard ratio; HR = 3.1, 95% CI 1.2–7.4), presence of hoof lesion (HR = 33.1, 95% CI 17.6–62.5), and there was variation between farms. Aside HT, lower parity (OR = 0.4, 95% CI 0.2–0.8), normal hock condition (OR = 0.06; 95% 0.01–0.29), and absence of overgrown hoof (OR = 0.4; 95% 0.2–0.7) were protective against non-infectious hoof lesions. Functional HT is beneficial as a lameness preventive strategy during lactation; however, ensuring older cows are in good body condition and free from hock injuries are equally important.

Keywords: lameness, hoof lesions, hoof trimming, animal welfare, dairy cows

INTRODUCTION

Lameness is amongst the 3 most common health issues affecting dairy cows after mastitis and infertility (1, 2). It remains a financial burden to dairy farmers, with significant economic loss attributed to impaired milk yield, low reproduction performance, high culling risk, and treatment costs (3–5). Most lameness cases involve hoof pathologies, although their presence may not elicit detectable changes in cows' gait (4, 6). Thus, lameness may be seen as an indicator of hoof lesion, which is often painful and contributes to the poor welfare of dairy cattle (7, 8).

Animal-based welfare measures (ABWM) are vital parameters in assessing the well-being and performance of dairy cows (9). For instance, individual cow characteristics such as body condition, leg hygiene, hock condition, and lying behavior have been associated with lameness levels on dairies (6, 7, 10). Cow-level factors relating to body condition including combined depth of the digital cushion and corium influenced the development of hoof horn disruptive lesions during lactation (11, 12). However, there is a scarcity of data regarding the variation of these measures with intervention targeted on lameness control. Moreover, ABWM need to be monitored during lactation to identify the variation of welfare outcomes and developing appropriate management plans (13).

Lameness levels vary under different management systems, with studies reporting higher prevalence in non-grazing or confined cows than those kept on pasture-based herds (14, 15). Factors such as prolonged standing time, reduced lying time, and little exercise were suggested to enhance the development of hoof lesions in confined and non-grazing dairy cows (16). These events contribute to detrimental hoof traits including disproportionate heel height, too-long dorsal wall, greater imbalance in weight distribution between the front and hind hooves (3, 17), and increasing the need for preventive or curative hoof trimming (HT) (18).

The five-step Dutch method (i.e., functional trimming) is widely practiced in the dairy industry (19). The HT technique advocates for relatively leveled abaxial and axial walls of the claw, and they are presented perpendicular to the metatarsals (17). Few authors have reported the benefits of functional HT as a lameness control strategy on dairies. Cows that were trimmed at mid-lactation had a lower incidence of lameness (20) and lower odds of hoof lesions (21) compared to control groups. The incidence of hoof horn lesions was significantly lower in farms conducting preventive HT compared to farms lacking such practice (20), while cows trimmed at late lactation had lower odds of developing sole ulcers in the subsequent lactation (21). However, based on the study designs and lack of information on previous lameness events, lesion history, and characteristics of the enrolled cows, attributing better hoof health to preventive HT is limited. Additionally, the benefits of preventive HT as a lameness management strategy in cows managed under confined and grazing conditions have not been widely investigated. By enrolling cows with reliable information on lameness history and monitoring of ABWMs, variation in either lameness or hoof lesion levels can be identified, and targeted welfare management plans can be implemented. Therefore, the objectives of this study were to, (1) investigate the impact of the Dutch five-step HT technique on time to lameness and lesion prevalence in grazing (GR) and non-grazing (NGR) dairy cows, and (2) determine the animal-based factors that may influence the potential benefits of HT during lactation.

MATERIALS AND METHODS

Selection of Farms

This study is part of a large project including several observational studies on the epidemiology and effective preventive measures for lameness control in Malaysian dairy farms. A total of 14 dairy farmers from six states in Peninsular Malaysia were contacted *via* a phone and email directory obtained from the Divisional Department of Veterinary Services (DVS), and they were briefed about the study objectives, inclusion criteria, and methodology. The inclusion criteria entailed farmers' consent to participate, large herd size (>150 cows), adequate farms' health and production records, availability of HT chute (manual or hydraulic system), and either confined housing or provision of pasture access. Eight of the farmers agreed to participate. Upon farm visits, the herds were re-evaluated for compliance with the inclusion criteria, and five farms located in five states in Peninsular Malaysia (Selangor, Negeri Sembilan, Melaka, Johor, and Pahang) were finally enrolled in the study. The data collection took place from October 2018 to December 2019.

Farm Management Practices

Farm characteristics and management practices (e.g., herd size, number of milking cows, and number of staff, HT services, feeding pattern, cleaning frequency, milking technique, footbath usage, and animal source) were assessed through a structured interview with the farm owner or manager. The interview session was conducted by a single researcher in all the selected farms. Factors related to barn design (e.g., flooring type, floor cleanliness, and slipperiness, distance to milking point, stocking density) were evaluated using a modified farm inspection protocol developed by Grandin (22) and Solano et al. (23) as presented in **Supplementary Table 1**.

Two of the farms (Farm C and D) were categorized as GR herds based on the provision for external grazing for 3–6 h daily all year round. The other three farms (Farm A, B,

TABLE 1 | Herd level factors of the non-grazing ($n = 3$) and grazing ($n = 2$) farms enrolled in the study.

	Non-grazing			Grazing	
Factors	**Farm A**	**Farm B**	**Farm E**	**Farm C**	**Farm D**
Stocking density	0.8 cow/stall	1.0 cow/stall	1.3 cow/stall	0.94 cow/stall	1.2 cow/stall
Milking technique	Milking machine	Milking machine	Milking machine	Milking machine	Milking machine
Cleaning frequency	Twice/day	>Twice/day	Twice/day	Twice/day	Twice/day
Outdoor exercise	No	No	No	No	Yes
Flooring-related factors					
Barn floor type	Concrete/RM	Concrete/RM	Concrete/RM	Concrete/RM	Concrete/RM
Walkway floor type	Concrete/RM	Concrete/RM	Concrete/RM	Concrete/RM	Concrete/RM
Slipperiness	Non-slippery	Non-slippery	Non-slippery	Non-slippery	Non-slippery
Cleanliness	Clean	Dirty	Clean	Clean	Clean
Cleaning method	Vehicular manual	Manual	Manual	Manual	Automated scraper
Average distance from barn to milking point	30 m	45 m	50 m	40–60 m	65–80 m
Source of replacement cows	Australia	Australia	Australia and local	Australia, Thailand, and local	Australia
Hoof trimming practice	Except during treatment	Except during treatment	1/year	Except during treatment	2/year
Footbath	Yes	Yes	Yes	Yes	Yes
305-day milk yield	4,230	3,355	3,050	3,250	5,490
Herd size	125	180	252	350	2,800
Number of milking cows	92	121	154	174	1,210

RM, rubber mats.

and E) practiced the NGR system as cows were completely housed indoor all year round (**Table 1**). In addition to pasture access, Farm D had an outdoor exercise area with compost bedding. All the farms used fans as a heat-abatement strategy. Also, concrete floors with installed rubber mats were present at the holding, resting, and milking pens in all the enrolled farms. The herd size ranged from 125 to 2,800 cows, while the 305-day milk yield/cow ranged from 3,050 to 5,490 kg. Professional hoof trimmers were invited on a timely basis for the management of lame cows in four farms (Farm A, B, C, and E), and only one farm (Farm D) had on-farm hoof care and trimming unit.

Study Design and Sample Size Calculation

This study employed a prospective longitudinal approach including 4 cohort groups; hoof-trimmed from GR farms (HGR), hoof-trimmed from NGR farms (HNGR), non-trimmed/control from GR (CON-GR), non-trimmed/control from NGR (CON-NGR). Hence, each farm had a proportion of trimmed and non-trimmed cows. The cows were enrolled at early (within 30 DIM) or late lactation (≥200 DIM). The follow-up period was 9 months from enrollment, which was sufficient for the cows to be observed during the high-risk period for lameness in the present and subsequent lactation. Also, the period was selected considering the scheduled time for preventive HT in farm B. The required sample size per group was calculated by assuming a precision level of 5%, power of 80%, and expected lameness incidence of 40 and 20% in control groups from NGR and GR farms, respectively. The estimated sample size per group was increased from 110 to 120 animals to adjust for loss to follow-up, by assuming that 10% of the animals will be culled during the study period.

Cow Selection and Enrollment

The inclusion criteria entailed sound locomotion score (LS < 3), moderate BCS (2.5–4.0) based on the 5-point scoring scales developed by Sprecher et al. (24) and Vasseur et al. (25), respectively, presence of healthy hooves, and indications for maintenance HT (overgrown hoof, unbalanced sole surface, and disproportional heel height). On the first day of visits, all the lactating cows in each farm were assessed for locomotion scores. The cows were observed and assessed for locomotion scores one at a time while they exited the milking parlor. LS was recorded when cows completed a minimum of four steps and undisturbed while walking on a flat and non-slippery floor surface. Cows were considered for selection when presented with sound mobility (LS < 3) and farmers' consent for such animals remaining in the herd until the next lactation. Thereafter, lame cows and those affected with other health issues or had been treated using non-steroidal anti-inflammatory drugs and/or antibiotics 2 weeks before the visit were excluded.

The hoof health of the cows was examined. A multi-purpose HT chute was present in four of the enrolled farms (Farms A, B, C, and D). To enable the cows to get accustomed to the trimming chute, they were allowed to walk through the facility when returning to resting barns after milking. This was conducted twice daily for 3 days before enrollment. In Farm E, cows were examined on a tilting table HT facility (hydraulic system). The cows' limbs were restrained and their hooves were assessed to ensure the absence of lesions before enrollment. The dorsal wall length was measured using a claw check device based on the distance from the proximal aspect of the perioplic horn to the distal end of the dorsal wall. Values ranging from 7 to 9 cm were considered normal, whereas values >9 cm were recorded as overgrown (26).

Other cows' characteristics such as leg hygiene and hock condition were recorded. Leg hygiene was measured using the 3-point scoring scale described by Vasseur et al. (27) where 1 = clean (absence of manure flecks on the lower limbs, upper limbs, and upper flank region), 2 = dirty (distinct splash of manure around the area), and 3 = very dirty (confluent plagues of manure). Hock condition was assessed by scoring (3-point scale) the fore and hind limbs based on the condition of the area around the tarsal (hock) and carpal (knee) joints, where 1 = healthy, 2 = balding or mild swelling, 3 = swollen or open wound (28).

Hoof Trimming and Animal Placement into Groups

Upon tossing a coin, the selected cows in each farm were randomly allocated into either the trimmed or control group. Cows selected for trimming were restrained in the HT facility and their hooves were trimmed using the five-step Dutch method (28). Briefly, overgrown hooves were identified, marked using a claw check, and reduced to normal length using a hoof nipper. The inner claw (medial claw) of the hindfoot was trimmed before proceeding to the outer claw (lateral claw) and vice versa for the front foot. A HT knife (Kruuse®) was used to pair and level the sole and heel region. For standardization, a little modification of the model described by other authors (29) was applied to pin-point the paring from axial to abaxial aspects of the claw (40 mm away). The cow enrollment and HT in farms A, C, D, and E were completed after 3 visits to each farm by the same veterinarian. In farm B, HT was conducted by the veterinarian and farms' professional hoof trimmers trained on how to apply the trimming technique employed in this study. For the control cows, indications for HT such as disproportionate heel height and unbalanced sole were not corrected.

Data Collection

The cows were assessed for locomotion scores, hock condition, BCS, and leg hygiene every month for 9 months study period. Lameness was defined as the manifestation of two successive locomotion scores of 3 or the first score of 4 or 5 (30). Cows fulfilling the lameness definition were immediately (on the day of lameness diagnosis) examined in the HT facility for hoof health. Thereafter, the lame cows were treated according to the farms' management protocol, which entailed a therapeutic trim, administration of a non-steroidal and anti-inflammatory drug, placement of hoof block on the healthy claw, and local antibiotic agent depending on the lesion type. The ICAR claw health atlas (31) was used as a guide for the lesion diagnosis. Lesions such as sole ulcer, sole hemorrhage, double sole, white line disease, toe ulcers, and thin sole were categorized as non-infectious lesions, whereas infectious lesions included digital dermatitis, interdigital dermatitis, heel horn erosion, and swollen coronet. Lesions such as corkscrew claw, interdigital hyperplasia, and wall fissures were categorized as "others." All the non-lame cows that remained in the study were examined for hoof lesions after the follow-up period.

Data Analysis

All statistical analyses were conducted using the IBM SPSS version 24.0 (Armonk, NY, USA: IBM Corp.). Descriptive statistics were used to simplify the characteristics of the enrolled cows. Mean and standard deviations were used to summarize the continuous variables, whereas categorical variables were presented in median, interquartile range, and percentages. The cows with complete locomotion scores and other animal-based measures either before censoring or throughout the study period were included in the final analysis. Those with missing data or culled before censoring or the end of the study period were not included. The outcomes (lameness incidence and lesion prevalence) were determined at cow levels using descriptive statistics. The incidence rate of lameness was calculated as the number of new cases divided by the total number of cows at risk multiplied by the time at risk. Lesion prevalence was calculated based on the total number of cows affected with either one or more hoof lesions to the total number of observed cows at the end of the study.

Survival analysis was used to evaluate lameness incidence in the trimmed and control groups. The time of lameness diagnosis was the date of the second successive lame locomotion score (LS = 3) or the first severe lameness score (LS = 4). The difference in time to lameness (measured in months) between the study groups was evaluated using Kaplan-Meier analysis. Univariable Cox proportional regression models were first constructed to evaluate the relationship between lameness incidence and the covariates: parity (first, second and greater parity), breed, DIM (≥200 DIM and within 30 DIM during enrollment), HCS (normal, hair loss, and swelling/ulcer), leg hygiene (clean, dirty and very dirty) and BCS (≤2.5, 2.6–3.4, and ≥3.5) and hoof overgrowth (present or absent). For the four latter covariates, records used in the regression analysis were the respective scores or observations either at the point of censoring (for lame cows) or at the end of the study period (for non-lame cows). Farms were introduced in the model as random effects. In the next stage, covariates were introduced into the multivariable cox proportional regression model if the *P*-value was <0.10. A forward conditional method was applied and changes in the remaining coefficients were checked as factors were added into the model. *P*-value <0.05 was used for the final model. Risk estimates were presented as hazard ratios with a 95% confidence interval. Interaction between farm groups and other predictors was checked. The proportional hazard function in the final model was assessed based on the Schoenfeld residuals test (32).

Hoof lesion prevalence was analyzed by including all the cows diagnosed with lameness during and at the end of the study period. The outcome was the odds for any hoof lesion either during or at the end of the follow-up period. Due to the low prevalence of infectious hoof lesions (<10%), analysis was only conducted for non-infectious types. Therefore, cows having a non-infectious hoof lesion and those without lesions were included in the regression models. Binary logistic regression models were conducted for all the enrolled cows. A similar two-step model building process described earlier was used to construct the final multivariable logistic regression models. Farms were considered as a random effect in the final model, whereas groups (trimmed vs. control) were treated as a fixed

TABLE 2 | Characteristics of all the enrolled cows ($n = 520$) in grazing and non-grazing dairy farms.

	Non-grazing farms				Grazing farms			
Factors	**Farm A Freq.**	**Farm B Freq.**	**Farm E Freq.**	**Total (%)**	**Farm C Freq.**	**Farm D Freq.**	**Total (%)**	**Overall (%)**
Breed								
Friesian Sahiwal	52	75	69	196 (84.4)	79	90	169 (69.3)	365 (76.6)
Jersey Friesian	36	–	–	36 (15.6)	33	42	75 (30.7)	111 (23.4)
Parity								
1	40	61	18	119 (51.2)	29	37	66 (27.0)	185 (38.8)
2	40	14	43	97 (41.8)	60	63	123 (50.4)	220 (46.2)
≥3	8	0	8	16 (7.0)	23	32	55 (22.6)	71 (15.0)
BCS								
2.5–2.9	7	7	0	14 (6.0)	4	2	6 (2.4)	20 (4.2)
3.0–3.4	66	63	47	176 (75.8)	80	105	185 (75.8)	361 (75.8)
3.5–4.0	15	5	22	42 (18.1)	28	25	53 (21.7)	95 (19.9)
Hock condition								
Normal	68	65	56	189 (81.5)	104	110	214 (87.7)	403 (84.6)
Hair loss	19	10	12	41 (17.7)	8	21	29 (11.8)	70 (14.7)
Swelling/ulcer	1		1	2 (0.8)	0	1	1 (0.4)	3 (0.7)
Leg hygiene								
Normal	50	36	58	144 (62.1)	89	78	167 (64.3)	311 (65.3)
Dirty	24	35	11	70 (30.2)	22	40	62 (21.3)	132 (27.7)
Very dirty	14	4		18 (7.7)	1	14	15 (61.5)	33 (6.9)
Hoof overgrowth								
Absent	36	52	33	121 (52.2)	86	63	149 (61.6)	270 (56.7)
Present	52	23	36	111 (47.8)	26	69	95 (38.9)	206 (43.3)
Group								
Trimmed	45	35	38	118 (72.8)	89	71	160 (65.6)	278 (58.4)
Control	43	40	31	114 (27.2)	43	41	84 (34.4)	198 (21.6)
Trimmed foot (per cow)								
All	28	38	11	77 (65.3)	24	73	97 (60.1)	174 (36.5)
Both rear	9	–	24	33 (27.9)	46	9	55 (34.4)	88 (18.4)
Both rear and one front foot	8	–	–	8 (6.8)	–	8	8 (5.0)	16 (3.4)
Indication for HT								
Dorsal wall length	28	35	17	80 (67.7)	23	41	64 (40.0)	144 (51.7)
Heel height	4	2	1	7 (5.6)	22	37	59 (36.9)	66 (23.7)
Unbalanced sole	13	1	17	31 (26.3)	25	12	37 (23.1)	68 (24.4)
Days in Milk (Mean ± SD)	92.0 ± 102.7	130.6 ± 115.3	148.2 ± 111.7	109.1 ± 111.4	117.8 ± 118.8	115.8 ± 113.4	126.5 ± 115.3	

Freq., frequency; SD, standard deviation; HT, hoof trimming; BCS, body condition score.
Indications for HT and trimmed foot per cow were only presented for the trimmed cows in grazing and non-grazing farms.

effect. Biologically plausible interactions were checked in the main effects, however, none was retained (all predictors had $P > 0.05$). The final model fit was selected based on the lowest Akaike's information criterion.

RESULTS

Descriptive Analysis

A total of 520 cows were enrolled in the study (HGR = 170, CON-GR = 97; IS; HNGR = 124, CON-NGR = 129). However, 44 cows were either culled or had missing data during the study and were not included in the final analysis. Descriptive statistics of the cows ($n = 476$) with complete data are presented in **Table 2**. The total number of enrolled cows in NGR herds was 232 with 72.8 and 27.2% in trimmed (HNGR) and control (CON-NGR), respectively. In GR herds, the proportion of cows in HGR and CON-GR were 65.6% ($n = 160$) and 34.4% ($n = 84$), respectively. The majority of the cows in both groups were Australian Friesian Sahiwal breed (GR vs. HGR; 69.3 vs. 84.4%), and equal proportions (75.8%) had BCS between 2.6 and 3.4 during enrollment. Also, both groups had similar proportions of cows with normal hock conditions, clean legs, and overgrown hooves. Overall, the major indication for HT was overgrown dorsal wall length (51.7%).

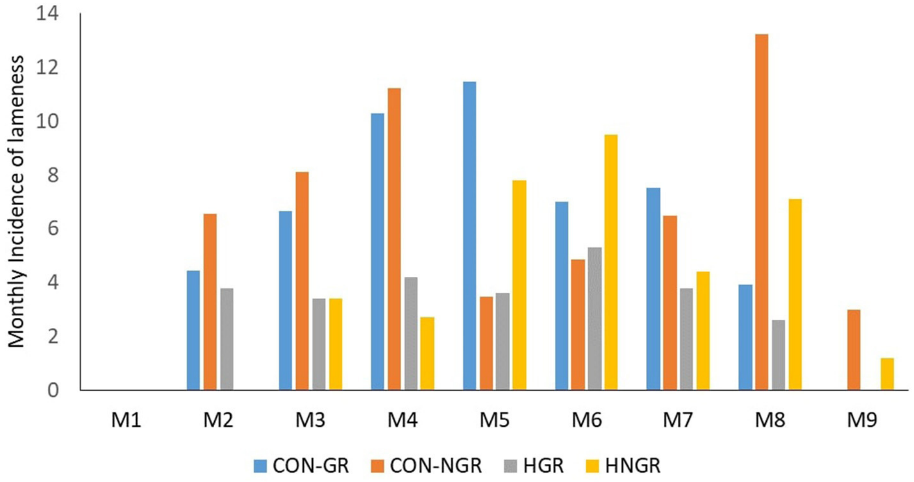

FIGURE 1 | Incidence rate of lameness in hoof-trimmed and control cows in non-grazing and grazing herds during the 9 month study period (M1 = first month post-enrollment, M9 = ninth month post-enrollment).

TABLE 3 | Time to first lameness event and lameness incidence rate in trimmed and control cows from grazing (*n* = 2) and non-grazing (*n* = 3) dairy farms.

	Time to first lameness (months)			**Mantel-Cox**				
	Mean	**S.E**	**95% CI**	**Chi-square**	***P*-value**	**Lame cows**	**ATE**	**Incidence rate**
Grazing cows								
HGR^{a}	8.12	0.15	7.69–8.32	6.37	0.01	39	142.3	27.4/100
CON-GR^{b}	7.36	0.26	6.86–7.87			33	68.1	48.4/100
Non-grazing cows								
HNGR^{a}	8.05	0.16	7.74–8.35	3.76	0.05	34	106.5	31.9/100
CON-NGR^{b}	7.39	0.23	6.95–7.83			45	98.6	45.8/100
Overall^{c}	**7.75**	**0.09**	**7.56–7.95**	**10.61**	**0.001**	**151**	**415.5**	**36.3/100**

^{a,b}Groups with significant difference in time to first lameness have different superscripts.
ATE, animal time events; SE, standard error.
^{C}Comparison between trimmed and control cows irrespective of management systems.
Total number of cows in trimmed cows: HGR = 160, NGR = 118.
Total number of cows in Control cows: CON-GR = 84, CON-NGR = 114.

Lameness Analysis

The monthly incidence rate of lameness in HGR and HNGR ranged from 5 to 9% throughout the study period; however, CON-GR and CON-NGR had higher monthly lameness incidence rates (12 vs. 13%) in the fourth and 7th month into the study (**Figure 1**). Overall, the incidence rate of lameness in the enrolled cows all through the study period was 36.3 cases/100 cows/per month. The corresponding rate of lameness in HGR, HNGR, CON-GR, and CON-NGR were 27.4, 31.9, 48.4, and 45.8 cases/100 cows/per month, respectively (**Table 3**). Time to first lameness event measured in months was significantly higher in HGR (mean ± S.E; 8.12 ± 0.15, $P = 0.04$) compared to CON-GR (7.36 ± 0.26), and in HNGR (8.05 ± 0.16, $P = 0.03$) compared to CON-NGR (7.39 ± 0.23). The majority of the lameness events in trimmed and control cows were observed during early lactation (50%) and mid-lactation (40.3%), respectively (**Figure 2**).

Factors in the univariable Cox regression model included BCS, hock condition, lesion presence, and farms, but hock condition was not retained in the final multivariable model. Cows with thin BCS (≤2.5) had a higher risk for lameness (Hazard ratio; HR = 3.05, 95% CI 1.24–7.46) compared to those with good BCS (**Table 4**). The risk of lameness was higher in cows affected with hoof lesions (either infectious, non-infectious or both) than those with a healthy hoof. Overall, lameness risk varied between farms with farm C recording higher risk compared to farm E.

Hoof Lesions Analysis

Table 5 shows the prevalence of hoof lesions in all the enrolled cows (i.e., both lame and non-lame) at the end of the study. The prevalence of hoof lesions in the enrolled cows was 36.9% (176/476), with a higher prevalence in CON-GR (48.8%) than HGR (23.2%). Likewise, CON-NGR recorded a higher prevalence (52.6%) of hoof lesions compared to HNGR (32.2%). The majority of hoof lesions were non-infectious in grazing (HGR vs. CON-GR; 21.3 vs. 33.3%) and non-grazing herds (HNGR vs. CON-NGR; 25.0 vs. 40.4%). The prevalence of infectious hoof

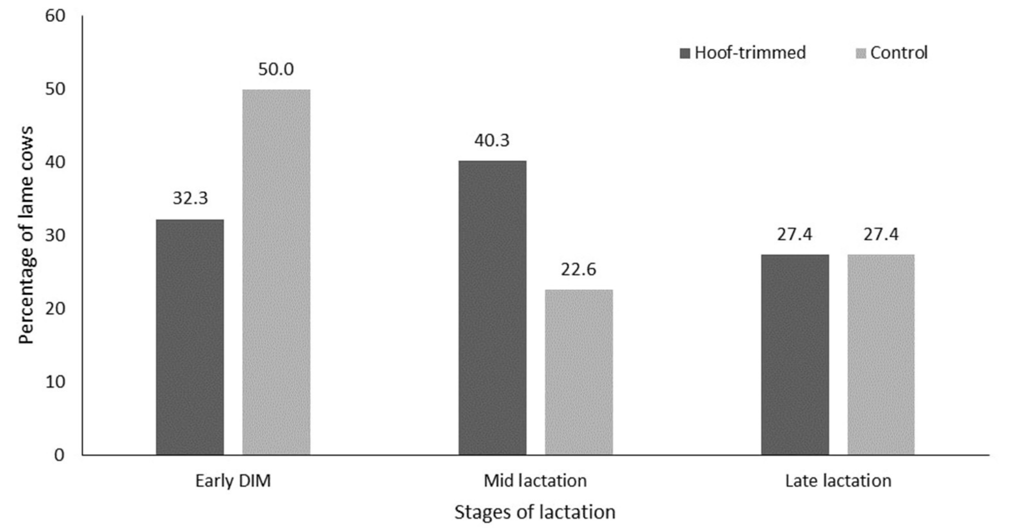

FIGURE 2 | Percentage of lame cows among the hoof-trimmed and control cows at various stages of lactation during the study.

TABLE 4 | Final multivariable cox regression models for factors associated with time to first lameness event in 476 cows from five farms in Peninsular Malaysia.

Factors	B	SE	Wald	*P*-value	HR	95% CI	
BCS			18.35	<0.001			
≤2.5	1.15	0.45	5.96	0.01	3.05	1.24	7.46
2.6–3.4	0.70	0.45	2.44	0.11	2.02	0.83	4.83
3.5–3.9	0.08	0.46	0.03	0.86	1.08	0.43	2.63
≥4.0					Ref		
Lesion			126.72	<0.001			
Non-infectious	3.50	0.32	117.26	<0.001	33.18	17.61	62.55
Infectious	3.66	0.37	97.60	<0.001	38.99	18.85	80.65
Both	2.99	0.48	37.74	<0.001	20.02	7.69	52.08
No lesion					Ref		
Farms			10.22	0.043			
Farm A	0.23	0.29	0.61	0.43	1.26	0.70	2.26
Farm B	0.03	0.30	0.01	0.92	1.03	0.57	1.85
Farm C	0.72	0.28	6.24	0.01	2.04	1.16	3.58
Farm D	0.19	0.29	0.44	0.50	1.22	0.67	2.19
Farm E					Ref		

BCS, body condition score; HR, hazard ratio; SE, standard error; CI, confidence interval; Ref, reference category.

lesions in HGR and CON-GR was 1.9 and 15.4%, respectively. However, similar proportions of infectious hoof lesions were recorded in HNGR (6.8%) and CON-NGR (7.0%).

Table 6 shows the estimated associations between covariates and having a non-infectious hoof lesion. Factors in the multivariable model included parity, BCS, hock condition, hoof length and treatment groups. Lower parity (Odds ratio; OR = 0.41, 95% CI 0.19–0.88), normal hock condition (OR = 0.06; 95% 0.01–0.29) and absence of overgrown hoof (OR = 0.47; 95% 0.28–0.79) were protective against non-infectious hoof lesion. Factors that increased the odds of non-infectious hoof lesions included low BCS (BCS ≤ 2.5) (OR = 19.71, 95% CI 6.39–60.81) and belonging to the control group (OR = 3.25; 95% 1.92–5.53) relative to those with BCS ≥ 4.0 and trimmed cows, respectively.

DISCUSSION

This study adds to the body of knowledge on the impact of the five-step Dutch trimming method on lameness incidence and lesion prevalence in grazing and non-grazing cows. Overall, the incidence rate of lameness in hoof-trimmed cows from GR and NGR was 27.4 cases/100 cows/month (3.6 cases/cow/year) and 31.9 cases/100 cows/month (4.2 cases/cow/year), respectively. The present result is consistent with the lameness incidence rate reported in cows from freestalls during dry periods (4.2 cases/cow/year) (33) but lower compared to 7.4 cases/cow/year recorded in lactating cows from a freestall herd (34) and pooled lameness incidence in grazing herds (64.6/100 cow-years) (16). The high lameness incidence rate could be attributed to the enrollment of primiparous and older cows, which are more susceptible to lameness episodes, management practice, and the presence of control groups that were not trimmed during lactation.

High milk-yielding cows are at higher risk of becoming lame (10, 23), but this was not the case in the present study. Although the enrolled cows in this study are expected to be high producers based on their genetics, the milk yield was relatively lower compared to the same breed of cows in other related studies (19, 29). Factors such as poor heat abatement strategies and nutrition may contribute to the low milk yield in the study population (9, 13). Based on the high lameness incidence rates, especially in the untrimmed cows, the finding suggests that other management factors may play a role in gait disturbance and the onset of hoof lesions on

TABLE 5 | Hoof lesion prevalence in trimmed and control cows from grazing (*n* = 2) and non-grazing (*n* = 3) dairy farms.

	Frequency of hoof lesions											
	SU	**SH**	**WLD**	**TU**	**TS**	**DD**	**SC**	**Others**	**Total**	**Cows**	**Prevalence (%)**	**NIF**	**IF**
Hoof-trimmed													
HGR	14	5	6	8	1	3	0	0	37	37	23.2	21.3	1.9
HNGR	17	7	10	5	1	6	2	2	50	38	32.2	25.0	6.8
Control													
CON-GR	11	5	9	4	1	10	3	4	47	41	48.8	33.3	15.4
CON-NGR	19	7	16	3	2	6	2	6	61	60	52.6	40.4	7.0
Overall	**61**	**24**	**41**	**20**	**5**	**25**	**7**	**12**	**195**	**176**	**36.9**		

SU, sole ulcer; SH, sole hemorrhage; WLD, white line disease; TU, toe ulcer; TS, thin sole; DD, digital dermatitis; SC, swollen coronet; NIF, non-infectious hoof lesions; IF, infectious hoof lesions.
Total number of cows in trimmed group: GR = 160, NGR = 118.
Total number of cows in Control group: GR = 84, NGR = 114.
More than one lesion per cow was included.

TABLE 6 | Univariable and multivariable logistic regression models showing the significant factors associated with non-infectious hoof lesions identified at the end of study period.

	Univariable model						Multivariable model		
Factors	***B***	**SE**	***P*-value**	**OR**	**95% CI**		**OR**	**95% CI**	
Parity			0.03						
1	−0.94	0.40	0.01	0.39	0.17	0.85	0.41	0.19	0.88
2	−0.95	0.38	0.01	0.38	0.18	0.82	0.40	0.19	0.84
>2				Ref			Ref		
BCS			0.001						
≤2.5	2.99	0.57	0.001	20.0	6.43	62.37	19.71	6.39	60.81
2.6-3.4	1.63	0.55	0.003	5.10	1.71	15.18	5.21	1.76	15.43
3.5-3.9	0.18	0.57	0.744	1.20	0.39	3.69	1.20	0.39	3.69
≥4.0				Ref			Ref		
Hock condition			0.005						
Normal	−2.58	0.81	0.002	0.07	0.01	0.37	0.06	0.01	0.29
Hair loss	−2.19	0.82	0.008	0.11	0.02	0.56	0.09	0.01	0.44
Ulcer/swelling				Ref			Ref		
Hoof length									
Normal	−0.73	0.26	0.006	0.48	0.28	0.81	0.47	0.28	0.79
Overgrown				Ref			Ref		
Group									
Not trimmed	1.23	0.27	<0.001	3.44	2,00	5.92	3.25	1.92	5.53
Trimmed									

BCS, body condition score; OR, odds ratio; SE, standard error; CI, confidence interval; Ref, reference category.

Malaysian dairies. A recent study found that floor designs around the milking pen, walkways, and resting pen, poor herd hygiene, and lack of hoof care influenced the risk of foot lesions (35). Hence, the application of HT as a management strategy may reduce the occurrence of hoof lesions such as sole ulcers and white line disease, which are the predominant causes of lameness in dairy cows in Peninsular Malaysia (35). Nevertheless, a more holistic strategy involving improved housing conditions, stall designs, and routine hoof inspection and care is required.

The time to first lameness event was significantly higher in trimmed groups compared to their respective controls in grazing and non-grazing herds. The result highlights the efficacy of the HT technique as a preventive measure for lameness in lactating dairy cows. Previous studies have investigated the impact of functional trimming on lameness prevention (19, 29, 36). Mahendran et al. (36) reported no significant difference in the odds of lameness and time to first lameness event between cows that underwent pre-calving and post-calving foot trim. In another study, cows trimmed at mid-lactation had a cumulative incidence of lameness of 18% compared to control groups (24%) during late lactation (19). Findings from the reviewed studies could not be solely attributed to HT, since the studied population either had hoof lesions or were not observed for lesions presence before enrollment. Although the authors used an adaption of the functional trimming, two studies reported that preventive trimming reduced the risk of lameness during lactation in freestall and pasture-based farms (20, 37). Daros et al. (33) did not state the HT technique used in their study, but primiparous cows trimmed before enrollment had lower odds of lameness during lactation. These findings are consistent with our results when comparing the incidence rate of lameness between trimmed and control cows. However, our result gives more insight on the impact of HT, since all cows in the present study were evaluated for healthy hooves and sound locomotion before enrollment. Application of functional trimming in the present study might have assisted to better the cows' gait due to improvement in weight distribution (38), frictional properties at the floor-claw interface, and preserving hoof dimensions after achieving the proper hoof length and sole thickness (17, 39).

The majority of lameness events in trimmed and control cows were observed during mid-lactation and early lactation (within 120 DIM), respectively. The reason for this finding is not fully understood. Nevertheless, early lactation is identified as a high-risk period for lameness due to factors such as negative energy balance, peri-calving hormonal changes affecting the hoof horn tissues, and challenges associated with peak lactation (40, 41). This might explain the higher lameness events in control cows at early lactation, whereas lameness episodes in trimmed cows might be delayed until mid-lactation due to the protective effect of HT.

Lesion prevalence at the end of the study period was lower in HGR (23.2%) compared to CON-GR (48.8%), as well as in HNGR (32.2%) compared to CON-NGR (52.6%). This result reflects the potential benefits of HT in grazing and non-grazing cows. For instance, the incidence of hoof lesions was lower in herds where trimmed cows spent more time on pasture (42). Pasture access as short as 4 weeks was associated with a higher tendency to bear weight on the affected claw, improved tracking up, and improved gait score of lame cows (43). In addition, HT enhances even weight distribution between the medial and lateral claws and restores proper sole thickness (17, 38, 39). These events might explain the delay in the onset and lower prevalence of hoof lesions in trimmed compared to non-trimmed cows in the present study.

Specifically, the majority of the lesions were non-infectious (i.e., hoof horn lesions) which corroborates the results from other related studies conducted in Malaysia (44, 45). Another indication of the benefits of HT in delaying the onset of hoof horn lesions was the lower prevalence in trimmed cows compared to controls under both management systems. This result is consistent with the findings of Manske et al. (20) and Gomez et al. (37), where an adaptation to functional trimming was protective against hoof horn lesions. Cows trimmed around drying off were found to have lower odds of sole ulcers (20% lower) in the subsequent lactation (21). The final step in the functional HT is the formation of a hollow dish (i.e., increased paring) around the solar area adjacent to the axial aspect of the hoof, which is regarded as the typical sole ulcer site (15, 17). Although we did not assess the longitudinal changes of sole thickness, the last step in the HT procedure might have reduced the pressure directed unto the corium during the risk period for hoof horn lesions (17).

In contrast, the prevalence of infectious hoof lesions was low in all the trimmed and control groups. Previous studies have highlighted low prevalences of digital dermatitis, heel horn erosion, and foot rot in Malaysian dairy farms (44, 45), which could play a role in the present findings. Besides, all the enrolled farms used footbaths as a lameness control practice. However, the HT may also contribute to the finding, as such intervention was reported to reduce the occurrence of digital dermatitis in pasture-based herds (46), while trimmed cows provided with pasture access had a lower prevalence of infectious foot lesions (43). The HT technique might have restored a proper heel height; thus, reducing the exposure to slurry, interdigital irritation, and subsequent lower odds of infectious lesions. A more controlled study is required to elucidate such speculation since the present study enrolled multiple farms with varying levels of herd hygiene. Furthermore, the fact that both trimmed and untrimmed cows had low prevalences of infectious hoof lesions limits our understanding of the effectiveness of the HT procedure.

The risk of lameness was higher in underconditioned cows and those affected with hoof lesions, with the incidence varying between farms. Previous researches have consistently demonstrated that low BCS predisposes cows to lameness and the other way around (47, 48). In addition, loss of BCS and increase of BCS at calving could influence the risk of future lameness events and the chance of recovery from lameness (49). BCS loss promotes thinness of the digital cushion and instability of the pedal bone; thus, supporting the pathogenesis of hoof horn lesions causing lameness (11, 12). These events are further augmented by the absence of HT, improper trimming, and long intervals between trimmings (50). Good BCS was a criterion for enrollment of cows in this study, however, the observation points (every month) limit our knowledge on the event direction. Cows could have been underconditioned prior to lameness onset or the other way around.

Lameness risk varied between farms in this study. Farm C had a higher risk of lameness compared to Farm E, but the risk was not different on other farms. The reasons for this finding are not clearly understood since important animal-based factors such as BCS, DIM, hock condition that could influence the risk of lameness were considered during enrollment. Nevertheless, a higher number of cows on farm C received HT on both hind hooves compared to other farms. Hoof traits such as overgrown dorsal wall length, uneven sole thickness, and disproportionate heel height may affect weight distribution between the medial and lateral claws, and heighten the risk of future lameness event (11, 17). A previous study reported a significant increase in LS in cows from few days to 2 weeks after preventive HT (51). However, the cows in the present study were observed at monthly intervals, which suggests that aside from HT, herd-level factors beyond the scope of this work might have contributed to the onset of high locomotion scores on farm C.

Lower parity was a protective factor against hoof horn lesions in the present study. Chronic degeneration of body structures such as ligaments, bone, and digital cushion are mainly associated with increasing age (52, 53). However, such changes are not common in first parity cows probably due to fewer lactations and less exposure to high-risk periods of lameness (24). Other factors that may play a role in higher risks of hoof horn lesions in older cows include relapse of such lesions in subsequent lactation (53), the onset of exostosis on the caudal aspect of the distal phalanx, and reduced protective capacity of the digital cushion following replacement by connective tissue (54, 55). For instance, previous lameness episodes were not considered as criteria during cows' enrollment in the present study. These factors may contribute to the increased odds of hoof horn lesions observed in underconditioned cows.

Cows with normal hock conditions had a lower prevalence of non-infectious hoof lesions compared to those with hock injuries. The result highlights the importance of cow comfort in lameness management. Lame cows affected with either sole ulcers or white line disease may lay down for longer periods, which may promote the onset of hock injuries especially when lying surfaces are abrasive (56). Moreover, environmental and housing factors relating to poor stall designs may influence the concurrent onset of hock injuries and hoof lesions (57, 58). A longitudinal assessment of cows with normal hock conditions and at shorter time intervals is more appropriate to ascertain the event's direction.

We acknowledge the strengths and limitations of the present study. The enrolled cows were randomly selected and placed into treatments and control groups in each farm, assessed for hoof health status before enrollment, and detailed explanation and standardization of HT technique. All the assessments were conducted by a single veterinarian. HT was conducted by the researcher in 4 of the dairy farms, whereas both professional hoof trimmers and the researcher carried out the procedure in farm D. The hoof trimmers in farm D also apply the Dutch Five-Step HT method for lameness management. Nevertheless, a hoof trimming training session involving the researcher and professional hoof trimmers ($n = 3$) was conducted before the onset of the study. The training entailed the steps and landmarks of the HT procedure and to have a standardized format. No separate training was conducted for locomotion scoring; however, a high level of agreement (Kappa coefficient = 0.80) was observed between the researcher's estimate of gait scores and available records conducted by the hoof trimmers on the same cows. These factors assisted in reducing issues related to low inter-rater reliability, confounding factors, and increased the chances of attributing changes in gait and hoof health to HT.

Nevertheless, aside from the management systems, some of the on-farm routine practices might influence lameness events during lactation. The assessment of previous lameness history in the study population was based on farm health records, which is insufficient to ascertain the true scenario before the study. Regarding the environmental conditions, data on the climatic conditions such as temperature and humidity were not collected. Although the climatic conditions in the various study areas are not expected to vary widely since they are within Peninsular Malaysia, they may impact differently on the outcomes. Furthermore, our results could not elucidate the influence of timing and frequency of preventive HT in dairy cows. Future studies might focus on these areas to improve our knowledge on the impact of HT as a preventive strategy for lameness management on dairies.

CONCLUSION

This study revealed that early and late-lactation functional HT contributed to lower lameness incidence, higher survival time to first lameness event, and lower prevalence of hoof lesions compared to non-trimmed cows from grazing and non-grazing farms. Factors that also increased the risk of lameness during lactation were low BCS, presence of hoof lesion, and there was variation in lameness risk between farms. The predominant causes of lameness were non-infectious hoof lesions. Aside absence of trimming, the odds of non-infectious hoof horn lesions were higher in older and underconditioned cows, as well as those with hock injuries. Functional HT is beneficial as a lameness preventive strategy when conducted at late lactation; however, parity, body condition, and hock condition are equally important.

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 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 conception of the work 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 revision of the drafted manuscript. All authors approved the final version of the paper and agree to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.631844/full#supplementary-material

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Low-Intensity, High-Frequency Grazing Positively Affects Defoliating Behavior, Nutrient Intake and Blood Indicators of Nutrition and Stress in Sheep

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The intensity and frequency of grazing affect the defoliating strategy of ruminants, their daily nutrient intake, thus nutrition and physiological status. Italian ryegrass (*Lolium multiflorum* Lam.) pastures were grazed by sheep either under a low-intensity/high-frequency grazing strategy (Rotatinuous stocking; RN) with nominal pre- and post-grazing sward heights of 18 and 11 cm, respectively, or under a high-intensity/low-frequency strategy (traditional rotational stocking; RT) with nominal pre- and post-grazing sward heights of 25 and 5 cm, respectively. Treatments were arranged under a complete randomized design and evaluated over two periods, in different years. In 2017, the aim was to depict the type of bites that sheep perform during the grazing-down and associate them to the grazing management strategy according to their relative contribution to the diet ingested. In 2018 we estimated the total nutrient intake and evaluated blood indicators of the nutritional status and immune response to stress of sheep. The bite types accounting the most for the diet ingested by RN sheep were those performed on the “top stratum” of plants with around 20, 15, and 25 cm, whereas the type of bites accounting the most for the diet of RT sheep were those performed on “grazed plants” with around 10, 5, and $\leq$ 3 cm. In 2018, the RN sheep increased by 18% the total organic matter (OM) intake and by 20–25% the intake of soluble nutrients (i.e., crude protein, total soluble sugars, crude fat), digestible OM and of metabolizable energy, and had 17.5, 18, and 6.1% greater blood concentration of glucose, urea nitrogen (BUN) and albumin, respectively, but 17% lower blood neutrophil-to-lymphocyte (N:L) ratio. Sheep grazing vegetative Italian ryegrass pastures under the low-intensity/high-frequency grazing strategy (RN) ingested a diet of better quality from bites allocated on the top stratum of plants, had greater intake of soluble nutrients and blood parameters positively associated with nutritional status and immune response to stress.

Keywords: animal welfare, nutritional status, grazing management models, sward height, ingestive comfort

INTRODUCTION

Criteria used to define the limits of sward depletion affect the foraging strategy of ruminants, thus the herbage intake and diet quality. Carvalho (1) proposed a low-intensity/high-frequency grazing approach that defines the management limits of the sward based on animal behavioral responses, i.e., a pre-grazing sward height allowing animals to maximize the intake rate and a grazing-down of 40% to sustain it high at any time while grazing. Although maximizing the intake rate is a natural foraging strategy of ungulates (2), applying this grazing approach is non-sense in most commercial farms, as orientations to maximize intake rate and individual animal intake are thought to reduce harvest efficiency and farm profit (3, 4). Conversely, for increasing herd forage intake and making full exploitation of the area, traditional guidelines propose starting grazing when the balance between herbage accumulation and its quality is optimized (5), and low residual sward height or mass as depletion limit (6–8), through high-intensity/low-frequency grazing strategies (9).

High-intensity/low-frequency grazing force animals to extend sward depletion to bottom parts of plants, preventing them from allocating bites on leaf laminas of the top stratum, which restrict the individual intake of a more digrestible diet (1). The metabolic profile of animals is directly affected by the intake of digestible organic matter [OM; (10)]. For instance, when blood glucose is low, other products coming from lipolysis of body reserves become available [e.g., non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB)], affecting the proliferation of immune cells [e.g., leukocytes; (11, 12)]. Therefore, apart from being against the natural preference for leaf laminas and of ingestive comfort associated with high and profitable intake rates (13), lower daily intake of a less digestible diet could threaten some of the domains of animal welfare [e.g., nutritional status and immune response; (14)], even when pastoral systems claim to promote it (15).

If the defoliating behavior of non-supplemented grazing animals affects their physiology, pastoral systems could improve or impair their nutrition and welfare depending on grazing management. We hypothesized that sheep grazing Italian ryegrass (*Lolium multiflorum* Lam.) pastures under a low-intensity/high-frequency grazing strategy and composing a diet from bites performed preferentially on the top stratum of plants, have greater intake of a diet with better quality and blood parameters positively associated with nutritional status and immune response to stress, compared to animals under a high-intensity/low-frequency traditional management (RT), composing a diet with lower quality from bites performed on both top and grazed parts of plants. To test this assumption, we conducted two grazing trials over two consecutive years. In 2017, we characterized, at the bite scale, the diet ingested by sheep grazing Italian ryegrass pastures, and in 2018, we determined the daily nutrient intake and compared the impact of the grazing management strategy on nutrition- and stress-related blood parameters of sheep.

MATERIALS AND METHODS

All procedures on animals followed the guidelines of the law of procedure for the scientific handling of experimental animals and were approved by the Ethics Committee for the Use of Animals (CEUA) of the Federal University of Rio Grande do Sul (UFRGS; protocol 3571).

Experimental Area and Pasture Establishment

The grazing trials were conducted at the Experimental Station of the Faculty of Agronomy of the UFRGS, in Southern Brazil (30°05”22’ S latitude, 51°39”09’W longitude and 46 m above sea level), with a subtropical humid “Cfa” climate with an average annual temperature of 18°C. Italian ryegrass pastures were established on April 20th in 2017 and May 23th in 2018, through conventional soil preparation (plowing and disking), mechanical spreading of 35 kg of seed per hectare and 250 kg of the formula (NPK, 5-30-15) per hectare at seeding and 200 kg of nitrogen (urea) 30 days later.

Treatments and Experimental Design

Two grazing management strategies in rotational stocking were evaluated under a completely randomized design, with two paddocks per treatment in 2017 and with four in 2018, over two evaluation periods. The Rotatinuous stoking (RN), with nominal pre- and post-grazing heights of 18 and 11 cm, respectively, was compared with a traditional rotational stocking (RT), with nominal pre- and post-grazing heights of 25 and 5 cm, respectively. The combination of pre- and post-grazing sward heights results either in low-intensity/high-frequency (RN) or in high-intensity/low-frequency (RT) grazing strategies (9). For the RN, the pre-grazing height aims to maximize intake per unit of grazing time on Italian ryegrass pastures, while the post-grazing height (40% reduction of the initial height) aims to sustain the intake rate at any time while grazing (1, 16, 17). For the RT, the pre-grazing height aims to initiate grazing at maximum net herbage accumulation and the post-grazing height to maximize herbage harvest efficiency (9, 18).

Animals and Pasture Management

The number of animals, paddocks and the duration of the stocking period differed between 2017 and 2018, according to year-specific objectives. Twelve Texel sheep (35 ± 4.3 kg of live weight; LW) in 2017, and 24 Texel × Corriedale castrated males (41.1 ± 3.4 kg LW) in 2018, were randomly allocated, respectively, to four and eight paddocks of 0.21 ha (three test animals per paddock). Pasture management was similar in both years. Briefly, sheep grazed in strips, changing to another daily between 14:00 and 15:00 h. Thereby, each paddock was subdivided into strips of variable size (130 and 47 m^2 on average for RN and RT, respectively), according to treatment targets and herbage growth; strips provided enough forage for animals to deplete the sward within the preestablished pre- and post-grazing sward heights.

The pre- and post-grazing sward heights were measured at two-day interval during the treatment adaptation period and daily during evaluations, by taking 150 random readings per strip at the "leaf horizon" on the top of the sward, with the aid of a sward stick (19). To maintain sward height targets, a variable number of put-and-take sheep, accompanied the three test-sheep on each strip (20). Sheep entered to paddocks before the pre-grazing sward height of both treatments was achieved, to complete a grazing cycle on all paddocks, while creating a sward height gradient. This allowed the first grazed strip within each paddock to reach the treatment pre-grazing sward height, just before it was grazed again. Thereby, from the second grazing cycle onwards on paddocks of each treatment, animals grazed on regrown sward strips within preestablished treatments heights; at this moment, the period of adaptation to treatments of 35 days in 2017 and of 16 days in 2018, started. Afterwards, in both 2017 and 2018, two evaluation periods took place on vegetative swards. Animals always had free access to water.

The Continuous Bite-Monitoring and Bite-Scale Hand-Plucking

In 2017, we implemented the continuous bite-monitoring (CBM) methodology consisting of (1) animal-observer familiarization, (2) bite-code grid elaboration, (3) observer training the bite-code grid monitoring and (4) real-time bite-monitoring evaluation [for details see (21–23)]. We used this method during two periods, with 3 days of observation, as schematized in **Figure 1A**. Briefly, during the first 10 days of adaptation to the treatment, four observers accustomed animals to the proximity of humans (<1 m) and during the following 25 days, the observers trained the bite-coding grid (**Figure 2**) previously elaborated for sheep grazing vegetative Italian ryegrass swards, and only when they were able to encode, in real time, each bite without hesitation, observations initiated. Each observer evaluated a different animal each day, alternating the treatment each day. Despite precautions, in period one, a sheep of each treatment had to be discarded from the analysis as they presented unusual behavior in the presence of the observer. Indeed, in period two, the 3rd day of observation was not conducted due to unsuitable weather conditions; thus, only two out of the three test animals within a paddock were evaluated during two observation days. Overall, 18 out of 24 possible observations were obtained. The bite encoding was recorded with a digital Sony recorder Icd-PX240® device. Recordings of the bite-monitoring were analyzed using the software JWatcher® (http://www.jwatcher.ucla.edu/, verified 10 December 2019; The Observer, Noldus Information Technology®, The Netherlands). While animals were not performing any eating activity during the CBM, the observers simulated at least 20 times each bite type (**Figure 2**), as detailed in Bonnet et al. (24); fresh samples were put on a paper bag. The total dry mass (g DM) of each bite type was calculated by oven drying the mass gathered per bite, at 55°C for 72, and dividing the dry weight on an electronic scale (0.0001 g precision) over the number of simulations; this information was used to compose the diet ingested.

Calculation of the Dry Matter Intake per Bite Type (Diet Ingested)

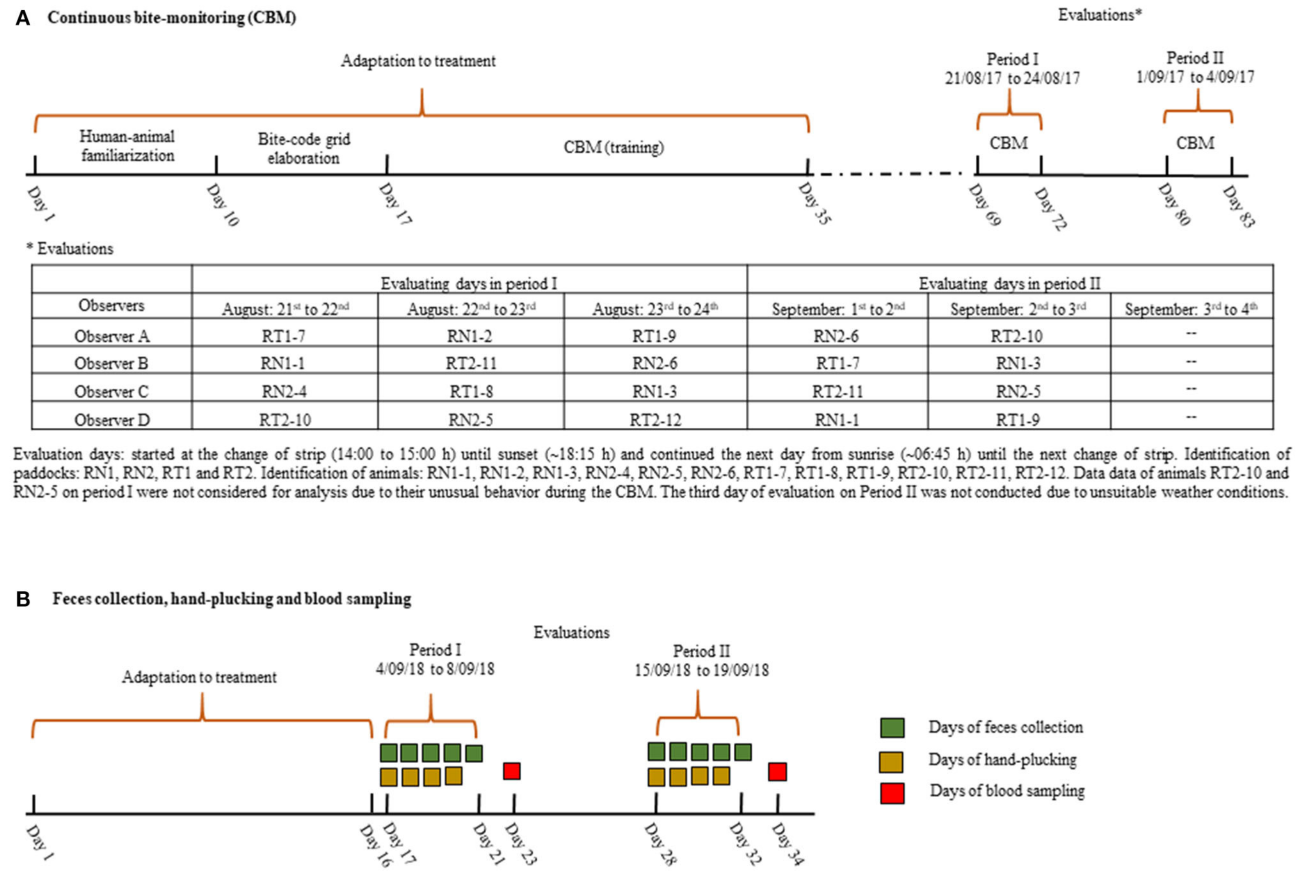
To determine the sheep DM intake per bite type, we multiplied the number of times that each bite type was recorded during the CBM by their individual dry mass (g DM). Afterwards, we summed up the DM ingested by all the bite types to estimate the intake during the CBM. Finally, the DM intake per bite type was divided by the intake of each animal to obtain the relative proportion that each bite type accounted for in the DM intake; herein referred as the diet ingested.

Total Organic Matter Intake and Herbage Digestibility

In 2018, the daily OM intake was estimated on the three test-sheep per paddock in two periods (**Figure 1B**). We used the fecal crude protein (CP) technique (25), as described by Savian et al. (18). The equation proposed by Azevedo et al. (26) for Italian ryegrass was used: OM intake = 111.33 + 18.33 × fecal CP (g/sheep/day). Each period consisted in total feces collection during five consecutive days. Sheep were fitted with feces collecting bags, which were emptied once per day (from 07:00 to 08:30 h) and the feces were weighed and homogenized to take sub-samples of 20% of the total. Fecal samples were dried at 55°C for 72 h, pooled per sheep, grounded and stored until analysis; afterwards, they were put on an oven at 550°C for 4 h to obtain the ash content. The OM content was calculated by subtracting the ash content from the dried mass of samples. The nitrogen content was obtained by the Kjeldahl method (27), and the CP content was calculated by multiplying the nitrogen content by 6.25. The OM digestibility was calculated as follows: OM digestibility = 1–total amount of feces (g DM/sheep/day)/OM intake (g DM/sheep/day). The digestible OM intake was calculated using the OM intake and OM digestibility. The metabolizable energy (ME) intake was estimated using the model proposed by CSIRO, [(28); ME = 0.169 × OM digestibility−1.986].

Nutrient Content of the Diet Ingested and Total Daily Nutrient Intake

In 2018, each bite of the bite-code grid (**Figure 2**) was hand-plucked, as described above, during the first 4 days of the feces collection (**Figure 1B**). The simulation was performed by one evaluator after the main morning and afternoon meal bouts, completing one paddock per treatment per day; all bite types were simulated once on each paddock on each period. The fresh mass



* Evaluations

Observers	Evaluating days in period I			Evaluating days in period II		
	August: 21st to 22nd	August: 22nd to 23rd	August: 23rd to 24th	September: 1st to 2nd	September: 2nd to 3rd	September: 3rd to 4th
Observer A	RT1-7	RN1-2	RT1-9	RN2-6	RT2-10	--
Observer B	RN1-1	RT2-11	RN2-6	RT1-7	RN1-3	--
Observer C	RN2-4	RT1-8	RN1-3	RT2-11	RN2-5	--
Observer D	RT2-10	RN2-5	RT2-12	RN1-1	RT1-9	--

Evaluation days: started at the change of strip (14:00 to 15:00 h) until sunset (~18:15 h) and continued the next day from sunrise (~06:45 h) until the next change of strip. Identification of paddocks: RN1, RN2, RT1 and RT2. Identification of animals: RN1-1, RN1-2, RN1-3, RN2-4, RN2-5, RN2-6, RT1-7, RT1-8, RT1-9, RT2-10, RT2-11, RT2-12. Data data of animals RT2-10 and RN2-5 on period I were not considered for analysis due to their unusual behavior during the CBM. The third day of evaluation on Period II was not conducted due to unsuitable weather conditions.

FIGURE 1 | Experimental scheme during the continuous bite-monitoring (CBM) in 2017 **(A)**, and feces collection, hand-plucking and blood sampling in 2018 **(B)**.

of bites was put on a cooler with ice immediately after sampling, and within 4 h stored at −20°C until freeze-drying (freeze-dryer Martin Christ DELTA 1-24 LSC, Germany) and grounding (1 mm screen). From these bite samples, we compounded 16 diets of 10 g of lyophilized samples (2 treatments × 4 paddocks × 2 periods), considering the proportion that each bite type accounted for to the diet ingested, as estimated in 2017. The herbage nutrient contents (g kg/DM) of diets were estimated by NIRS scanning (XDS NIRS system, FOSS—Denmark, 1,100–2,498 nm of wavelength by 2 nm steps and absorbency data expressed as log 1/R), using calibrations developed at the Walloon Agricultural Research Center (CRAW), Belgium (29). The daily OM intake estimated by the fecal CP technique was converted to DM intake (g/animal/day) by dividing the daily OM intake over the OM content of the diet, derived from NIRS. From this, we calculated the daily nutrient intake, as follows: daily nutrient intake = total DM intake (g/animal/day) × nutrient content (g/kg DM).

Biochemical and Hematological Blood Parameters

In 2018, sheep blood samples (4 mL) from the jugular vein were collected on tubes containing heparin (EDTA K2) and on non-heparinized tubes (Inforlab, São Paulo, Brazil), 2 days after the last fecal collection day of each period from 07:00 to 08:30 h (**Figure 1B**). Samples were transported in a cooler at 5°C, within 4–6 h, to the Department of Clinical Veterinary Pathology of the UFRGS. Packed cell volume was assessed from heparinized samples with the micro hematocrit method; samples (duplicate) were diluted with Turks's solution and centrifuged (Heraeus Pico 17, Thermo Scientific) on capillary tubes at 17,000 *g* for 5 min at room temperature (17°C) to perform total leukocyte counting (hemocytometer Neubauer Improved, New Optics). Blood smears were dried and stained with Diff Quick to perform differential leukocyte counting (i.e., total, neutrophils, lymphocytes and blood neutrophil-to-lymphocyte ratio; N:L). Non-heparinized samples were brought to room temperature and centrifuged (Heraeus Megafuge 8, Thermo Scientific) at 1,700 *g* for 10 min. The serum obtained was analyzed for glucose, urea, albumin, alkaline phosphatase, fructosamine and cholesterol by enzymatic colorimetric analysis using commercial kits (Glucose HK, Urea Color 2R, Albumin AA, ALP 405 AA, Fructosamine AA and Colestat enzymatic, respectively, Wiener Lab., Rosario, Argentina) in a Wiener Lab CM 200 auto-analyzer (Wiener Lab., Rosario, Argentina). Considering that the molecular weight of urea is 2.14 times that of urea nitrogen, the

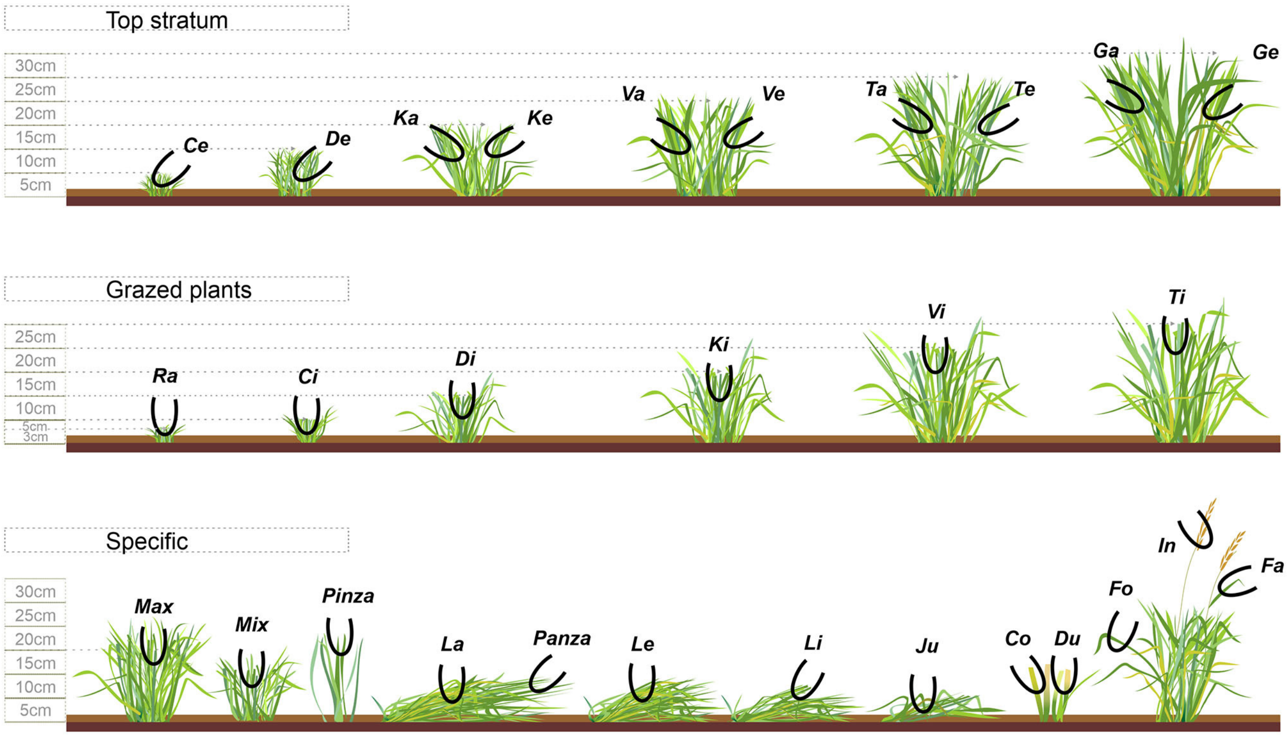


FIGURE 2 | Bite-code grid of sheep grazing vegetative Italian ryegrass pastures under contrasting grazing management strategies (RN and RT) in rotational stocking. The pictogram illustrates the bite types classified into three categories: top stratum, grazed plants or specific. Among the bites performed on "top stratum" are those coded with the consonants "C, D, K, V, T and G," indicating plants of around 5, 10, 15, 20, 25 and 30 cm (± 2.5 cm), respectively; yet, the vowels "a" and "e" after these consonants indicate, respectively, "dense" and "less dense" bites ($\geq$4 or $\leq$3 intact leaf laminas, respectively). Among the bites performed on "grazed plants" are those coded with the vowel "i" after the consonants, which also indicates leaf laminas with presence or not of stems; the bite "Ra," within this category, is performed on plants of $\leq$3 cm with minimum or no presence of leaf laminas. Among the "specific" type of bites, the bite "Max" is allocated on both grazed and intact leaf laminas, with the presence or not of stem, on plants with $\geq$15 cm; the bite "Mix" is similar, but on plants with $\leq$15 cm; the bite "Pinza" is allocated at the tip (superficial) of a single or no more than two intact or grazed leaf laminas, and at any sward height; the bite "Panza" is also a superficial bite, but on lying plants; the bite "La" is allocated on 2-to-3 lying intact leaf laminas with $\geq$20 cm length, while the bite "Le" is similar, but on plants with $\leq$20 cm length; the bite "Li" is allocated on lying plants gathering 2–3 grazed leaf laminas of any horizontal length; the bite "Co" and "Du" are performed on one and two defoliated stems, respectively, on plants with 5–15 cm; the bite "Fo" is performed on a single intact or grazed leaf lamina within the canopy; the bite "Fa" is performed on the leaf lamina below the inflorescence, intact or grazed; the bite "In" is allocated on the inflorescence; the bite "Ju" is allocated on a trampled plant, in which animals manipulate and gather 2–4 intact or grazed leaf laminas.

blood urea nitrogen (BUN) was estimated from serum urea as follow: BUN (mg/dL) = serum urea (mg/dL)/2.14. An aliquot of centrifuged serum was stored in Eppendorf tubes (1.5 mL) and frozen at −20°C until analyzed separately by enzymatic colorimetric analysis for NEFA (Randox, Antrim, UK) and BHB (Ranbut, Randox, Antrim, UK).

Statistical Analysis

Pasture data in 2017 and 2018 was subjected to ANOVA, at 5% of significance, considering the fixed effect of treatment, period and year, and their interactions, and the random effect of the paddock (experimental unit). The diet ingested by animals, estimated in 2017 from the CBM, was subjected to a multivariate analysis of variance (MANOVA), at 5% of significance, to compare the relative proportion that each bite type accounted to the diet ingested between grazing management (RN and RT), considering the fixed effect of treatment and period, and their interaction. Moreover, a principal component analysis (PCA) was performed to order the types of bytes performed by sheep grazing Italian ryegrass pastures under both grazing management strategies, according to their relative contribution to the diet ingested. The percent of the variance explained per axis was used as a selection criterion. Data of 2018, describing the nutrient content of the diet, the daily nutrient intake, OM digestibility and metabolizable energy content and blood parameters, was subjected to ANOVA at 5% of significance. The model included the fixed effects of the treatment, the random effects of the period and of the animal nested within the paddock (experimental unit), and of the treatment × period interaction (lmer function), except for the data of the nutrient content of the diet, whose model excluded the effect of the animal nested within the paddock. The statistical models in both years were selected considering the best fit model according to the AICs' criteria. Means were compared using the least-squares mean linear hypothesis test adjusted for Tuckey comparison. All analyzes were performed using the R software [(30), version 3.6.0].

TABLE 1 | Sward surface height and sward height depletion of vegetative Italian ryegrass pastures grazed by sheep under contrasting grazing management strategies (RN and RT) in rotational stocking.

Variables	RN	RT	SEM	*P-value*
Pre-grazing, cm	19.4	27.3	0.4	<0.0001
Post-grazing, cm	12.2	6.9	0.3	<0.0001
Sward height depletion, %	37.3	74.7	1.8	<0.0001

RN, Rotatinuous stocking; RT, Traditional rotational stocking; SEM, standard error of the mean.

TABLE 2 | Bite-scale characterization of the diet ingested by sheep (relative proportion that each bite type accounted to the diet ingested) grazing vegetative Italian ryegrass pastures under contrasting grazing management strategies (RN and RT) in rotational stocking.

Bite type	RN	RT	SEM	*P*-Value
Top stratum (less dense)				
Ce	0.69	3.48	0.87	0.134
De	3.30	2.18	0.35	0.092
Ke	11.19	5.23	1.52	0.031
Ve	24.07	5.76	2.88	0.000
Ge	3.54	2.25	0.77	0.472
Te	11.3	3.67	1.31	0.001
Top stratum (dense)				
Ka	0.65	0.45	0.24	0.768
Ta	1.51	1.16	0.28	0.425
Va	2.81	0.96	0.50	0.062
Ga	0.66	1.47	0.30	0.188
Grazed plants				
Ra	0.40	9.24	1.73	0.004
Ci	3.44	12.66	1.48	0.000
Di	7.23	13.34	1.22	0.008
Ki	5.93	6.70	1.01	0.698
Vi	3.61	3.19	0.69	0.824
Ti	0.99	1.425	0.42	0.585
Specific				
Mix	10.77	10.77	1.64	0.983
Max	6.30	4.215	0.85	0.271
Pinza	0.35	0.275	0.10	0.256
Panza	0	0.16	0.06	0.736
La	0	1.68	0.37	0.020
Le	0.57	3.53	0.69	0.027
Li	0.13	3.86	0.66	0.001
Jun	0	1.565	0.30	0.004
Co	0.01	0.305	0.05	0.000
Du	0	0.085	0.02	0.059
Fa	0.21	0.215	0.04	0.981
Fo	0.08	0.135	0.03	0.296
In	0.01	0.005	0.005	0.391

RN, Rotatinuous stocking; RT, Traditional rotational stocking. SEM, standard error of the mean.

RESULTS

Sward Canopy Height and Bite-Scale Characterization of the Diet Ingested

Table 1 shows the difference in pre- and post-grazing sward heights between treatments. The effects of year and period, and their interaction with treatments did not affect the pre- and post-grazing sward canopy height or the sward height depletion ($P > 0.05$). The management of the sward height affected the proportion that some, but not all bite types, accounted to the diet ingested ($P < 0.001$; **Table 2**). Neither the effect of the period nor its interaction with treatment was significant. **Figure 3** shows

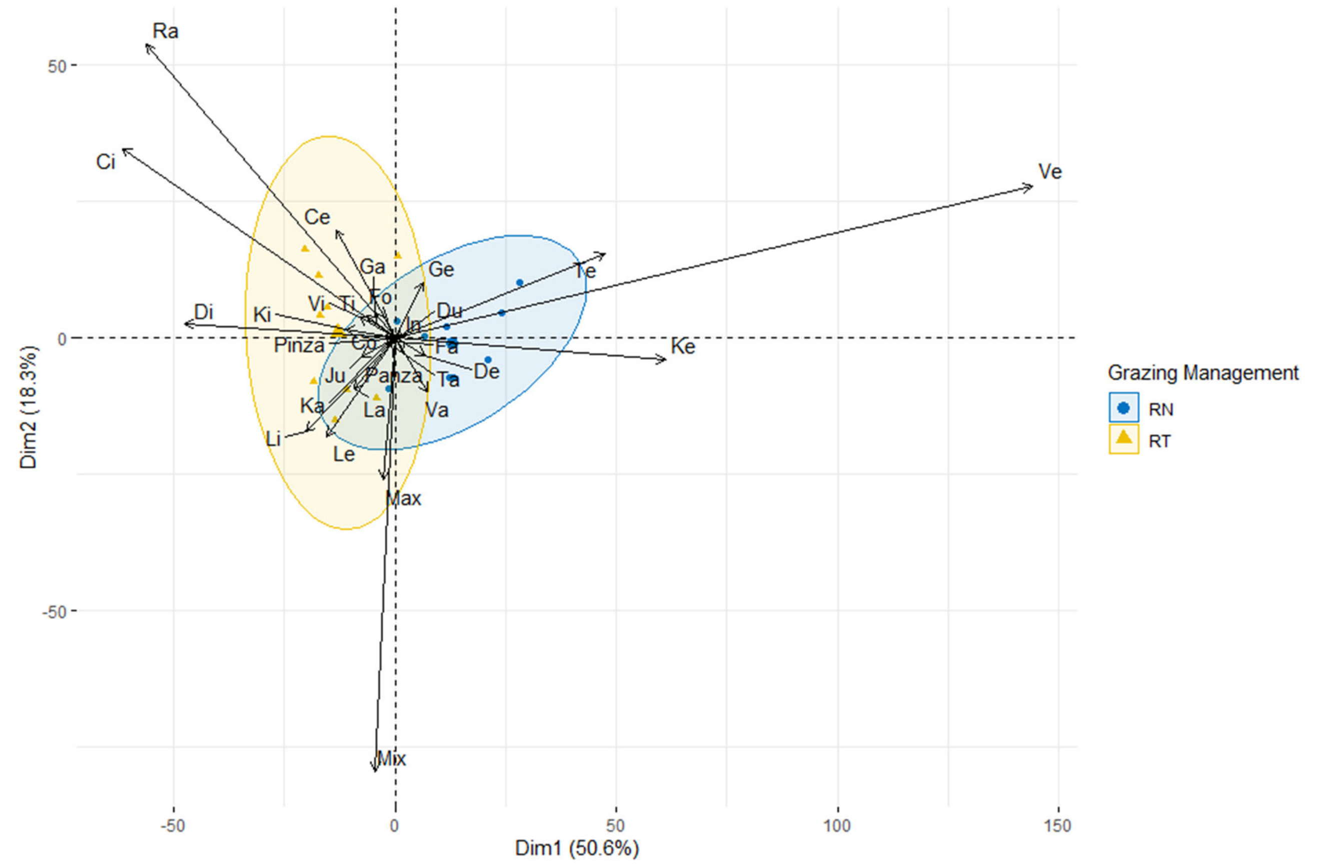


FIGURE 3 | Principal Component Analysis (PCA). Ordination diagram of the types of bytes performed by sheep grazing vegetative Italian ryegrass pastures under different grazing management strategies (RN: blue circles, RT: yellow triangles), according to their relative contribution on the diet ingested.

the first two dimensions of the PCA, explaining 69% of the total variation of data. The bite *Ve*, performed on the top stratum of plants with ∼20 cm, was the most associated with the RN management, followed by the bite *Te* and *Ke*, performed also at the top stratum of plants with ∼25 and ∼15 cm, respectively; these three bite types accounted for 47 and 15% of the diet ingested by the RN and RT sheep, respectively. Overall, the bites of the top stratum of plants accounted for 60 and 27% of the diet ingested, respectively, by the RN and RT sheep (**Table 2**). Bites that associated the most with the RT management were *Ci*, *Di* and *Ra*, performed on grazed plants with ∼5, ∼10, and ≤3 cm, respectively; these three bite types accounted for 11 and 35% of the diet ingested, respectively, by the RN and RT sheep. Overall, these three and the other bite types performed on grazed plants accounted for 22 and 47% of the diet ingested, respectively, by the RN and RT sheep (**Table 2**). Other specific type of bites whose contribution in the diet differed between treatments, were those performed on lying plants, trampled plants or steams, namely *La*, *Li*, *Le*, *Jun,* and *Co*, and accounted for 1 and 11% of the diet ingested by the RN and RT sheep, respectively; the contribution to the ingested diet of some of the bite types performed on the top stratum, grazed plants or specific, did not differ between the RN and RT strategies (**Table 2**).

Nutrient Content of the Diet Ingested and Daily Nutrient Intake

Table 3 shows the effect of treatments on the nutrient content of the diet ingested and on total daily nutrient intake; neither the effect of the period nor its interaction with treatment was significant ($P > 0.05$). In the RN management, the CP and total soluble sugar contents of the diet ingested were greater ($P < 0.001$), the crude fat content tended to be greater ($P = 0.056$), while the fibrous compounds such as ADF and ADL contents were lower ($P < 0.001$), with no difference for NDF content between treatments ($P > 0.05$). OM digestibility and ME content greater in the RN diet ($P < 0.0001$). The intake of OM, digestible OM, and all nutrients, except ADF ($P > 0.05$), were greater for the RN management ($P < 0.001$).

Blood Biochemistry and Hematology

Table 4 shows blood biochemical and hematological parameters of sheep as affected by the grazing management strategy. Neither the effect of the period, nor its interaction with treatment

TABLE 3 | Nutrient content of the ingested diet and total nutrient intake by sheep grazing vegetative Italian ryegrass pastures under contrasting grazing management strategies (RN and RT) in rotational stocking.

Variable	RN	RT	SEM	*P-value*
Nutrient content, g/kg DM				
Organic matter (OM)[a]	896	883	3.4	0.036
Crude protein (CP)[a]	254	220	5.5	<0.0001
Neutral detergent fiber (NDF)[a]	358	367	9.7	0.4086
Acid detergent fiber (ADF)[a]	259	321	13.9	0.009
Acid detergent lignin (ADL)[a]	23	33	2.7	0.027
Total soluble sugars[a]	144	126	3.4	0.0086
Crude fat[a]	41	37	1.9	0.056
OM digestibility, g/kg OM	771	755	2.2	<0.0001
Metabolizable energy, MJ/kg DM	11.05	10.77	0.04	<0.0001
Daily intake, g/animal				
Organic matter (OM)	835.95	680.90	54.3	<0.0001
Crude protein (CP)	237.1	169.5	7.2	<0.0001
Neutral detergent fiber (NDF)	333.8	281.6	8.9	<0.0001
Acid detergent fiber (ADF)	241.7	248.4	7.4	0.586
Acid detergent lignin (ADL)	21.5	25.3	1.1	<0.0001
Total soluble sugars	135.2	96.9	4.0	<0.0001
Crude fat	38.1	28.6	1.2	<0.0001
Digestible OM	643.5	513.6	16.9	<0.0001
Metabolizable energy, MJ/day	10.3	8.3	0.3	<0.0001

RN, Rotatinuous stocking; RT, Traditional rotational stocking. SEM, standard error of the mean. [a]Values estimated by NIRS.

TABLE 4 | Biochemical and hematological parameters of sheep grazing vegetative Italian ryegrass pastures under contrasting grazing management strategies (RN and RT) in rotational stocking.

Variables	RN	RT	SEM	Reference values	*P-value*
Biochemical					
Albumin (g/dL)	3.76	3.53	0.04	2.4–3.0[a]	0.011
Alkaline phosphatase (U/L)	260.1	231.4	10.8	68–387[a]	0.335
Fructosamine (umol/L)	254.3	233.3	4.5	–	0.175
Glucose (mg/dL)	61.0	50.4	1.3	50–80[a]	0.001
BUN (mg/dL)	30.8	25.3	0.7	8–20[a]	0.003
Cholesterol (mg/dL)	75.6	84.5	2.4	52–76[a]	0.478
BHB (mmol/L)	0.327	0.309	0.02	0.55[a]	0.170
NEFA (mmol/L)	0.12	0.24	0.02	–	0.092
Hematological					
Total leukocytes (cells/μL)	6853.0	6231.1	166.7	4,000–8,000[b]	0.11
Neutrophils (cells/μL)	1183.6	1272.8	71.3	700–6,000[b]	0.76
Lymphocytes (cells/μL)	3755.8	3358.7	127.7	2,000–9,000[b]	0.05
Neutrophils (%)	17.46	20.44	1.1	10–50[b]	0.133
Lymphocytes (%)	55.3	53.9	1.4	40–55[b]	0.109
N:L	0.315	0.379	0.03	–	0.046

RN, Rotatinuous stocking; RT, Traditional rotational stocking. [a]Reference value from Kaneko et al. (31) or [b]Byers and Kramer (32). SEM, standard error of the mean.

affected blood variables ($P > 0.05$). The serum concentration of albumin, glucose and urea (BUN) were greater in the RN sheep ($P < 0.011$), while the NEFA tended to be greater on RT animals ($P = 0.09$), and the alkaline phosphatase, fructosamine, cholesterol and BHB were unaffected by treatments ($P > 0.05$). The hematology profile shows lower lymphocyte counting ($P = 0.05$) and greater neutrophil-to-lymphocyte ratio (N:L; $P < 0.046$) on the RT sheep, and unaffected total leukocytes and neutrophil number, and neutrohpils and lymphocytes percentages ($P > 0.05$).

DISCUSSION

The RN stocking stresses the central role that the sward height has over the intake rate and daily forage intake of grazing ruminants. Boval and Sauvant (13) mentioned that foraging decisions driving the intake rate provide information of ingestive comfort, appetite, gut health and welfare. Indeed, Mellor (14) suggests that eating enough of a high-quality diet and experiencing postprandial satiety are components of a good mental and nutrition state, thus of welfare. Thereby, the RN stands as a grazing management that might promote welfare, as it mimics the "time minimizing" nature of grazers by allowing them to graze at high intake rates and to "take the best and leave the rest" of plants (1). We show this by finely depicting the diversity of bites that sheep can perform to cope with contrasting grazing strategies and compose their diet, and the effect that the resulting amount and quality of the diet ingested have over some nutrition- and stress-related blood parameters of sheep grazing vegetative Italian ryegrass pastures.

The Biting Behavior and Nutrient Content of the Diet Ingested

Bergman et al. (2) suggest that ungulates are "time minimizers" or the opposite, intake rate maximizers, this is, that they opt to graze on sites allowing them to harvest the greater amount of food in the least possible time. This could be a foraging strategy adopted by grazers for fitness (i.e., surviving until reproductive age, finding a mate and producing an offspring). From this assumption, our observation that RN sheep composed their diet preferentially from bites performed on the top stratum of plants (i.e., leaf laminas), especially on Italian ryegrass plants with ∼20 cm (**Table 2**) was expected, as around this sward height sheep display high intake rate on vegetative Italian ryegrass pastures (1), and also by assuming that herbivores defoliate swards from the top to the bottom of the canopy (33). Moreover, animals select leaves with greater soluble content (34) when they are not forced to explore the bottom parts of plants. As pointed out by Savian et al. (18), we also observed 14% greater CP content in the RN diet, with values within the range reported for ryegrass pastures grazed by sheep (35, 36). The 12% greater content of soluble sugars in the RN diet, but similar NDF content in both treatments, probably resulted from the low fiber content of the vegetative stage of Italian ryegrass in this study. The 2.2 and 2.5% greater OM digestibility and ME intake, respectively, by the RN animals (**Table 3**) could result in greater LW gain (9, 35) or milk yield (3). The 13% increase in crude fat was expected, with values within the range of other grazing trials with ryegrass (37–39). Several experiments showing the vertical quality gradient of forages (40–42) support our results indicating better nutritive value of the RN diet in response to preferential biting behavior on the top stratum of plants.

Daily Nutrient Intake

According to Dove (43), as farm profit derives from outputs per hectare, balancing nutrient supply with demands should not be attempted on an individual-animal basis, instead, a daily penalization of ∼10% of individual DM intake should be targeted (3); in dairy systems where the herbage utilization reaches 93% (6) restriction could arguably surpass 10%. Nonetheless, we suggest that optimizing individual nutrient intake from grazing would occur without the trade-off of reducing per hectare harvest efficiency and farm profit, provided that both primary (i.e., total herbage production and harvesting per stocking season) and secondary production (i.e., individual and per hectare LW gains), are both optimized with the RN stocking, with respect a RT applied on Italian ryegrass grazed by sheep (9). While setting sward heights for maximizing the intake rate does not mean that animals will always defoliate plants at such sward height or warrant maximum daily intake, the conditions are ideal for this to happen (1, 16, 17, 44), thus for reducing the supplementation with high-grain diets or silages. This is timely for high yielding animals [e.g., cows whose forage intake capacity increases by 0.18 kg/kg of milk at lactation peak on good quality pastures; (45)], under time-limiting scenarios. Clearly, the daily competence of grazing with other time-consuming behaviors (i.e., ruminating, idling, socializing, walking), human interventions (i.e., nocturnal housing, milking) or weather conditions (i.e., rain and fouling, heat stress, low forage growth), could reduce eating time and accentuate intake restrictions under scenarios of low intake rate (1).

Blood Biochemistry and Hematology

The management of grazing affects the amount and quality of nutrients that animals ingest, thus their metabolic status. Blood glucose is a short-term proxy of energetic metabolism (46, 47), and in ruminants, propionic acid is its main precursor, although amino acids make a minor contribution to gluconeogenesis (48). Therefore, its greater concentration on RN animals could be explained by their greater intake of digestible OM and CP (**Table 4**). In line with this, Raja et al. (10) suggested that glucose responds positively to digestible OM intake. Glucose concentration in both RN and RT sheep (**Table 4**) is within reference values of adult sheep (31), and values in RN animals compare well with those of sheep grazing ryegrass at low intensity (49). Moreover, other reports coincide with this study in that animals with restricted feed intake, thus with limited supply of gluconeogenic substrates, have lower blood glucose concentration than better feed animals (50–53).

Fructosamine is formed from glucose and mainly albumin and owing to albumin's half-life of around 2 weeks (54), it serves as a blood marker of glycemia of the previous weeks. The non-significant increase in fructosamine in RN animals, despite higher blood glucose and albumin, could indicate the non-sensitivity of this proxy to acute changes of glucose (55), as observed on cows with less energy deficit after 30 days in lactation (56), or that a single glucose sampling did not allow an accurate referencing of glycemia of the previous weeks, because of day-to-day variation in DM intake in grazing conditions. Nonetheless, the reduction by half in blood NEFA concentration ($P = 0.09$) in RN sheep, is consistent with a better energy balance compared to RT sheep.

In this latter regard, energy intake restriction reduces cholesterol levels (57), triggers the mobilization of fatty acids from adipocytes and increases the serum concentration of

NEFA (47), limits propionate production (58) and stimulates the synthesis of ketone bodies, mainly BHB (59, 60). In this study, cholesterol and BHB blood concentrations were within values previously reported for sheep (49, 52), but lower than values reported by Kaneko et al. (31) for BHB; however, as the grazing management did not affect their concentration on non-metabolically challenged adult sheep, we suggest that substantial changes of these energy metabolites are more likely to occur when imposing energy intake restrictions below maintenance (50), which was not the case of our study, as animals of both treatments put on weight (data not shown), as demonstrated by Schons et al. (9) in a similar experiment. Likewise, alkaline phosphatase can be reduced under feed intake restriction, as occurred with sheep at a high stocking rate (61). Nonetheless in our study, its decrease in RT animals was not significant. As with energy metabolites, it is perhaps necessary a severe feed restriction to affect its concentration.

As explained above, albumin is indicative of mid-term protein status (54), while BUN is of readily dietary protein intake (62). Both metabolites were above the superior limit of 30 g/dL and 20.7 mg/dL, respectively, reported by Kaneko et al. (31) for adult sheep. As these metabolites respond positively to dietary CP, its excessive intake by sheep of both treatments is evident. BUN concentration on RN and RT sheep are comparable to values reported on sheep grazing temperate pastures with 25% of CP [blood urea equivalent to 33.1 mg/dL of BUN; (63)]. High BUN may cause reproductive inefficiency in sheep at values around 14.6 mg/dL (64–66). Such inefficiencies have been noticed on temperate pasture-based dairy herds (67, 68). Moreover, high concentration of nitrogen in the rumen could lead to less efficient ATP-producing fermentation pathways (69), high energy expenditure due to ureagenesis in the liver (70) or boost the emission of nitrous oxide from manure. In this latter regard, despite Savian et al. (71) noticed greater nitrous oxide emissions from feces, these represented <1% of the CO_2-eq emitted as enteric methane (g/ha/day), which was 61% lower in the RN, with respect RT grazing (18). To avoid inefficiency associated to excessive dietary CP, it becomes necessary to test optimal levels of energetic supplementation on animals grazing under the RN management and assess the trade-off between the emission of greenhouse gases via urine and the carbon stock in soil on RN paddocks, hypothesized to be enhanced due to greater herbage growth (9).

Low energy intake can activate the pituitary-adrenal axis, suppress animals' immune response and impair their welfare. Within leukocytes or immune cells, neutrophils participate in phagocytosis, produce reactive oxygen species (highly toxic for engulfed bacteria) and antibacterial enzymes (72), and lymphocytes confer cell-mediated immunity through immunoglobulins (73). As under long-term stress the blood N:L ratio increases (11) in response to greater levels of glucocorticoids (32, 74), this ratio is a good proxy of long-term stress (75). Sub-optimal feeding (76), monotonous diets (77) or delaying feeding (78), affect leukocytes formation and function (47). In this study, the 17% higher N:L ratio on RT sheep, support our hypothesis that the grazing strategy penalizing individual OM intake by 18% could impair animal welfare via depressing the immune response. This is also suggested from the greater NEFA concentration, as animals with high circulating NEFA can have their immune system suppressed (Ingvartsen and Moyes, 2012). A greater N:L ratio (52) or the expression of hepatic proteins involved in immune response and inflammatory cytokines (53), as indicators of welfare impairment were also observed on sheep under sub-optimal grazing conditions.

Overall, RN sheep exhibited a metabolic and hematological profile that could be associated to a better nutritional status and immune response to stress, thus to welfare. Nonetheless, it is advisable that given the subjective nature of the welfare concept, no single physiological measurement is conclusive and that complementary assessments over the whole grazing season (e.g., inflammatory responses, oxidative stress, behavioral responses to the grazing environment denoting ingestive comfort or the opposite), should be considered for a broader evaluation of the well-being of grazing animals.

CONCLUSION

Pastoral systems must be evaluated not only by their productivity and environmental impact, but also by how much they promote animal welfare. This study shows that the low-intensity/high-frequency grazing strategy (RN) allowed sheep to compose their diet mostly from bites performed on the top stratum of plants, contrary to a traditional strategy (RT) of high-intensity/low-frequency grazing, forcing sheep to compose diets mostly from bites performed on top and grazed parts of plants. The biting behavior of sheep grazing vegetative Italian ryegrass pastures under the RN strategy allowed them to have a greater intake of a diet with better quality and a biochemical-hematological profile positively associated with nutrition and immune response to stress, which means that well-managed pastures (e.g., RN) could be a good strategy to promote welfare in grazing conditions.

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 Ethics Committee for the Use of Animals (CEUA) of the Federal University of Rio Grande do Sul (UFRGS; protocol 3571).

AUTHOR CONTRIBUTIONS

AZ: conceptualization, writing original draft, investigation, review, editing, and field data collection. AM: field data collection and review and editing. JS: conceptualization, field data collection, and review and editing. AS: conceptualization, field data collection, and review and editing. JR: data curation, formal analysis, and review and editing. JB: writing, review and editing. MB: field data collection and laboratory analysis. CB: formal analysis and review. LQ: review and editing and laboratory analysis. SV: resources and review. VD: writing, review and editing and resources. PdF: conceptualization, supervision, funding acquisition, review and editing, resources, and project administration. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.631820/full#supplementary-material

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