RUMINANT GRAZING BEHAVIOR: A TOOL TO IMPROVE PRODUCT QUALITY AND ECOSYSTEM SERVICES

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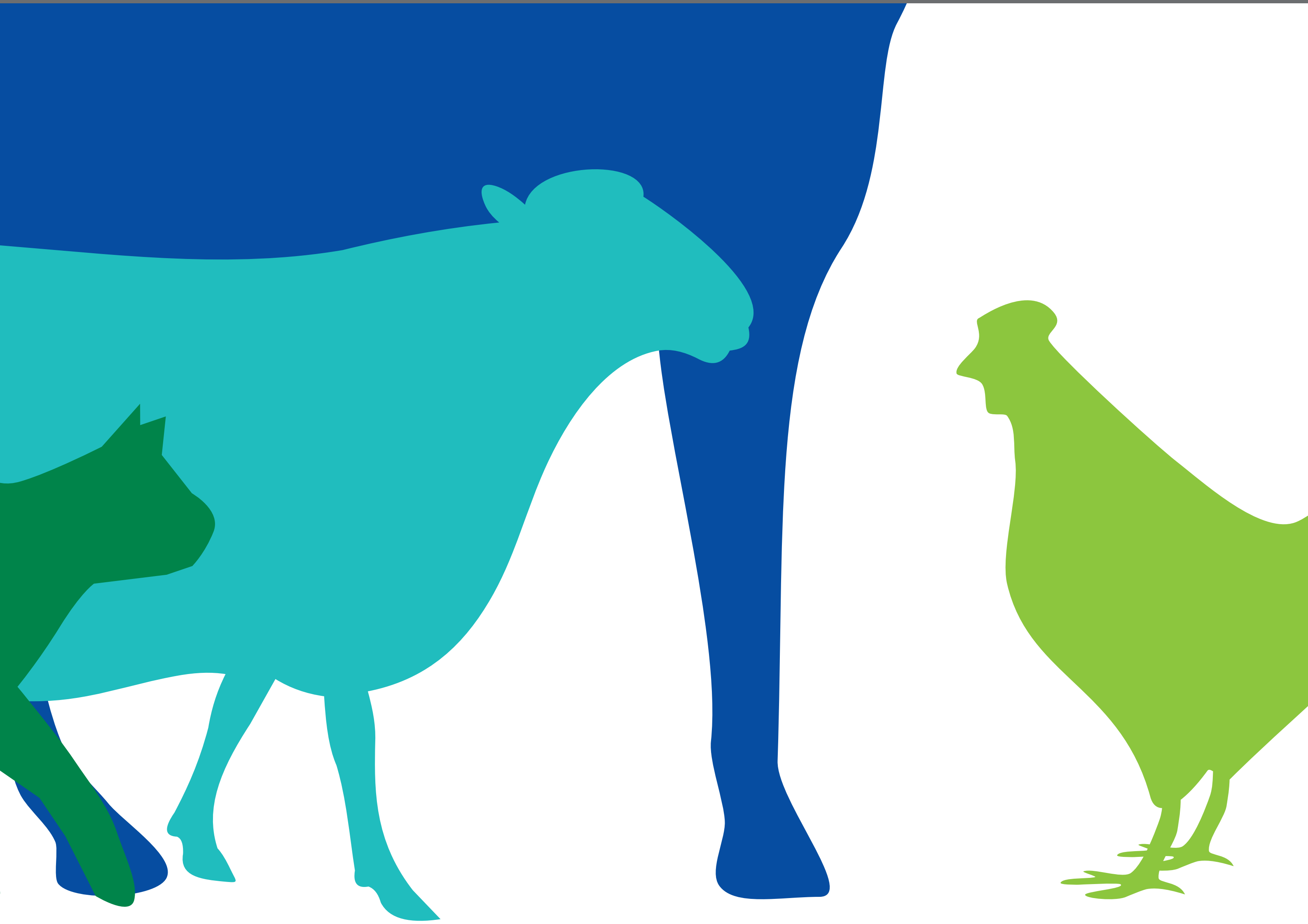

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RUMINANT GRAZING BEHAVIOR: A TOOL TO IMPROVE PRODUCT QUALITY AND ECOSYSTEM SERVICES

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Table of Contents

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EDITORIAL
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Editorial: Ruminant Grazing Behavior: A Tool to Improve Product Quality and Ecosystem Services

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Keywords: pasture grazing management, cows, sheep—lamb, goats, milk

Editorial on the Research Topic

Ruminant Grazing Behavior: A Tool to Improve Product Quality and Ecosystem Services

This Research Topic presents a series of original research and one review article that reveals the latest approaches to ruminant grazing behavior management associated with product quality and traceability. This collection embodies 12 original articles from eight countries, including Europe (Mediterranean, continental, and alpine regions) and North and South America. Articles were mainly focused on three axes: (i). pasture and grazing management and its relationship with ecosystem services, (ii). effect feeding behavior on animal's products, and (iii). genetics and grazing behavior.

Pasture and grazing management are some of the most powerful tools to manage and orient grazing behavior for both lambs and cattle, shown through studies conducted in controlled experiments or on field studies. In the studies published under this Research Topic, herbage allowance/structure, stocking rate, time allocation, and differences in grazing behavior between breeds were the investigated variables to set optimal management of both mono and multi-specific grasslands. Silva et al., in a subtropical environment, evaluated the ingestive behavior of young lambs considering the sward height. This study led to management recommendations for lamb production on tropical grasses under the ingestive behavior perspective, considering a maximum pasture height and a minimum lamb body weight to meet satisfactory intake parameters for optimizing lamb production. Poli et al. reviews the main available technologies for lamb production on pasture-based systems in subtropical regions. The proposed approaches are similar to classical pasture recommendations in other environments (i.e., controlling pasture availability, sward height, and structure), however, the authors identified a lack of studies on strategies to better manage herbage growth and minimize intense parasitic infections, which are common problem in subtropical regions.

Nicolao et al. evaluated if early-life dam-calf contact can influence post-weaning grazing behavior. The authors found that calves that experienced grazing with their dams until weaning immediately start to graze when turned out to pasture and expressed a grazing behavior more typical of adult cows than calves that were separated from their dams immediately after birth or to those that has not experienced previous dam contact. In temperate regions and on biodiverse pastures, Lind et al. described lamb performance and discussed opportunities and challenges for future sustainable sheep grazing on an island. The authors conclude that an adaptative management strategy must be adjusted to find an optimal compromise between animal production and restoring and conserving these environments, and therefore provide ecosystems services. Hamidi et al. monitored cow activity in a semi-natural grassland in Germany, under three levels of herbage

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allowance. At a lower herbage allowance, higher cattle activity is observed and at moderate allowances, a higher spatial distribution of cattle during grazing peaks is observed, with potential practical implications in the ecosystem. Finally, Noelle et al., in desert conditions, determined the optimal grazing time to avoid a second defoliation of swards and therefore indicates management targets to avoid degradation of grasslands in this fragile ecosystem. Pauler et al., in a controlled grazing experiment in the Swiss Alps, observed breed-specific differences in the behavior intake which can be used to improve pasture management and contribute to grassland conservation. These differences can be associated with allometry and anatomy of breeds adapted to graze nutrient-poor pastures. Having a general overview of the illustrated studies, their results highlighted that grazing behavior and management play a key role in pasture utilization and animal performances and welfare. Furthermore, grazing management and behavior can also be a powerful tool to drive ecosystem conservation and management, especially when exploited by adapted breeds, and more generally, to improve ecosystem services of pasture-based farming systems.

This Research Topic also explored the effect of grazing behavior and management on the characteristics of animal products, focusing on cow, ewe, and goat dairy products. Claps et al. in two case studies developed in the Italian mountain regions, provides information about milk quality, and discusses the relationship between grazing systems in biodiverse pastures and milk products with favorable characteristics for human nutrition, able to give high added value prices to dairy products. Studying milk from cows of breeds of different levels of specialization, Koczura et al. observed little differences in fatty acid composition according to breeds when grazing on the same pasture, suggesting that different grazing behaviors of adapted breeds could have consequences on dairy product characteristics. Molle et al. used fatty acids and n-alkane composition to trace feeding systems of ewes' milk. This study used mathematical models, such as Genetic Algorithms (GA) combined with Linear discriminant analysis (LDA), which allowed to discriminate products according to the amount of ingested pasture when using appropriate chemical markers. Complementary, Molle et al., used Fourier transformed mid-infrared spectroscopy (FT-MIR) to estimate milk FA composition, and the results obtained to discriminate milks sourced from dairy sheep rotationally grazing for 2, 4, or 6 h per day in Italian ryegrass or berseem clover. The authors conclude that is possible to discriminate milk from ewes grazing Grasses or Legumes using the GA-LDA of their FA profile. However, lower accuracy of the model was detected when they considered the different time allocations, probably also because of adaptation of grazing behavior according to the time restriction by ewes. At a genetics axis, Davis et al., explored the effect of breeding strategy on low input and organic production in dairy systems in the United Kingdom. The results of this study bring out the weakness of the breeding programs when animals face organic and low input production systems, in which grazing is the most common practice, and highlights the need to explore genotype x environment interactions to better guide breeding selection in this scenario. Thus, generally, grazing behavior and management can have an impact on diary product characteristics. Adapted breeds, exerting a different grazing selection than specialized dairy breeds, consume vegetation type, or patches which influence dairy product composition, particularly fatty acids. Differences in milk composition related to grazing management can be detectable for authentication purposes, with important perspectives for the valorization of dairy products issued from grazing systems.

Even if this Research Topic proposed important advancements in the research on grazing behavior and management as tools to improve product quality and ecosystem services, we can identify a lack of studies relating grazing behavior of ruminants on meat and wool products. Social studies and evaluation of environmental impacts and ecosystem services of grazing systems are just recently becoming more prominent as object of study, thus further research will be required in the future on these topics. Finally, new methodologies for monitoring feeding behavior and estimating herbage intake at pasture (especially on species rich and heterogeneous pastures) should be better explored in future research.

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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Lamb Performance on Island Pastures in Northern Norway

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The Norwegian sheep industry is based on utilization of "free" rangeland pasture resources. Use of mountain pastures is dominating, with about two million sheep grazing these pastures during summer. Regional challenges related to e.g., loss of sheep to large carnivores make farmers think differently. The Norwegian coastline is among the longest globally and is scattered with islets and islands. Alone along the coast of Nordland county, it is estimated more than 14,000 islands. Use of islands for summer pasture is an alternative but there is a limited knowledge about such a management system. In this study, we examined lambs' average daily gain on island pastures at the coast of Norway. In total 230 lambs on three islands (Sandvær, Sjonøya, and Buøya), with varying pasture quality and stocking rate, for 3 years (2012, 2013, and 2014). At Sandvær as much as 92% of the island was characterized as high nutritional value while at Sjonøya and Buøya only 15%, was characterized high nutritional value. We found an average daily lamb growth rate of 0.320 kg d^{-1}. Lambs on Sandvær had a higher daily gain ($P < 0.05$) than those on Sjonøya and Buøya, and lambs' average daily gain was significantly lower ($P < 0.05$) in 2013 compared to 2012 and 2014. We conclude that with a dynamic and adaptive management strategy there is a potential to utilize islands for sheep grazing during summer.

Keywords: daily gain, vegetation types, stocking rate, grazing quality, sheep

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INTRODUCTION

The Norwegian sheep industry is based on utilization of spatially diverse rangeland pasture resources as reflected in different management systems and local adaptations. Only 3% of Norway is used for crop production, but more than half of the land area has potential value as livestock pasture. Rekdal (1) estimated that harvesting of rangeland vegetation by livestock could be doubled and in a White paper from the Norwegian Government from 2016 (2) an increase in rangeland grazing is encouraged for all regions of the country. In Norway, ∼2 million sheep are released onto extensive pastures for summer grazing (3). Most sheep are grazing rangeland pastures in mountainous areas but challenges due to high mortality to e.g., large carnivores have increased the interest in utilizing pastures on islands and islets along the coast.

The coastal line of Norway's mainland is estimated to about 30,000 km, but including islands, the length increases to about 103,000 km (4). Nordland county, stretching from 65 to 69°N, has a surface area of about 38,000 km^2 and constitutes 12% of the total area of Norway (5). Nordland coastal line is estimated to be about 27,000 km of which 21,000 km are island coastal lines. The coast is scattered with some 18,000 islands of all sizes, from small islets of ∼1 ha to inhabited islands up to 500 km^2 (5). Many of the smaller islands were previous inhabited but are now abandoned and traditional farming with meadow harvesting and livestock grazing has ceased. Indeed, the open and grazing-induced semi-natural pastures rich in biodiversity and pleasing to the human's eye are at risk. In Nordland county, farmers are therefore offered a diverse package of subsidies and incentives for restoring and maintaining this unique semi-natural coastal landscape (6).

Most of these islands are flat (rising to 40–50 m above sea level) and natural fresh water supply can be limited during summer. The phenological development of the plants is more uniform on islands than in mountain areas. Vegetation types, their proportion, and distribution and thus pasture value varies substantially between islands (7). A management of stocking rate customized to available pasture resources is therefore necessary to ensure animals' performance and welfare (8). However, appropriate stocking rates are defined by decades-long experience by farmers, while little scientific knowledge exists about sheep performance on these coastal pastures.

In a field study we investigated lamb performance during three consecutive summer grazing seasons (2012–2014) on three islands, with highly variable grazing values and stocking rates at the coast of Helgeland, Nordland county. The aim of the study was to describe lamb daily weight gain and to evaluate and discuss opportunities and challenges for future sustainable sheep grazing on island pastures.

MATERIALS AND METHODS

Ethics Statement

The study was performed at commercial farms and the only extra handling of animals was through weighing. The animals were collected by help of sheep dogs per normal practice at the farms. We followed the regulation for use of animals in experiments,

FIGURE 1 | Map of Norway and the islands (insert) Sandvær (south), Sjonøya and Buøya (north).

adopted by the Norwegian Ministry of Agriculture and Food and approved by the administrative officer for animal trials of NIBIO (Approved Animal welfare unit no 171). Ethical review and approval was not required for the animal study because the study was performed at commercial farms. Written informed consent was obtained from the owners for the participation of their animals in this study.

Study Area

The three islands studied have been used for sheep grazing during many years. The islands are situated in Lurøy and Rødøy municipalities, at the coast of Nordland county in Norway (**Figure 1**). Sandvær (66°20′35 N, 12°43′55 E) covers 39 ha and range up to 20 meters above sea level (m.a.s.l.). Sjonøya (66°21′51 N, 12°52′42 E) covers ∼208 ha and range up to 40 m.a.s.l while Buøya (66°37′31 N, 12°56′35 E) covers 36 ha and range up to 40 m.a.s.l. The total livestock unit (LU) at Sandvær, Sjonøya, and Buøya were 1.26, 3.60, and 2.70, respectively, in all 3 years (2012, 2013, and 2014). At Sjonøya, an additional 40 sheep of the Old Norwegian breed (5.6 LU) grazed and was included when stocking rate was calculated. The weather is typical coastal climate with mild winters and wet summers, with mean temperature during winter around 0°C and during summer around 12°C (9). Annual precipitation is around 2,000 mm.

Vegetation

The vegetation was mapped using the system of Rekdal and Larsson (10) and a total of 19 different vegetation types, both natural and semi-cultivated, were identified on the islands (**Table 1**). We classified the vegetation types into four main classes based on value for sheep grazing: "Not Suitable" (no grazing value or inaccessible), "low," "medium," or "high," following the vegetation classification system of Rekdal (7). The "Not Suitable" class including barren land and exposed bedrock was omitted from all analyses. Rekdal (7) evaluate the grazing value of the different vegetation types based on plant production and the grazing habits of the livestock species. At present, there is no systematic information on nutritional values such as energy, protein, and fiber for different vegetation types (11) and such values would necessarily be highly uncertain due to varying plant species composition within a vegetation type, site-specific phenological development for each plant species, and the impact on the vegetation from the grazing animals both within year and historical.

In the study area, vegetation types of high nutritional value, contain species such as common bent (*Agrostis capillaris*), sweet vernal grass (*Anthoxanthum odoratum*), kentucky bluegrass (*Poa pratensis*), and red fescue (*Festuca rubra*). Wavy hair-grass (*Deschampsia flexuosa*), blueberry (*Vaccinium myrtillus*), and sweet vernal grass (*Anthoxanthum odoratum*) are found in medium nutritional value classes while the low nutritional value class is dominated by crowberry (*Empetrum nigrum*), heather (*Calluna vulgaris*), and purple moor-grass (*Molinia caerulea*).

Table 1 shows the distribution and proportion of the vegetation types and their nutritional classes on the three study islands. **Figure 2** shows the vegetation maps of the three islands.

TABLE 1 | Distribution of vegetation types and nutritional value in area (ha) and percentage (%) at Sandvær, Sjonøya, and Buøya.

Vegetation type	Grazing value	Sandvær		Sjonøya		Buøya	
		Ha	%	Ha	%	Ha	%
Dwarf shrub heath	Medium			0.25	0		
Low herb meadow	High	14.7	38	5.1	2		
Tall forb meadow	High	8.2	21				
Lichen and heather birch forest	Low			0.1	0		
Bilberry birch forest	Medium			5.3	3		
Meadow birch forest	High			2.6	1		
Pasture land forest	High			1.2	1		
Meadow spruce forest	Medium			0.3	0		
Poor swamp forest	Low			0.8	0		
Rich swamp forest	Medium			0.7	0		
Bog	Low			4.1	2	3.7	10
Fen	Low	1.3	3	6.1	3	0.4	1
Sedge marsh	Low						
Coastal heath	Low	1.9	5	64.5	31	8.6	24
Damp heath	Low			85.3	41	18.2	50
Moist meadows	High	1.5	4	2.3	1		
Pasture	High	10.8	28	19.9	10	5.0	14
Barren land							
Exposed bedrock		0.3	1	9.2	4	0.4	1

Animals

The study animals were of the dominating sheep breed in Norway, the cross-bred, prolific Norwegian White Sheep. Ewes and lambs were recruited from two commercial sheep farms that had used the islands for summer grazing during several years prior to the study. We asked the farmers to randomly select adult ewes (>2 years of age) with two lambs at foot. Twins are the most common litter size in the breed. The farmers selected the animals post-lambing to ensure that all ewes and lambs were healthy and distributed the animals randomly to the islands (**Table 2**). The animals had access to all vegetation types within each island, and did not receive any supplement feeding during the grazing period.

Due to missing data the number of lambs used in the performance analysis was 11, 13 and 14 at Sandvær for 2012, 2013, and 2014, respectively. For Sjonøya, 39, 39, and 40 lambs are included in the analyses and for Buøya 28, 28, and 18 for 2012, 2013, and 2014, respectively.

Lambs were born in May and were between 1 and 4 weeks old when released to the island pastures. All ewes and lambs were individually ear-tagged for identification. The ewes were weighed before released to and when collected from the islands. The lambs ($n = 230$) were weighted at birth (average 4.90 Standard deviation, $SD = 0.86$ kg), when released to the island (average 9.98 $SD = 3.51$ kg), and when collected (average 38.1 $SD = 7.90$ kg) as normal routine done by the farmers. The animals were on average released to the islands in week 21 (end of May) and collected in week 37 (beginning of September).

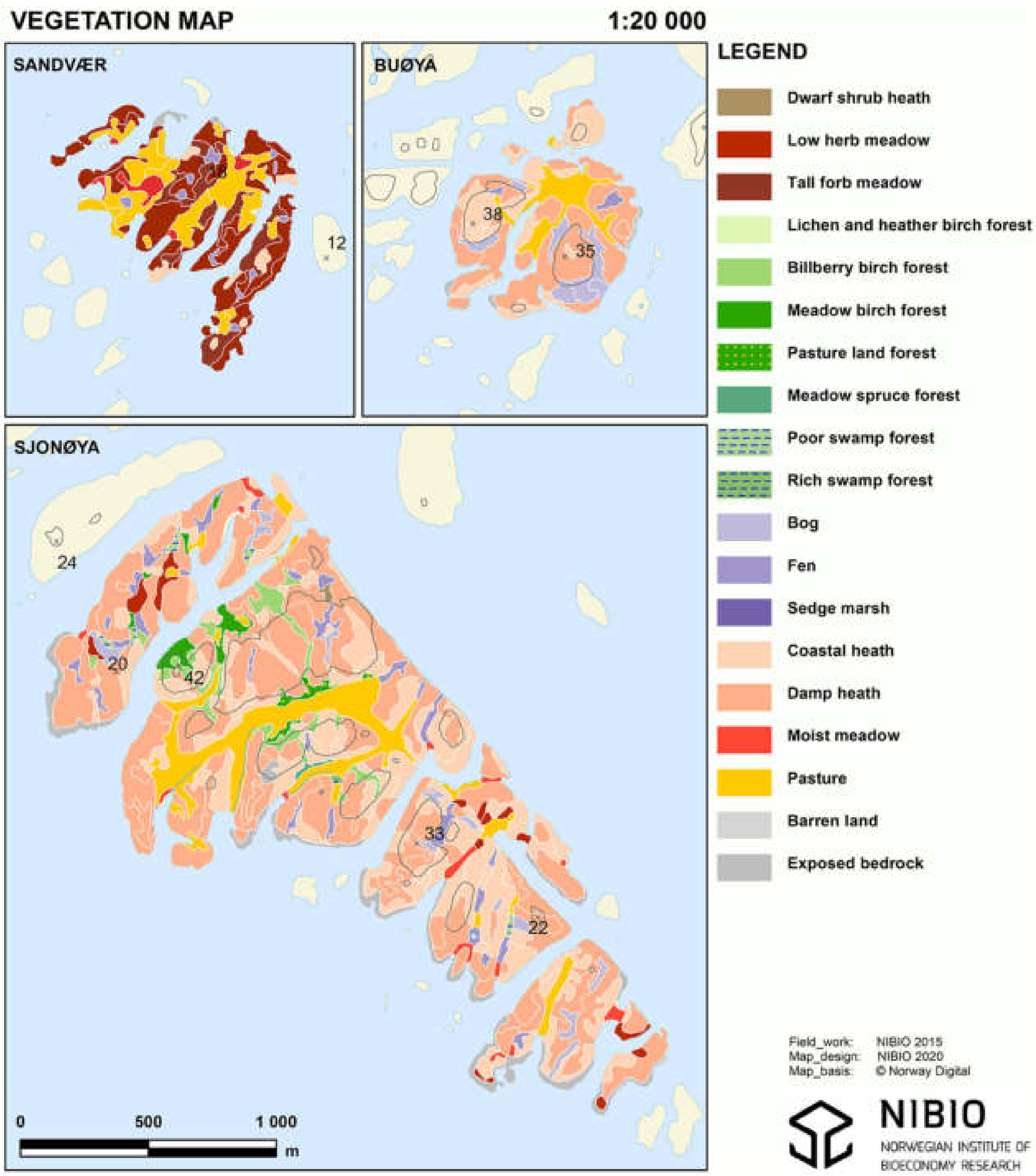

FIGURE 2 | Vegetation maps of Sandvær, Sjonøya, and Buøya.

Weather Conditions

As a proxy for the daily average temperature and precipitation at the three islands, data was collected from the weather station at the mainland at NIBIO Tjøtta in Nordland County (65°49'22N, 12°25'37E); the information is shown in **Table 3** for June, July, and August of the years 2012, 2013, and 2014.

Statistics

Data on a total of 230 twin lambs were analyzed by fitting a general mixed linear model in Proc Mixed of SAS statistical software (12), using the Satterthwaite option for estimation of denominator degrees of freedom. The model used was

$$y = Xb + Zu + e,$$

where y is the observation of individual lamb body growth (kg d^{-1}) on island pasture; *b* is a vector containing fixed demographic and environmental effects, and *X* is the incidence matrix relating the observations to the effects in b. The random effect of ewe by year is *u*, related to observations by incidence matrix *Z*. Finally, *e* is the residual variance.

The effects in *b* are

- the overall average daily weight gain μ.
- regression variables lamb age (days) at start of island grazing (2–58; mean 15.8), early lamb growth (kg d^{-1}) from birth to start of island grazing (−0.05-0.77; mean 0.33), and ewe weight (kg) at the start of island grazing (41–101; mean 73.9),
- class variables lamb sex (female or male, sex ratio 0.5), ewe age in years (1, 2, ...,6; mean 2.5), island (Sandvær, Sjonøya, or Buøya), year (2012, 2013, or 2014), and the interaction effect between year and island (nine levels).

To account for dependency within litters, the interaction effect of individual ewe by year was fitted as random, with 121 classes (98 ewes). Seventeen ewes were observed in more than 1 year; but the litters of these were still treated as independent of each other. Least square means were estimated for all significant fixed class variables and pair-wise *t*-tests were performed. Effects were considered significant when $P < 0.05$.

RESULTS

As much as 92% of the area of Sandvær is characterized as high nutritional value which here includes the vegetation types of low herb meadow, high forb meadow, moist meadow, and pasture. At Sjonøya, about 80% of the area is characterized as low nutritional value with the island dominated by coastal heath (31%) and damp heath (41%). Most of the remaining area is classified as medium to high nutritional value (low herb meadow, meadow birch forest, and pasture). At Buøya six vegetation types were present and the island is dominated by low nutritional value classes (86%). On this island, high nutritional value is only found on patches of pasture (14%). Exposed rock is found on all islands, 1% at both Sandvær and Buøya and 4% at Sjonøya.

Lambs' average daily gain on the island pastures was 0.320 kg d^{-1} ($SD = 0.067$ kg d^{-1}), and they spend on average 89 days on the islands ($SD = 13$ days). From the mixed model (**Table 4**) all variables in the model were significantly affecting lamb growth at $P < 0.05$, except for lamb age (days) at release on the islands ($P = 0.66$).

Least square means (*LS* means) for the class variables island, year, interactions of year * island, lamb sex, and ewe age are shown in **Table 5**.

T-tests between *LS* means showed that lamb daily gain (**Table 5**) differed between islands ($P < 0.01$) and that lambs at Sandvær had the highest daily gain (0.372 kg d^{-1}) mainly caused by the high growth rate in 2012. Across islands, lamb daily gain was higher in 2012 and 2014 compared to 2013 ($P < 0.01$). The interaction between year and island show that the lambs' growth on Sandvær in 2012 was higher than that of all other year * island classes ($P < 0.01$); no other significant differences were found. Male lambs had a higher average daily gain than female lambs ($P < 0.01$).

Daily weight gain of lamb from birth to release on the islands was fitted as a regression variable, with 0.076 (*SE* 0.029) kg d^{-1}, i.e., an increase in early growth of 0.1 kg d^{-1} would give an increased growth on island of 0.0076 kg d^{-1}. Given 89 days grazing period on the islands, this increase gives an extra ≈

TABLE 2 | Number of Norwegian White ewes and lambs at Sandvær, Sjonøya, and Buøya in 2012–2014.

	Sandvær		Sjonøya		Buøya	
	Ewes	Lambs	Ewes	Lambs	Ewes	Lambs
2012	7	13	20	40	15	30
2013	7	14	20	40	15	30
2014	7	14	20	40	12	23

TABLE 4 | Effect of lamb age (d) at release, lambs average daily gain (g d^{-1}) from birth to release, lamb sex (male or female), age of ewe (year), ewe weight (kg) at release, islands (Sandvær, Sjonøya, Buøya), year (2012, 2013, 2014), and the interaction between year and island, their nominator Degrees of Freedom (NDF), denominator Degrees of Freedom (DDF), *F* and *P*-values.

Effect	NDF	DDF	*F*-value	*P*
Lamb age	1	101	0.19	0.663
Early growth	1	209	6.64	0.011
Sex	1	206	27.81	<0.001
Ewe age	5	104	2.69	0.025
Ewe weight	1	104	13.37	<0.001
Island	2	101	20.88	<0.001
Year	2	106	10.79	<0.001
Year*Island	4	102	32.07	<0.001

TABLE 3 | Average temperature (°C) and total precipitation (mm) in June, July, and August for 2012, 2013, and 2014 at Tjøtta weather station.

	June		July		August	
	Average temperature	Total precipitation	Average temperature	Total precipitation	Average temperature	Total precipitation
2012	10.9	15.0	12.4	102.3	12.2	47.2
2013	13.0	84.1	13.3	169.2	13.9	65.6
2014	12.0	36.8	18.8	63.8	15.1	70.5

TABLE 5 | Least squared means (LS means) corrected for the other effects in the model for lambs average daily gain (kg d^{-1}) on island pasture, with standard error (SE), for class variables island, year, year * island, and sex in the mixed model.

Effect	Level	LS means	*SE*
Island	Sandvær (Sa)	0.372	0.013
	Sjonøya (Sj)	0.285	0.007
	Buøya (Bu)	0.326	0.007
Year	2012	0.344	0.009
	2013	0.303	0.008
	2014	0.337	0.008
Year*Island	2012*Sa	0.472	0.016
	2012*Sj	0.230	0.010
	2012*Bu	0.331	0.014
	2013*Sa	0.301	0.023
	2013*Sj	0.302	0.011
	2013*Bu	0.306	0.013
	2014*Sa	0.345	0.015
	2014*Sj	0.324	0.008
	2014*Bu	0.342	0.013
Sex	Male	0.343	0.006
	Female	0.313	0.007

0.7 kg live weight per lamb. The regression on ewe weight was 0.002 kg d^{-1} (*SE* 0.0005) per kg ewe live weight; meaning that 1 kg higher ewe weight corresponds to an increased lamb growth of 2 g per day or 178 g during the 89 days grazing period on the islands. The variance component of year by ewe, of 0.0005 (*SE* 0.0002) was significantly different from zero (Wald-test: $Z = 2.50$, $P = 0.006$); the residual variance was 0.0013 (*SE* 0.0002) and different ($Z = 7.45$, $P < 0.001$) from zero.

DISCUSSION

In the present study, we investigated lambs' performance when grazing semi-natural pastures on islands to evaluate the quality of these pastures. Further, we corrected for age of ewe, weight of ewe, and sex of lamb. All these effects significantly influenced lambs' average daily gain, as expected (13, 14) and are therefore not considered in the following discussion.

The proportion of vegetation types of high nutritional value differed between the islands. The vegetation type pasture is mainly former managed permanent grassland for forage production, now abandoned, and has a high nutritional value with an estimated grazing capacity of 0.75 LU per ha per year (7). At Sandvær, 35.5 ha, around 92% of the total area was classified as high value according to Rekdal (7). Pasture alone, covering around 12 ha, could sustain around 3.6 LU. In addition to pasture, the high nutritional value vegetation classes low herb meadow (covering 38%) and tall forb meadow (covering 21%) were found on this island. During the 3 years experiment, only 21 sheep (1.26 LU) grazed the island every summer. When vegetation is grazed at an optimum stocking rate the forage quality maintains. However, when the number of animals is too low to maintain the quality, the non-grazed areas will degrade. At Sandvær, the tall forb meadow vegetation type was dominated by meadowsweet *(Filipendula ulmaria)* resulting in a degradation of its grazing value. Meadowsweet has little grazing value for sheep and is often seen dominating areas with zero or low grazing pressure (15). The higher lamb daily gain at Sandvær compared to the two other islands, could be attributed to the high percentage of vegetation types with high nutritional value. A higher stocking rate combined with an earlier release would help improve the now low nutritional value of the vegetation type tall forb meadows and lead to an even higher weight gain of the lambs.

The stocking rate at Sjonøya was estimated to 9.2 LU including the flock of Old Norwegian (3.6 Norwegian White and 5.6 Old Norwegian). About 10% (20 ha) of the island was pasture which could carry about 10 LU (7). In addition, the high nutritional vegetation types of low herb meadow, meadow birch forest, pasture land forest and moist meadows (in total covering 5% of the area) was present allowing additionally 2 LU to graze the island. The number of grazing sheep at Sjonøya during the summer was equivalent to 9.2 LU thus close to estimated grazing capacity of 10 LU. However, lamb daily gain on Sjonøya was significantly lower than that of both Sandvær and Buøya. Sjonøya consists of four smaller islands connected only at low tide. Most of the cultivated pasture type is located on one of them and sheep could be temporary stranded at an island with mostly low nutritional value vegetation types. This could be one explanation for the lower average daily gain. The Old Norwegian sheep present at Sjonøya is a breed which can utilize coastal heath when higher nutritional value of other forages become scarce. However, during summer when higher nutritional value is available, the Old Norwegian breed graze the pasture as well.

The number of sheep at Buøya was estimated to 2.70 LU. Fourteen percent of the island (5 ha) was pasture and according to Rekdal (7) could carry around 2.5 LU. With a LU density of 2.70, density may be a limiting factor for lambs' growth, since the rest of Buøya is dominated by heath vegetation types and classified as having low nutritional value. Lambs' growth rate was significantly lower on this island compared to Sandvær, but higher than on Sjonøya. When the stocking rate is higher than the estimated capacity of the high nutritional value vegetations types, animals are forced to graze in medium and low nutritional value vegetations types. Species such as purple moor-grass (*Molinia caerulea*) and Viviparous sheep's-fescue (*Festuca vivipara*) were found in the coastal heath vegetation type at Buøya. These species could be important for animals grazing in areas dominated by low nutritional value classes (Haugen, unpublished). Comparing LU and cover of high nutritional vegetation types between Buøya and Sandvær, one could expect a higher difference in lambs' average daily gain. As discussed, parts of the high nutritional areas of Sandvær was not grazed due to the low stocking rate. We suspect that the total area was reduced in forage quality during the summer. On the other hand, the smaller area of high nutritional vegetation types at Buøya could be more intensively grazed and thus maintain a higher quality throughout the grazing period. The investigated islands all had a high degree of plant species diversity. Over a 3-months period, the nutritional value-change would be species-specific and influences by general phenological development as well as the within-year impact of grazing.

The climate along the Norwegian coast is dominated by mild winters and wet summers. The average summer temperatures on the islands are 1–2 degrees lower than that observed at the weather station of Tjøtta (Lind, not published). Steinheim et al. (16) and Nielsen et al. (17) found that local weather affected growth of the lambs over summer, but that the effects were area specific. Nielsen et al. (18) examined the relationship between weather and lambs' growth at Tjøtta farm for 17 years and found that a warm July had a positive effect on lamb growth. Precipitation did not seem to have any direct influence on lambs' growth (18). In the present experiment, 2012 was in general cooler (11.8°C in average during June, July, and August) than the years 2013 (13.4°C) and 2014 (15.4°C) with less precipitation (164, 319, and 171 mm for 2012, 2013, and 2014, respectively). Hatten et al. (8) in a 1999–2001 study in the Vega archipelago, situated about 80 km south of our study area, studied lambs' growth on four islands. The summer of 2001 was warm and dry and affected lambs' growth rate adversely. The animals in that study were collected from the islands late in August when available pasture and fresh water was inadequate and the average daily gain during the last month was negative for some lambs. The islands in our study were larger than the ones used in Hatten et al. (8) and thus likely not as sensitive to the summer weather conditions. We suggest that weather, within the range observed, did not strongly influence lamb growth rates in our study.

Similar challenges linked to phenological development of plants are not found in mountainous areas to the same extent (7). On the contrary, among the benefits of using mountain pastures are the diverse vegetation, the young phenological stages of plants, high in nitrogen, and low in fiber resulting from the snow line retreating upwards. This allows the animals to follow and graze on high quality pastures during the summer.

Lambs' daily gain during summer on mountain pastures varies and depends primarily on factors affecting available forage quality and stocking rate (19). Nielsen et al. (18) found lambs' daily gain both on lowland and mountain pastures to vary between 0.25 and 0.31 kg d^{-1}. Animalia (20) report average Norwegian White Sheep lamb daily gain during summer of 0.29 kg d^{-1}. This is in the same range as what we found, with an estimated daily gain of 0.32 kg d^{-1}. This figure concurs with Hatten et al. (8) who reported lambs' daily gain between 0.25 and 0.33 kg d^{-1} from islands in Vega archipelago.

A dynamic management plan when using island pastures is important. As the islands are flat, phenological development is uniform across the pastures and the stocking rate should ideally be higher in the spring and early summer than later. During the summer, the lambs' need for high-quality forage increase while at the same time the pasture quality declines, decreased digestibility, and crude protein content. However, the pasture quality can to some extent be maintained if the stocking rate is adjusted during the grazing season. To release and collect the animals at the right time are therefore critical for the production output.

CONCLUSION

In the present study we evaluated lamb performance on three islands with different grazing value and stocking rates. These lambs had a daily weight gain similar to the average weight gain for the Norwegian White breed on a national level. The homogenous topography and low altitude variation on the islands result in a uniform vegetation development and render the vegetation more sensitive to between and within summer climate variation. Adjustment of stocking rate, date of release, and collection of animals must be fine-tuned. With a dynamic and adaptive management strategy, there are high potential benefits for increasing the use of island pastures.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

VL was responsible for study design, data collection, analysis, and interpretation, and was the principle author of the manuscript. ØH was responsible for study design, data collection, analysis, and interpretation. F-AH was responsible for vegetation mapping and interpretation. GS was responsible for statistics, analysis, and interpretation. All authors contribute to manuscript revision and have read and approved the manuscript.

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Grazing Allometry: Anatomy, Movement, and Foraging Behavior of Three Cattle Breeds of Different Productivity

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Modern breeding has formed a multitude of cattle breeds ranging from undemanding, low-productive breeds to high-productive, specialized dairy, or beef cattle. The choice of breed has important implications for farm management, but its impact on pasture vegetation is underestimated. We hypothesized (i) that anatomy, movement, and foraging behavior of cattle are allometrically related on the individual level, (ii) that differences among cattle are not explained by individual variation alone but also by breed, and (iii) that anatomy, movement, and foraging behavior of a cattle breed is related to its productivity. In order to test these hypotheses, we conducted a controlled grazing experiment in which three cattle breeds simultaneously grazed three types of heterogenous, alpine pastures: low-productive Highland cattle (average weight: 358 kg); local, dual-purpose Original Braunvieh (582 kg); and high-productive Angus × Holstein crossbreed (679 kg). We measured body weight and claw base of nine cows per breed after 10 weeks of grazing alpine pastures. Over a period of 9 days, we recorded the step frequency and lying time by pedometer and space use by GPS. Moreover, we visually observed foraging behavior on three occasions per cow. Forage selectivity and quality were calculated for every cow's diet. Allometric relationships were analyzed on the individual level by fitting standardized major axes. For most parameters measured, we detected strong allometric relationships and clear differences among breeds that depended on the level of productivity. The claws of Highland cattle were relatively large compared to their body weight and thus they exerted less static pressure than other breeds. Moreover, the more productive a breed was, the higher its selectivity and step frequency were. For example, Highland cattle foraged shrubs and thistles more frequently than high-productive Angus × Holstein. The latter walked longer distances to select higher-quality forage, while Highland cattle used the space more evenly, visited steeper slopes, and moved further away from water points. Irrespective of breed, vegetation composition influenced cattle behavior: On pastures of low forage quality, animals walked more, foraged more selectively, and used space less evenly. In conclusion, the observed breed-specific differences can be used to improve pasture management and grassland conservation.

Keywords: alpine pastures, cattle breeds, claws, forage selection, GPS, movement behavior, pedometer, species diversity

INTRODUCTION

The domestication of wild aurochses (*Bos primigenius*) created a plethora of cattle breeds (*Bos taurus*) with different characteristics (1). While the aurochs slowly evolved to cope with environmental conditions (2), human breeding decisions enormously accelerated genetic transformation to meet agricultural needs, and adaptation to the natural environment became less important (3). During the mid-nineteenth century, different breeds emerged from pure-breeding, as motivated by ideas of Darwinism, Mendelism, and biometry. In recent decades, genetic improvements facilitated by artificial insemination, quantitative genetics, and molecular markers considerably increased productivity (4). Thereby, traits prioritized by humans, particularly milk yield, body weight, feed intake, and growth rate were enhanced. Records of historical livestock production in Austria indicate that at the beginning of nineteenth century cows weighed about 250 kg and produced 1,300 kg of milk per year (5). Today, specialized beef cattle, such as Charolais or Blonde d'Aquitaine, weigh about 700–950 kg (6), and specialized dairy cows, such as Holstein Friesian, produce up to 12,800 kg of milk per year when fed concentrates (7). In addition to these prioritized traits, which breeding controls, there are numerous characteristics that are not accounted for in selection and have co-evolved unnoticed. Some of these hidden traits recently have gained awareness, such as robustness (8), longevity, and feed efficiency (9), while others, such as claw size, movement, and foraging behavior, have long been ignored in herdbook breeding (10).

Such profound transformations of cattle are likely to have an impact on the vegetation of the sites they graze. Semi-natural pastures, which belong to the most diverse habitats on earth (11), were created by centuries of low-intensity grazing with low-productive animals (12). If the animals that formed these pastures undergo tremendous modifications within a few decades, vegetation may also change. Indeed, in a recent study we identified differences in vegetation when pastures were grazed by breeds of different productivity (13). In order to quantify the drivers of these differences, a follow-up study was designed: Strong changes in body weight, e.g., may exert increased pressure to the ground with negative consequences for vegetation, soil properties, and claw health. Cattle's claws are particularly interesting, because the base that is burdened by animal mass was not considered in breeding decisions and is, therefore, presumably disproportionately underdeveloped. Moreover, higher body weight, growth rate, and milk yield probably altered movement and foraging behavior. If modern cattle walk more, use the pasture differently, or forage other plants than their lower-productive ancestors, this could influence vegetation composition, as suggested by Pauler et al. (14).

Unfortunately, it is not possible to compare modern, high-productive cattle directly to their low-productive ancestors, which grazed pastures centuries ago, before production-oriented herdbook breeding began. However, there are modern, low-productive breeds, such as Highland cattle, which are less affected by breeding: Mason (3) postulated little difference between modern Highland cattle and sculptures of cattle made by ancient Etruscans. While other breeds annually broke records of beef and milk production, the main breeding aim of Highland cattle was to thrive under harsh environmental conditions and on the low forage quality of the Scottish Highlands. Consequently, these animals are lighter and grow more slowly; at the same time, however, they are more robust and less demanding than high-productive breeds (15).

If productivity of cattle has an impact on pasture vegetation, there are far-reaching consequences for habitat conservation of low-productive grasslands, which host many vulnerable and endangered plant species (11, 16). These species may be negatively affected by grazing with high-productive cattle breeds as suggested by Pauler et al. (13): Plant species resistant to selective foraging, such as thistles or shrubs, as well as species adapted to trampling become dominant on pastures of high-productive breeds, and thus biodiversity decreases (13, 17). Moreover, in contrast to Highland cattle, high-productive animals are insufficiently alimented by the forage present in low-productive grasslands (18).

In the present study, we hypothesized (i) that anatomy, movement and foraging behavior of cattle are allometrically related on the individual level, (ii) that differences among cattle are not explained by individual variation alone but also by breed, and (iii) that anatomy, movement and foraging behavior of a cattle breed are related to its productivity. These hypotheses were tested, for the first time, in a controlled grazing experiment on species-rich alpine pastures using three cattle breeds that differ widely in productivity.

MATERIALS AND METHODS

Three Breeds: Low-, Medium-, and High-Productive

We investigated anatomy, movement, and foraging behavior of three cattle breeds, representing a gradient from low to high productivity: The lower end of this gradient was represented by Highland cattle (HC), an undemanding and low-productive traditional breed, bred to thrive in the harsh environmental conditions of the Scottish Highlands, but widespread all over the world. Cattle of medium productivity were represented by Original Braunvieh (OB), a dual-purpose breed traditionally kept in the Swiss Alps, with body weight, and growth rate considerably higher than that of Highland cattle (6). The Original Braunvieh is not to be confused with Brown Swiss, a high-productive, but genetically less diverse dairy breed selected from the same original population (19). The most productive breed in our experiment was Angus × Holstein crossbreed (AH), which combines the strongly muscled, heavy body of Angus beef cattle with the large-framed body and elevated milk production of Holstein dairy cows.

The cows taking part in the experiment were randomly selected from their original herds. All cows were familiar with mountainous grasslands, since they originated from mountain farms and also, had experience grazing high-elevation, alpine pastures in preceding summers. At their home farms, all study animals were fed grass silage and hay only. They were kept in the stable during winter with regular access to pastures in spring. Due to similar previous forage experience and housing conditions, we assumed that breeds experienced similar pre-conditioning. Cows were aged between 2.8 and 10.3 years (HC: 80 months, range: 53–124; OB: 46 months, range: 34–75; AH: 92 months, range: 60–110). We tested all variables for correlations with age, but found only weak relationships (R^2: 0.08–0.31).

Over a period of 2 weeks before the experiment was started, all cattle were allowed to graze the pastures of the study area together to acclimatize to the alpine conditions. For each of the breeds

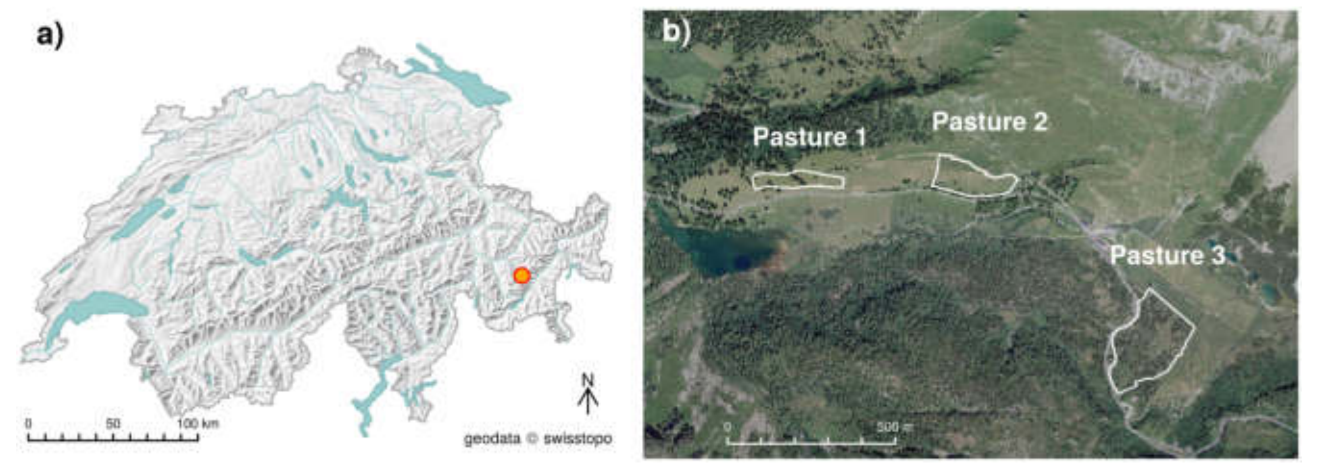

FIGURE 1 | (a) Overview map and **(b)** aerial image of the study area in Swiss Alps with the three pastures grazed by cattle in the experiment.

TABLE 1 | Characterization of the three pastures the cattle were grazed on.

	Pasture 1	Pasture 2	Pasture 3
Description	Nutrient-rich, flat	Heterogeneous, steep with few flat parts, nutrient-poor with few nutrient-rich parts	Steep wood pasture, flat fens, extremely nutrient-poor
Bedrock material	Calcareous	Calcareous	Crystalline
Slope (%)[a]	19.2; 0.3; 56.7	48.1; 1.2; 122.6	25.1; 0.3; 146.7
Size (ha)	1.05 (0.39; 0.39; 0.27)	1.83 (0.70; 0.69; 0.43)	4.38 (1.66; 1.71; 1.01)
Stocking density (LU/ha)	11.4 (12.3; 12.2; 9.8)	6.6 (6.8; 7.0; 6.1)	2.8 (2.9; 2.8; 2.7)
Stocking rate (LU/ha/a)	0.28	0.20	0.08
Forage quality	High (5.9)[b]	Medium (4.6)[b]	Low (2.7)[b]
Available biomass (kg DM)	3,380	2,440	4,860[c]
Vegetation type (**Table 2**)	• Fertile pasture	• Fertile pasture • Mat-grass community • Dwarf-shrub community	• Alpine fen • Larch-Pine forest

Table provides a short description of each pasture; the predominant bedrock; the average, minimum, and maximum slope (%)[a]*; the total size (ha) of the entire pastures and, in brackets, the average paddock size of Angus × Holstein, Original Braunvieh, and Highland cattle; the stocking density (LU/ha) normalized to the metabolic live weight (= weight$^{0.75}$) of a cow of 600 kg, on average and, in brackets, for the three breeds; the total stocking rate (LU/ha/yr); the forage quality relative to the other pastures and as averaged forage indicator value (21); the available biomass (kg dry matter); and the main vegetation type (20).*

[a] *SwissAlti3D, Federal Office of Topography swisstopo, Wabern.*

[b] *Average cover-weighted mean of forage quality indicator value (21) of all vascular plant species within 18 vegetation subplots per pasture, estimated before the first grazing in spring. For details, see Pauler et al. (14).*

[c] *Total standing biomass including woody structures in the herb layer (mainly dwarf shrubs).*

TABLE 2 | Characterization of the vegetation types.

Vegetation type	Association	Dominant plant species
Fertile pasture	*Poion alpinae*	*Trifolium pratense* L. *Trisetum flavescens* (L.) P. BEAUV. *Phleum rhaeticum* (HUMPHRIES) RAUSCHERT *Ranunculus acris* L. *Carum carvi* L. *Alchemilla xanthochlora* ROTHM.
Mat-grass community	*Nardion*	*Festuca rubra* L. *Nardus stricta* L.
Dwarf-shrub-community	*Juniperion nanae*	*Erica carnea* L. *Calluna vulgaris* (L.) HULL
Alpine fen	*Caricion fuscae*	Various mosses *Trichophorum cespitosum* (L.) HARTM. *Carex nigra* (L.) REICHARD *Carex panicea* L.
Larch-pine forest	*Larici-Pinetum cembrae*	*Larix decidua* MILL. *Pinus cembra* L. *Vaccinium myrtillus* L. *Vaccinium gaultherioides* BIGELOW *Juniperus communis* L.

Table provides the main vegetation types in the study area [classification according to Delarze and Gonseth (20)], the scientific name of these plant associations, and their dominant plant species.

studied, three subgroups of three suckler cows and their calves were formed, resulting in a total of 54 animals. The subgroups were developed by ranking cows breed-wise based on specific body weight and joining every third individual (1 heavy, 1 middle weight, 1 light cow per subgroup). Anatomy and behavior were quantified for the 27 cows, but not for the calves.

Study Areas: Three Types of Alpine Pastures

Movement and forage behavior were observed on three types of alpine pastures on Alp Weissenstein in the eastern Swiss Alps (2,026 m asl., 46.5816°N, 9.8002°E, **Figure 1**).

The three pastures differed in plant species composition, forage quality, and bedrock material (**Tables 1**, **2**). We calculated a total forage demand of 1,800 kg dry matter per pasture for all individuals during the experiment. In spring, the pastures already supplied 2,440–4,860 kg dry matter and there was additional regrowth during summer. Pasture size was set to provide excessive forage compared to the estimated forage demand and the actual biomass was measured by a rising plate meter (14). This amount of excess forage made sure that cattle selected plants based on preference rather than being pressured by shortage.

The three pastures were (i) a nutrient-rich, flat pasture, (ii) a steep, nutrient-poor pasture with few flat and nutrient-rich areas, and (iii) an extremely nutrient-poor, steep wood pasture with flat fens (for details, see **Tables 1**, **2**). Each pasture was subdivided into three paddocks with highly comparable conditions (14).

The three paddocks of a pasture were grazed simultaneously by three subgroups—one of each breed. The paddock size was adjusted to the breed to ensure similar stocking density despite the lower body weight and forage demand of Highland cattle (**Table 1**). Thus, additional space was added to the paddocks of Original Braunvieh and Angus × Holstein. Stocking density and stocking rate were calculated by normalizing metabolic body mass (= weight$^{0.75}$) to cows of 600 kg (22).

From the three paddocks of pasture 1, the three subgroups (= three cows plus calves per breed) were transferred to the paddocks in the second, and subsequently in the third pasture. The animals stayed 3–4 days on each pasture. This rotation procedure was repeated three times. Different subgroups and, therefore, different animals were used for each rotation to avoid pseudoreplication. Applying a Latin square design, a different breed grazed each paddock in each rotation. Thereby, each breed visited each paddock once. This procedure resulted in three independent repetitions to account for variation in social behavior, season, and weather. On each pasture, movement and foraging behavior of every cow in the subgroup were observed. During the rotations, the remaining animals of the other two subgroups per breed were kept on another pasture not included in the experiment.

The paddocks of pasture 1 were relatively small compared to other alpine farms (23). This was necessary to define homogeneous paddocks. Larger paddocks would have led to confounding effects due to larger heterogeneity. However, the system was not an intensive grazing system since the number of animals per paddock was small. Moreover, the fast rotation reduced stocking rate while allowing for independent replications with different individuals. In Switzerland, the 465, 000 ha alpine pastures are grazed by 300,000 livestock units (LU) for 100 days (23). This results in an average stocking rate of about 0.18 LU/ha/yr. Hence, with 0.08–0.28 LU/ha/yr, the stocking rate in our study was representative of alpine grazing systems in Switzerland (23, 24) and is applicable for extensive grazing systems.

Assessment of Anatomy: Body Weight and Claw Base

All cows were weighed at the beginning and at the end of the grazing experiment (Weighing System FX15, Texas Trading, Windach, Germany). The body weight after 10 weeks of grazing alpine pastures was used for analysis. The average change in body weight during the grazing period was calculated for each cow.

Two weeks prior to the experiment, the shape and health status of the claws of all 27 cows were inspected by an approved expert and claws were corrected if necessary. At the end of the grazing season, after 10 weeks under similar conditions, the claw base of each cow was measured using the left forefoot and the left hindfoot. Adapting the method of Nuss and Paulus (25) to living animals, we took a picture of the claw base in a scaled frame (**Figure 2A**) and rectified the photograph (software: Office Lens, Microsoft, Redmond, USA). Using the software "Measure pictures" (CAD-KAS Kassler Computer Software, Markranstädt,

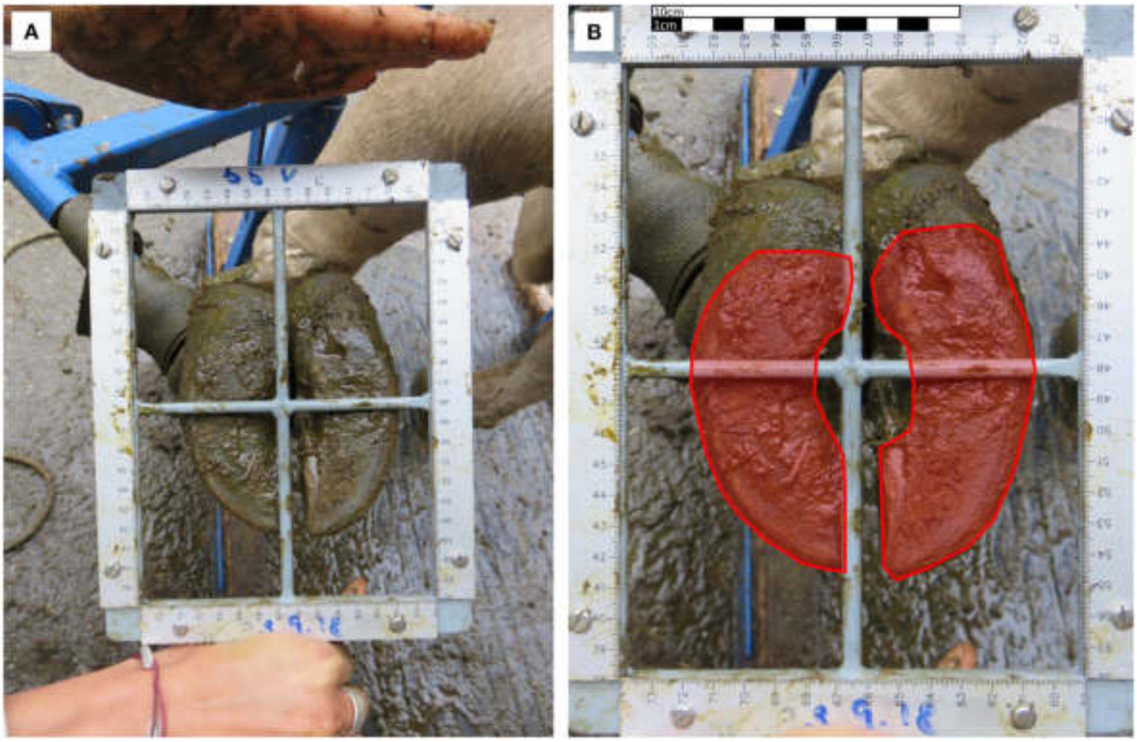

FIGURE 2 | Measurement of claw base: **(A)** Unedited photograph of the ground of a cow's left forefoot with scaled frame. **(B)** The same photograph after rectifying with scale and red polygons, drawn to measure the base of the lateral (on left here) and the medial (on right here) claw.

Germany), we traced the outline of the claw base and calculated the area of this polygon based on the scale included in the picture (**Figure 2B**). Thus, we measured the medial and lateral claws of both feet. Assuming the left claws as proxies for the right claws (26), we doubled the values and summed them. Static pressure to the ground was calculated by dividing the body weight by the summed claw base.

Assessment of Movement Behavior: Pedometer and GPS Logger

Movement behavior of cows was tracked by pedometers and GPS loggers, which recorded data for the entire duration cows were on the study pastures (9–10 days per cow). To quantify movement behavior, we used IceTag pedometers (IceRobotics, Edinburgh, UK). This device is a three-axis accelerometer that uses the force of movement to identify the number of times a cow lifts its leg and records these events as steps. The time the sensor is horizontal is recorded as lying time. A pedometer was fixed at the left hindfoot of six cows per breed for a total of 18 pedometers installed. The step counts and lying time were recorded for each cow in each pasture separately; from these data, average steps per hour and the proportion of time spent lying (lying ratio) were calculated.

In addition, all 27 cows were equipped with collars carrying a box with a GPS logger (Qstarz BTQ1000XT, Qstarz, Taipei, Taiwan) and 3.6 V lithium batteries (27). The GPS loggers recorded movement for the entire duration cows spent on the study pastures; for unknown reasons, however, 3 of the 27 GPS loggers inadvertently stopped recording prematurely. Positions were logged every 15 s, providing information about the distance covered during a certain time span. The median absolute position error of the GPS devices is 3.1 m (27). The accuracy of covered distance measured by GPS loggers was supported by visual observations and checked against the step count of pedometers, which is not GPS-dependent. The values recorded by pedometers and GPS loggers proved highly correlated ($R^2 = 0.90$).

For each cow in each pasture, the average hourly covered distance (i.e., speed in m/h) was computed. Furthermore, in order to find out how often cattle visited different portions of the entire available area of each pasture, we calculated the evenness of space use by counting the number of GPS positions within 5 × 5 m grid cells throughout the entire study time and by calculating Camargo's index of evenness across all cells (28). For all cells, two topographic covariates were calculated: percentage slope based on the swissALTI3D digital elevation model with 2 m resolution (Federal Office of Topography, Wabern) and the Euclidean distance to the water sources accessible within the paddock. The positions counted for each individual in each paddock were regressed against each covariate separately using a linear model with a spatially structured and a random error term, fitted using integrated nested Laplace approximation with prior specifications similar to Homburger et al. (23). Covariates were standardized into z-scores to make estimated coefficients comparable across paddocks and individuals.

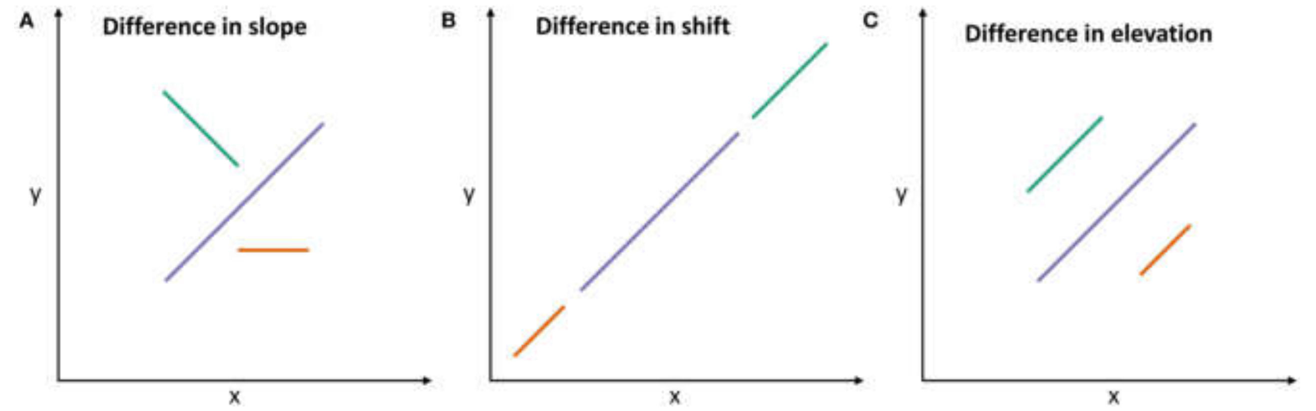

FIGURE 3 | Schematic illustration of differences among allometric lines of three exemplified breeds [adapted from Warton et al. (32)]: **(A)** Allometric lines differ in slope, i.e., the relationships of x and y differ among breeds. **(B)** Allometric lines are shifted along their common slope, i.e., the x and y vary consistently across breeds. **(C)** If allometric lines differ in elevation, they are shifted in parallel to each other, i.e., the values of x differ among groups at similar values of y. The length of allometric lines reflects the data range, but does not affect the allometry.

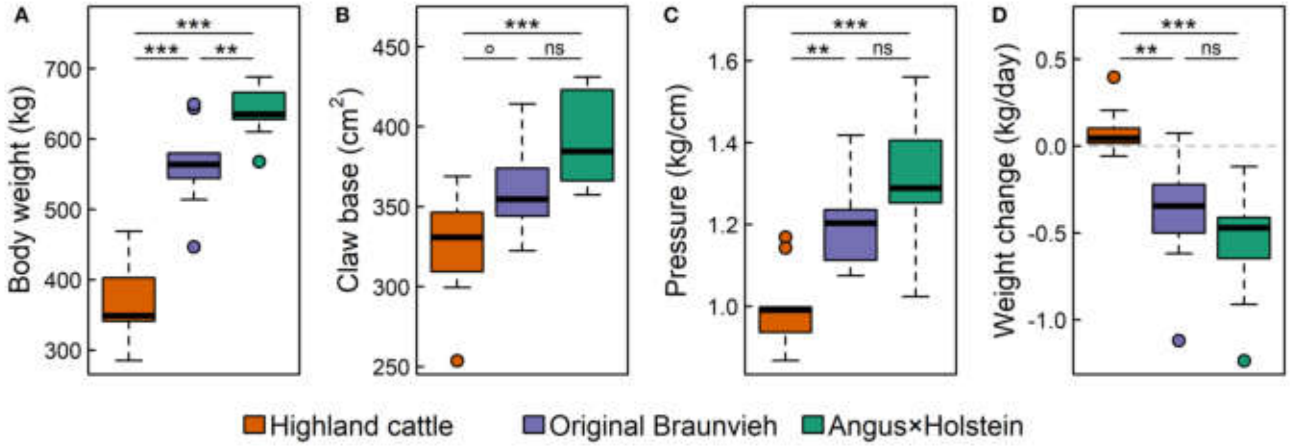

FIGURE 4 | Differences in **(A)** body weight, **(B)** claw base, **(C)** the static pressure of body mass on the ground, and **(D)** the average daily body weight change during 10 weeks on alpine pastures of three cattle breeds: Highland cattle, Original Braunvieh, and Angus × Holstein. Nine cows were measured per breed [box: 25th to 75th quartile range (IRQ); line: median; whiskers: max. 1.5 × IQR; points: outliers; $^{ns}p > 0.1$; $^{\circ}p < 0.1$; $^{**}p < 0.01$; $^{***}p < 0.001$].

Assessment of Foraging Behavior

The foraging behavior was assessed by direct visual observation of the plant species consumed by each cow on 3 different days—one at each pasture type. On each day, every cow was observed foraging for 15–41 min (mean: 26 min), depending on the foraging activity during observation. Before the experiment started, animals were familiarized with to the observer: after a few hours, there was no indication of unnatural behavior and the cows foraged as if they were unobserved. Hence, it was possible to follow the grazing cow in close proximity to the side of the cow's head (from 0.5 to 2 m away). For every second bite, the plant species with the highest share within a bite was recorded. Despite the short distance, it was not always possible to discriminate between some species with similar habitus in the short time available. We therefore combined a few plant species into groups: broad-leaved Poaceae (except *Deschampsia cespitosa,* which was easy to identify, and has much lower forage quality than other broad-leaved Poaceae); fine-leaved Poaceae (except *Nardus stricta*, for which the same applies as for *D. caespitosa*); yellow Asteraceae; *Carex* species; *Trifolium pratense* and *T. repens*; *Potentilla aurea* and *P. erecta*. All other plants were recorded at species level.

Subsequently, we calculated the relative consumption of each plant species or species group per cow and pasture. As a proxy for palatability to cattle, we used the indicator values of forage quality by Briemle et al. (21). The indicator values were multiplied by the relative consumption of all species to estimate the average quality of the consumed forage. For species groups, the relative abundance of the individual plant species within each group in each pasture was calculated based on 186 vegetation relevées (14). In order to reveal how strictly cattle select their forage, we also calculated Pielou's evenness of the selected plant species.

Statistical Analysis: Tukey Range Tests and Allometric Line Fitting

All calculations were conducted in R 3.6.1 (29). Differences among breeds and among pastures were tested using Tukey range tests as implemented in package *multcomp* (30). For movement variables and foraging behavior of each animal in each pasture, tests were conducted on the mean value per animal over all three pastures, as well as on separate mean values for each pasture. In the text, pairwise comparisons between breeds were labeled by the symbol ∼. The effects of paddock size, breed, and their interaction on movement behavior were analyzed by a linear regression model, followed by an analysis of variance.

Allometric relationships on the individual level were estimated by fitting standardized major axes (SMA) using the R package *smatr* (31). SMA is appropriate if there is no causal relationship between two variables x and y, and if x and y differ in variance (32). In contrast to linear regression, SMA minimizes residuals for both axes, not only the y-axis, i.e., both variables are assumed to produce errors. The allometric lines fitted for the three breeds were tested for differences in slope, shift, and elevation. In the case of differing slopes (**Figure 3A**), the relationship between x and y varied among the three breeds. In cases of a difference in shift (**Figure 3B**), breeds differed consistently in the levels of x and y. In such cases, breeds had similar values of x at similar values of y. If allometric lines differed in elevation (**Figure 3C**), the level of the relationship of x and y differed consistently among breeds. In the latter case, breeds had different values of x at similar values of y. For example, in order for the green breed to have a similar elevation as the blue breed, it would have needed to have either larger x or smaller y values.

RESULTS

Differences in Body Weight and Claw Base Among Breeds

The breeds differed significantly in body weight and claw size (**Figures 4A,B**). Highland cattle were the lightest breed on the smallest claw base, followed by Original Braunvieh. Angus × Holstein cattle were the heaviest breed and had the largest claws. However, the differences in the claw base were less distinct than the differences in body weight. Hence, claw base generally scaled with body weight, but there were significant differences among breeds beyond individual effects: Although Highland cattle had smaller claws compared to the other two breeds, their claw base was larger relative to their body weight (**Figure 4C**). Therefore, the static pressure of the body mass on each square centimeter of claw base was significantly lower in Highland cattle than in the other two breeds.

The cattle spent a total of 10 weeks on the alpine pastures, which were relatively nutrient-poor compared to the pastures of their home farms. During this period, Angus × Holstein and Original Braunvieh cattle lost, on average, 0.6 and 0.3 kg weight per day, respectively (**Figure 4D**). With an average positive daily weight gain of 0.08 kg, Highland cattle differed significantly from the other two breeds ($p_{\mathrm{HC}\sim\mathrm{OB}} = 0.002$ and $p_{\mathrm{HC}\sim\mathrm{AH}} < 0.001$, respectively).

Differences in Movement Behavior Among Breeds as Influenced by Pasture Conditions

The number of steps recorded by pedometer and the distance covered per hour showed similar patterns for the breeds and the pastures (**Figures 5A,B**): Original Braunvieh moved most (on average 4.6 km and 2,660 steps per day), followed by Angus × Holstein (4.1 km; 2,510 steps), which differed marginally from each other (steps: $p_{\mathrm{OB}\sim\mathrm{AH}} = 0.86$; distance: $p_{\mathrm{OB}\sim\mathrm{AH}} = 0.02$; displayed in black in **Figure 5**). Highland cattle (3.4 km; 1,880 steps) took significantly fewer steps than Angus × Holstein ($p_{\mathrm{HC}\sim\mathrm{AH}} = 0.04$) and Original Braunvieh ($p_{\mathrm{HC}\sim\mathrm{OB}} = 0.02$), covered less distance ($p_{\mathrm{HC}\sim\mathrm{AH}} = 0.09$; $p_{\mathrm{HC}\sim\mathrm{OB}} < 0.001$, respectively), and spent more time lying than the other two breeds (**Figure 5C**).

The linear regression model demonstrated that paddock size as well as the breed had a significant impact on the steps taken ($p_{\mathrm{breed}} < 0.001$; $p_{\mathrm{size}} < 0.001$) and the distance covered ($p_{\mathrm{breed}} < 0.001$; $p_{\mathrm{size}} < 0.001$), but interactions were not significant (steps: $p_{\mathrm{breed}\sim\mathrm{size}} = 0.72$; distance: $p_{\mathrm{breed}\sim\mathrm{size}} = 0.10$). All breeds were significantly less active on the small, nutrient-rich pasture 1 than on the large, nutrient-poor pasture 3 (steps: $p_{\mathrm{pasture}} < 0.001$; distance: $p_{\mathrm{pasture}} < 0.001$, displayed in gray in **Figure 5**) and spent more time lying there ($p_{\mathrm{pasture}} < 0.001$). However, apart from this general trend, Highland cattle moved least on all pastures. For instance, on pasture 3 Highland cattle took about as many steps (**Figure 5A**) and covered about the same average daily distance (**Figure 5B**) as the other two breeds on pasture 1, where Angus × Holstein and Original Braunvieh moved least.

Furthermore, the evenness of space use differed among breeds (**Figure 5D**): Highland cattle used the pastures most evenly, whereas the space use of Angus × Holstein was more tightly clustered. The latter explored the available area least. There were no significant differences in evenness of space use between Angus × Holstein and Original Braunvieh ($p_{\mathrm{OB}\sim\mathrm{AH}} = 0.2$), but both breeds differed significantly from Highland cattle ($p_{\mathrm{HC}\sim\mathrm{AH}}$ and $p_{\mathrm{HC}\sim\mathrm{OB}} < 0.001$). Similar to recorded steps and covered distance, the linear regression model demonstrated an impact of pasture size ($p_{\mathrm{size}} = 0.005$) indicating that animals spread more evenly across smaller pastures. Thus, the relatively homogeneous, flat pasture 1 was used more evenly than the heterogeneous pastures 2 and 3 (both $p_{\mathrm{pasture}} < 0.001$). However, when taking pasture size into account, the breed effect was more distinct ($p_{\mathrm{breed}} < 0.001$) than the pasture size effect. The interaction of breed and size was insignificant ($p = 1.0$).

Steep slope generally reduced space use, but its impact differed among breeds (**Figure 5E**). Highland cattle avoided steep areas least, Angus × Holstein most clearly ($p_{\mathrm{HC}\sim\mathrm{AH}} < 0.001$). Original Braunvieh took an intermediate position ($p_{\mathrm{HC}\sim\mathrm{OB}} = 0.07$ and $p_{\mathrm{OB}\sim\mathrm{AH}} = 0.001$). On the flat pasture 1, the breeds differed only marginally in their response to slope. On pastures 2 and 3, which offered both, steep and flat areas, Highland cattle differed significantly from the other two breeds (pasture 2: $p_{\mathrm{HC}\sim\mathrm{OB}} = 0.01$, $p_{\mathrm{HC}\sim\mathrm{AH}} < 0.001$; pasture 3: $p_{\mathrm{HC}\sim\mathrm{OB}} = 0.08$, $p_{\mathrm{HC}\sim\mathrm{AH}} < 0.001$).

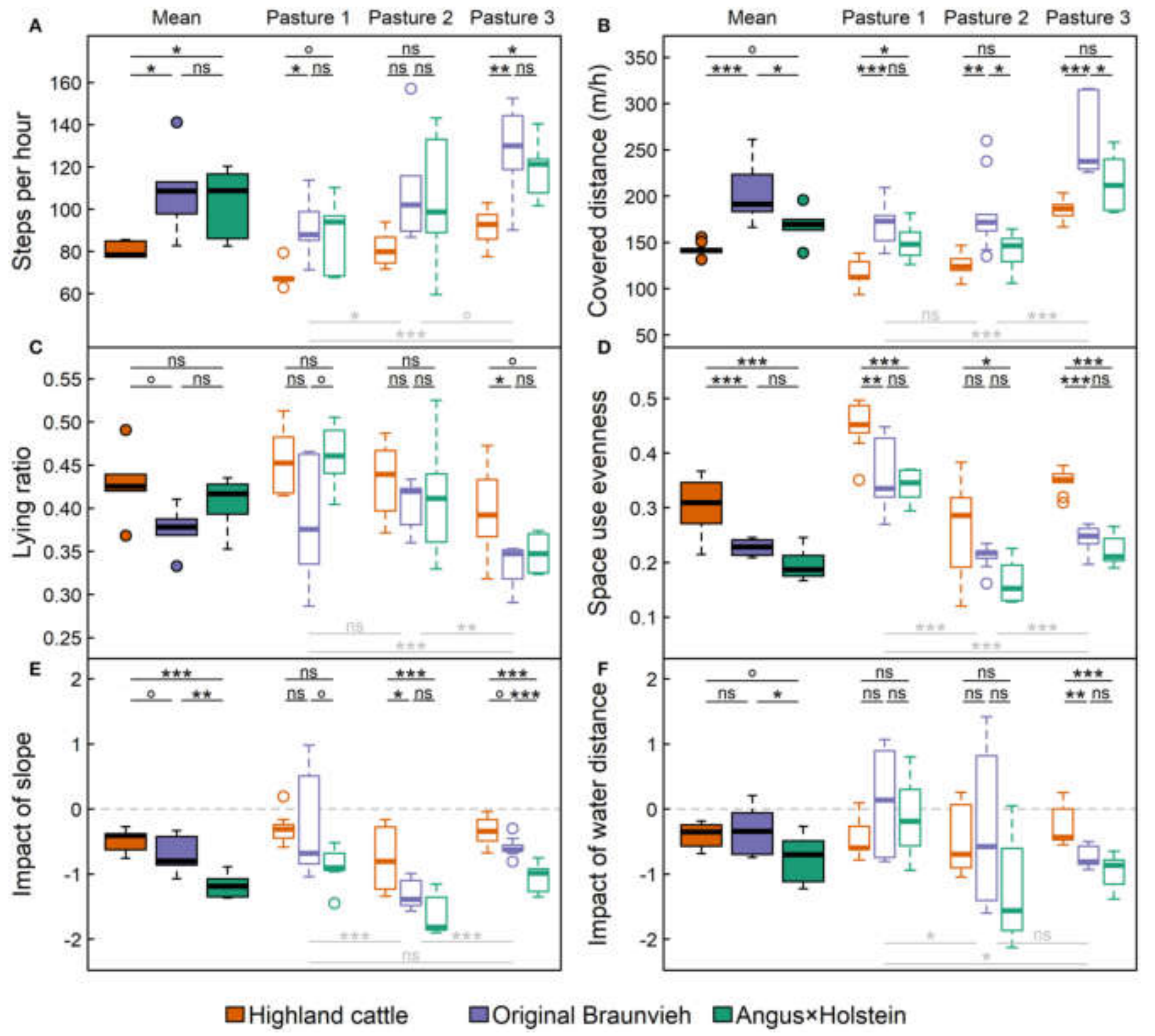

FIGURE 5 | Movement behavior of the three breeds Angus × Holstein, Original Braunvieh, and Highland cattle: **(A)** The average number of steps recorded per hour; **(B)** the average covered distance per hour (i.e., the speed); **(C)** the ratio of the time spent lying; **(D)** the evenness of space use; **(E)** the impact of slope, and **(F)** of the distance to water points on cattle movement behavior. Steps and lying ratio were recorded for six, covered distance and space use evenness for all nine cows per breed. Filled boxplots represent mean values, empty boxplots differentiate by the three types of alpine pastures: (1) nutrient-rich, flat pasture, (2) heterogeneous dwarf-shrub pasture, (3) nutrient-poor fen and wood pasture. Significances of differences among breeds are displayed above the boxplots in black, those among pastures below the boxplots in light gray [box: 25th to 75th quartile range (IRQ); line: median; whiskers: max. 1.5 × IQR; points: outliers; $^{ns}p > 0.1$; °$p < 0.1$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$].

Moreover, the space use of cattle was influenced by the distance to water points (**Figure 5E**). The further a location was away from water, the less frequently it was visited. The impact of the distance to water increased with pasture size: on the small pasture 1, cattle were less influenced by the distance to water than on the largest pasture ($p_{\text{pasture}} = 0.04$). Clearer differences among breeds were observed in larger paddocks. On pasture 3, breeds differed significantly in their response to water distance. Highland cattle moved further away from water than Original Braunvieh ($p_{\text{HC}\sim\text{OB}} = 0.002$) and Angus × Holstein ($p_{\text{HC}\sim\text{AH}} < 0.001$).

Differences in Foraging Behavior Among Breeds as Influenced by Pasture Conditions

We found differences in the evenness of forage selection and the forage quality of selected plant species among cattle breeds,

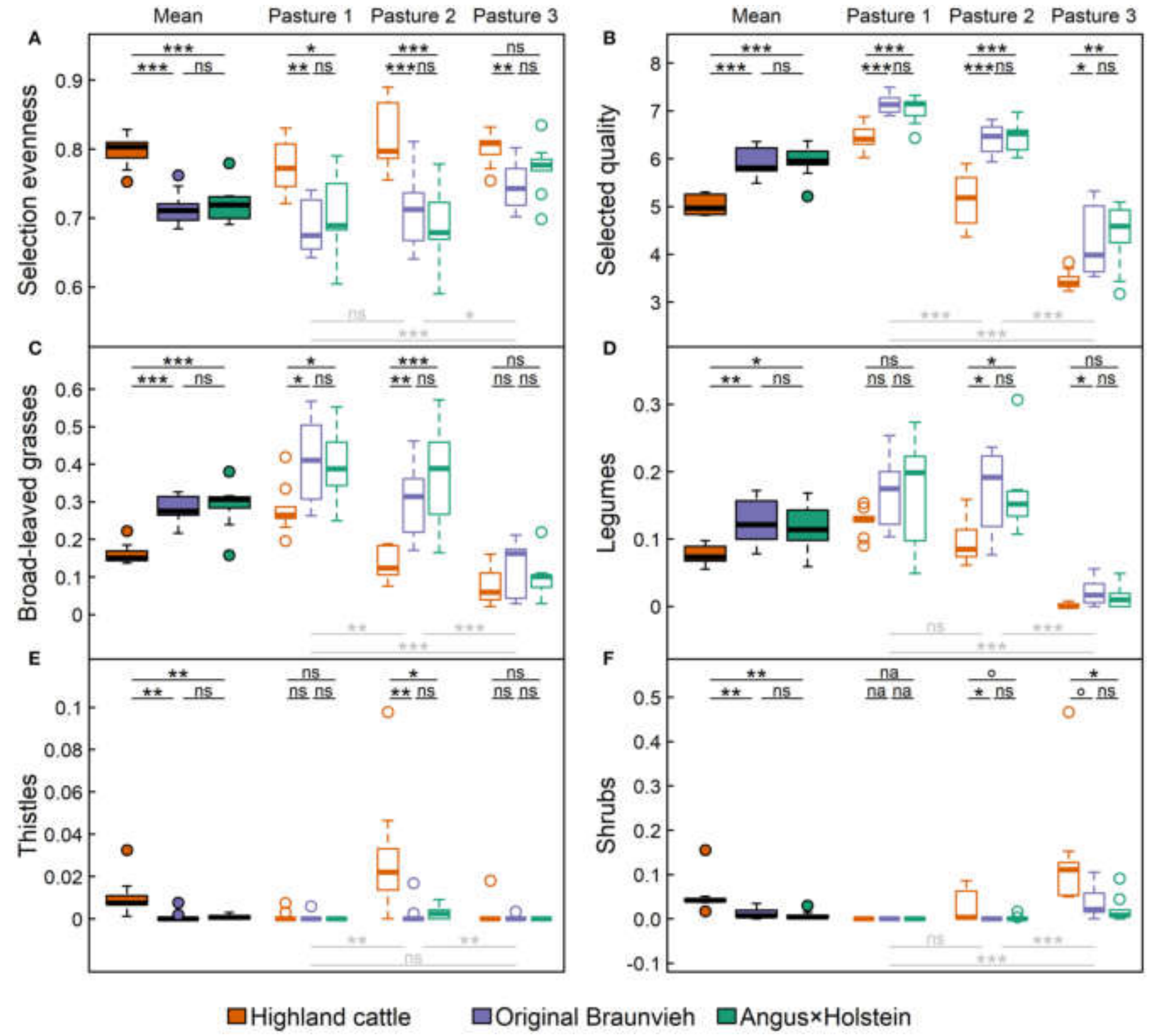

FIGURE 6 | Forage selection behavior of three cattle breeds Angus × Holstein, Original Braunvieh, and Highland cattle. For all the nine cows per breed **(A)** the evenness of forage selection, **(B)** the average forage quality of the selected plants (21), and the share of **(C)** broad-leaved grasses, **(D)** legumes, **(E)** thistles, and **(F)** shrubs within the selected forage plants were measured. Filled boxplots represent average values, empty boxplots differentiate by the three types of alpine pastures: (1) nutrient-rich, flat pasture, (2) heterogeneous dwarf-shrub pasture, (3) nutrient-poor fen and wood pasture. Significances of differences among breeds are displayed above the boxplots in black, those among pastures below the boxplots in light gray [box: 25th to 75th quartile range (IRQ); line: median; whiskers: max. 1.5 × IQR; points: outliers; $^{ns}p > 0.1$; $^{\circ}p < 0.1$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, na, not available].

indicating that different breeds preferred different groups of plants. For all averaged indicators, Highland cattle differed significantly from the other two breeds ($p < 0.02$). In contrast, no significant differences were found between Angus × Holstein and Original Braunvieh for any of the indicators of foraging behavior (p: 0.84–1).

Highland cattle foraged more evenly than the other breeds (**Figure 6A**), as observed in the overall average ($p < 0.001$), as well as in pasture-wise values. Only the evenness of forage selection by Highland cattle in pasture 3 did not differ significantly from Angus × Holstein cattle. Simply put, Highland cattle ate what was available. Thereby, they selected forage with significantly lower quality than the other two breeds ($p < 0.001$; **Figure 6B**). This was also reflected in breed-specific preference and avoidance of certain plant groups. Broad-leaved grasses and legumes were the plants with the highest forage quality in our study area. Angus × Holstein and Original Braunvieh had a stronger preference for these plants than Highland cattle

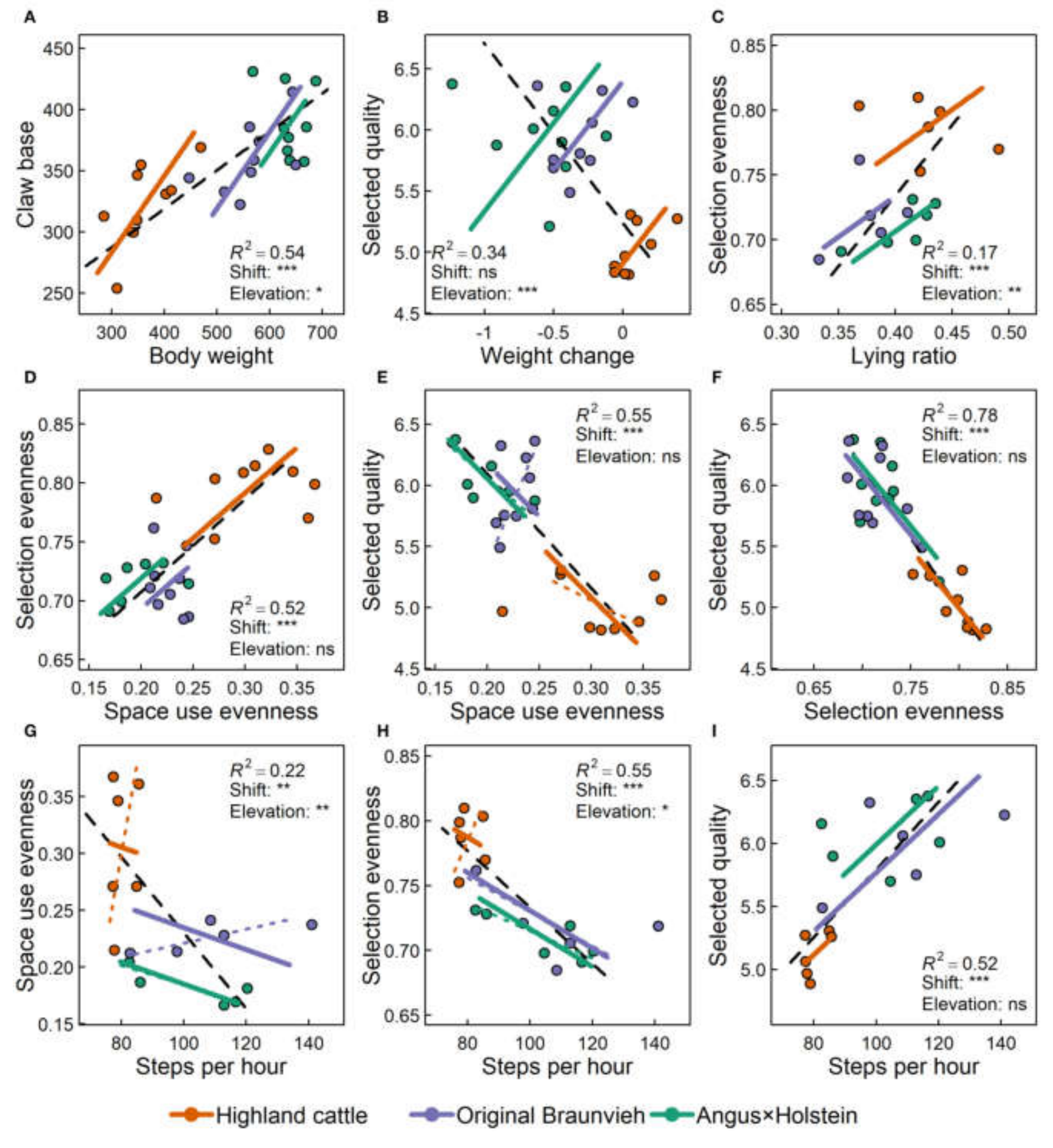

FIGURE 7 | Selected allometric relationships among variables concerning anatomy, movement and foraging behavior of three cattle breeds. Panels **(A–I)** show allometric relationships of body weight (kg), claw base (cm), average daily body weight change over 10 weeks on alpine pastures (kg/d), the average number of steps recorded per hour, the ratio of the time spent lying, the evenness of space use, the evenness of plant species selection, and the average forage quality of the selected plants (21). The number of recorded steps and lying ratio were available for six cows per breed and the other variables for nine cows per breed. Figures show the overall allometric line for all animals (dashed black) with their regression coefficient (R^2) as well as allometric lines for each of the three breeds. For all allometries where the slope differed significantly among breeds, the breed-specific allometric lines are provided (dashed lines) together with the forced common slope (solid lines). This was necessary for testing shift and elevation, for which significances of differences among breeds are given ($^{ns}p > 0.1$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

(**Figures 6C,D**). In contrast, thistles and shrubs had the lowest forage quality and were foraged much less by Original Braunvieh and Angus × Holstein than by Highland cattle (**Figures 6E,F**). Since thistles primarily grew on pasture 2 and shrubs on pastures 2 and 3, differences were only detectable on these pastures.

In addition to breed, the pasture type also influenced foraging behavior: Cattle selected their forage more evenly on the homogeneous, nutrient-rich pasture 1 than on the heterogeneous, nutrient-poor pasture 3 ($p_{\text{pasture}} = 0.001$). In contrast, the quality of selected forage was highest on pasture 1, where plants with the highest forage quality grew, and lowest on pasture 3, where only forage of low quality was available ($p_{\text{pasture}} < 0.001$). Plant groups were grazed most on the pastures where they were most abundant: broad-leaved grasses and legumes were foraged significantly more often on pasture 1 than on pasture 3 (both $p_{\text{pasture}} < 0.001$), thistles were foraged more on pasture 2 than on pasture 1 ($p_{\text{pasture}} = 0.003$) and on pasture 3 ($p_{\text{pasture}} = 0.004$), and shrubs were consumed most on pasture 3 ($p_{\text{pasture}} < 0.001$).

Allometry of Anatomy, Movement, and Foraging Behavior

There were various strong allometric relationships among the variables tested (**Figure 7**), indicating that characteristics are consistently related to one another within each individual. However, most allometries were better explained, when breed was taken into account. As described above, Highland cattle differed from Original Braunvieh and Angus × Holstein in all variables measured, as indicated by a significant shift along the allometric lines (i.e., data clouds in **Figure 7** are shifted along the direction of the lines). In addition to the simple positive or negative relationships, there were numerous effects of cattle breed on the specific allometries itself: We found significant differences in elevation among breeds' allometric lines (i.e., a parallel shift of the lines) for five out of nine allometries investigated.

Body weight and claw base (**Figure 7A**) were highly related to each other ($R^2 = 0.54$). The relationship was similar for all three breeds, as indicated by the lack of significant differences in slopes of the breeds' allometric lines. Thus, heavy animals consistently had larger claws than light animals, independent of breed. However, breeds significantly differed in weight and claw base as indicated by a significant shift ($p < 0.001$) of Highland cattle data along the allometric lines compared to the other two breeds, which did not differ significantly from each other. In addition, not only the position of the point clouds of the breeds along the allometric lines, but also the elevation of their lines differed ($p = 0.01$). Highland cattle had significantly larger claw base in relationship to the body weight than the other two breeds.

There was an overall negative relationship between the average daily change in body weight and the quality of the selected forage ($R^2 = 0.34$, **Figure 7B**): Animals that selected forage of higher quality lost more weight. Taking breeds into account reveals that this is primarily a breed effect, as indicated by the highly significant differences in elevation of the allometric lines ($p < 0.001$) and by the positive relationship within each breed, contrary to the overall negative relationship. In contrast to the other breeds, Highland cattle increased body weight despite low forage quality.

Breed also strongly affected the allometric relationship between selection evenness and lying ratio ($R^2 = 0.17$, **Figure 7C**). In general, animals that selected their forage more evenly, spent more time lying. Forage selection was most even for Highland cattle and they spent the most time lying (shift: $p < 0.001$); however, but relative to the evenness of their forage selection, the lying ratio was low (elevation $p = 0.005$).

Space use evenness showed a positive relationship with selection evenness ($R^2 = 0.52$, **Figure 7D**) and a negative relationship with the selected forage quality ($R^2 = 0.55$, **Figure 7E**), which in turn was negatively linked to selection evenness ($R^2 = 0.78$, **Figure 7F**). Animals that used space evenly also selected forage plants evenly, but they foraged plants of lower quality. Highland cattle used space and foraged most uniformly, but selected forage of lowest quality (shift of all allometries $p < 0.001$).

Over all animals, the average number of steps recorded per hour was negatively related with the evenness of space use ($R^2 = 0.22$, **Figure 7G**). Animals that walked a lot covered less space. However, within each breed, the linkage of steps and space use evenness was less clear, pointing to a breed effect instead of a real allometric relationship (elevation: $p = 0.001$).

Finally, the number of steps recorded had a negative relationship with the evenness of selection ($R^2 = 0.55$, **Figure 7H**) and a positive relationship with the quality selected ($R^2 = 0.52$, **Figure 7I**). Animals that moved a lot, selected their forage plants more strictly and ingested forage of higher quality, irrespective of breed. Highland cattle, the breed that walked least, selected plant species least strictly, and of lowest quality (shift of both allometries $p < 0.001$). The significant differences in elevation ($p = 0.02$) among breeds' allometric lines show that Highland cattle would have foraged more selectively or taken fewer steps, if the relationship of steps and selectivity only depended on the individual.

DISCUSSION

As initially hypothesized, this comparative study of cattle on alpine pastures identified several close relationships among anatomy, movement, and foraging behavior, as demonstrated by allometric line fitting (hypothesis 1). Moreover, a considerable part of the variation among individuals is explained by breed, as indicated by Tukey range tests and by tests for allometric shift and elevation (hypothesis 2). Finally, the gradient of productivity from low-productive Highland cattle to intermediate Original Braunvieh to high-productive Angus × Holstein was consistently reflected in almost all parameters analyzed (hypothesis 3).

Anatomical Differences Among Breeds and Consequences for Animal Health, Soil, and Vegetation

Body weight and claw base were closely related at the individual level: The heavier a cow was, the larger was the area of its claw base. However, breed did also matter: Relatively small claws were measured for the two high-productive breeds compared to those of Highland cattle. Therefore, the static pressure of body mass on every square centimeter of claw base was relatively high for Angus × Holstein, marginally less for Original Braunvieh, and significantly lower for Highland cattle. The similar weight-claw allometry of the two productive breeds goes along with Tuohy et al. (33), who found only small differences in weight-claw allometry between Holstein and Holstein × Jersey dairy cows. The relatively large claws of Highland cattle have been presumed (34), but have never been quantified in a comparative assessment. For this experiment, cows where kept under similar, but not identical housing condition over winter. To increase comparability, they grazed the same grounds over a period of 10 weeks prior to the claw measurement. An explanation for the differences observed among breeds may be that the breeding process increased cattle's body weights to a much larger extent than their claw bases—likely because nobody declared "large claws" as a breeding objective. These differences may strongly affect the animals as well as the pastures they graze.

On the one hand, huge body mass on a small base has the potential to affect claw health and may be an overlooked source of claw pathologies. Previous studies did not find differences in claw health among high-productive breeds (26, 35). However, testing a broader range of productivity, low-productive dairy breeds showed significantly fewer claw diseases than high-productive breeds (36). This may, at least partially, be explained by differences in allometry between body weight and claw base, since less weight burdens each square centimeter of claw. Correspondingly, many Highland cattle farmers reported that they almost never observe claw diseases and rarely need claw trimming or veterinary assistance at their home farms. Unfortunately, the relative frequency of claw diseases in Highland cattle has never been analyzed relative to other breeds.

On the other hand, claw pressure not only has an impact on animal welfare, but also on pastureland. Generally, heavy animals on relatively small claws compress the soil more forcefully, thereby promoting erosion (37). Herbin et al. (38) reported an increase in soil penetration resistance and a decrease in porosity on pastures grazed by heavy animals with relatively small claw base. Accordingly, we found more open ground susceptible to erosion in pastures of high-productive breeds than in those of Highland cattle in a previous study (13). If grazing intensity increases, to which trampling pressure contributes, soil organic carbon decreases, with negative consequences for greenhouse gas emissions (39). High trampling pressure comes along with structural deterioration and compaction of soil (40), whereas water storage capacity and pasture productivity decrease (41). The negative effects of trampling (42) are particularly notable where heavy animals are present on steep slopes (43). In contrast, light Highland cattle with large claws have the potential to minimize trampling-induced erosion effects, especially on shallow alpine soils that benefit notably from light and moderate grazing (44).

Moreover, trampling pressure is a driver of selection and thus affects vegetation composition by promoting plant species well-adapted to trampling (45, 46): (i) Short plants with caespitose, matted or rosette architecture and with elastic tissue are less damaged; (ii) prostrate or stoloniferous species with rooting stems or stolons can regrow from intact parts after trampling; (iii) species with high regenerative capacity can quickly rebuild damaged parts; (iv) early bloomers avoid being trampled by finishing their life cycle before the first grazing in spring. These plant species become dominant under high trampling impact (47). As a result, they are significantly more frequent on pastures of high-productive breeds than on Highland cattle pastures (13). On pastures of heavy animals with relatively small claws, trampling pressure is an important ecological driver of vegetation composition and trampling-adapted plants outcompete less adapted species, resulting in a decrease in plant species richness (13).

Movement Behavioral Characteristics Are Allometrically Related at the Breed Level

Soil and vegetation is not only affected by static pressure, but also by the frequency of trampling and its spatial distribution: The static pressure, as measured in the present study, only applies when the animal is standing, equally weighting all four feet. Since pressure concentrates onto three or even two claws while moving, trampling pressure increases as the cow walks and exerts additional destructive kinetic energy (42). As measured by pedometer and GPS tracking in our study, Highland cattle moved least and slowest (i.e., they covered least distance per time) on almost all pastures. Pressure on vegetation and soil is less intensive and less frequent and thereby, the negative impact of trampling on soil and vegetation described above may be reduced on Highland cattle pastures.

Generally, cattle do not cover available space evenly, especially on heterogeneous alpine pastures (10, 23, 48). It seems logical that animals that walk less visit fewer parts of the pasture and leave most places undiscovered. Yet, the opposite was the case: The fewer steps an animal took, the more evenly it occupied the available space. This unexpected negative allometry makes sense, if the breed effect is considered. Despite their slowness, Highland cattle visited the most distant and steepest places on the pastures. In contrast, Original Braunvieh and Angus × Holstein took many steps, but explored a smaller share of the available area. The sparse flat and nutrient-rich parts of the pastures, where they spent most time, provide plants of high forage quality and smooth terrain, which are both attractive qualities (23, 48), especially for cattle with high nutritive demand and large body size. The data suggest that both productive breeds moved more than Highland cattle, but within a smaller space, in flatter areas and closer to water points. Undemanding Highland cattle gathered less frequently on the attractive, flat parts of the pastures, although pasture size was large enough not to force them to forage on the poorer, steep parts of the pastures far away from water. A more even space use is expected in smaller paddocks and at higher stocking density (49), but Highland cattle spread more evenly than would be expected based on paddock size and stocking. The differences in movement behavior among breeds go along with Spiegal et al. (50), who found a traditional cattle breed visiting more different places than a high-productive breed, which preferred the hotspots more clearly. As Highland cattle spread more evenly, they comply with farmers' ambitions to utilize remote or unattractive parts of their land.

Although paddocks of Highland cattle were about one third smaller, it is unlikely that differences in movement behavior among breeds were caused by paddock size alone: Highland cattle in paddocks of pasture 2 moved less than Angus × Holstein and Original Braunvieh in the smaller paddocks of pasture 1. Moreover, in pasture 3, Highland cattle moved about as much (93 steps and 190 m per hour), and as evenly (Camargo's index of evenness: 0.35) as the other breeds did on pasture 1 (steps: 95 and 91; distance: 150 and 170 m per hour; Camargo's index: 0.35 and 0.34 for Angus × Holstein and Original Braunvieh, respectively), although Highland cattle had nearly three times more space in pasture 3 than the other breeds in pasture 1. If movement were inhibited by paddock size, Highland cattle would have taken more steps, covered more distance and spread less even across pasture 3. Additionally, if the movement were a function of paddock size alone, the breed differences should be expected to diminish with increasing available area. However, the opposite was observed: The breed effect on movement parameters was stronger in the large paddocks of pastures 3 than in the small paddocks of pasture 1. In pasture 3, where the differences were most significant, the three cows of each breeds had access to more than 1 ha pastureland and were thus hardly limited in their movement. Nevertheless, Highland cattle covered the least distance and spread most evenly there. Finally, the linear regression model clearly demonstrated a breed effect that goes beyond the effect of pasture size.

Foraging Behavior Depends on Breeds' Level of Productivity

Generally, animals that used space evenly also foraged evenly, as supported by Bailey et al. (51), and cattle that walked little also selected forage plants evenly. Independent of the breed, an individual cow that spread evenly, grazed many different plants, and took only few steps. This suggests that a highly selective cow needs to cover more distance to find the most palatable plants, while a less selective cow eats what is in close proximity of her mouth, not caring much about the quality. This assumption corresponds with the low quality of the selected forage for those animals that took only few steps. Highland cattle moved the least, thereby foraging most evenly and selecting a diet of lowest quality compared to the other two breeds. Original Braunvieh cattle took an intermediate position, but were much more similar to Angus × Holstein than to Highland cattle. Differences in the quality of the selected forage may be additionally explained by cattle's physical access to steep slopes (10), which typically offer forage of lower quality. While large body mass may hinder high-productive breeds' ability to visit steep areas, Highland cattle can reach them and forage the poorer forage there.

Through modern breeding, Original Braunvieh and Angus × Holstein have been selected for a higher growth rate and milk production than Highland cattle (15). Therefore, they are in need of high-nutritive forage, such as broad-leaved grasses and legumes (21) and move longer distances to reach these plants. In contrast, the lower nutritive demand of slow-growing Highland cattle were covered by forage of lower quality. Thus, they save steps while foraging.

In the long term, the higher selectivity of more productive breeds has important consequences for pasture vegetation (13). Unattractive plants co-evolved under grazing pressure and developed strategies to avoid foraging. Thus, toxic species (e.g., *Ranunculus*, *Aconitum*), plants of low forage quality (e.g., *Nardus stricta*), plants with physical defense mechanisms (e.g., thistles, *Deschampsia cespitosa*), or shrubs are avoided by cattle. The more selectively herbivores graze the more dominant these species become (52, 53). Since they outcompete other plants less-adapted to grazing, plant species richness decreases. Accordingly, significantly fewer plant species were found on pastures grazed by high-selective, high-productive breeds than on pastures of less selective Highland cattle (13).

Interestingly, cattle that foraged more evenly spent more time lying. A diet that is chosen evenly across the pasture contains more fiber-rich plants with higher leaf dry matter content and smaller specific leaf area than a strongly selected diet (14). Fiber increases the ruminal retention time and, hence, the time required to digest the forage (54). Therefore, an animal that forages evenly, selects a diet of lower digestibility and, subsequently, spends longer time ruminating, normally done while lying. Highland cattle that foraged most evenly and selected plants of lowest digestibility, spent the longest time lying due to increased ruminal retention time. In addition to the overall allometric relationship of selection evenness and lying time concerning all individuals, there was a clear breed effect as indicated by the difference in elevation: If the relationship were independent of breed, Highland cattle would have lain even more, indicating that Highland cattle digested relatively quickly with respect to the quality of their forage. This suggests that Highland cattle have a more effective food conversion than higher-productive breeds. A more efficient food conversion of less productive breeds was shown in previous, comparative experiments for beef breeds (18, 55, 56) and dairy cattle (57, 58). Morris and Wilton (59) showed that small beef and dairy cattle are more efficient in weight gain and milk production, respectively, than large cattle. Accordingly, Highland cattle seem to make use of fiber-rich and nutrient-poor forage more efficiently and may, therefore, be better adapted to the harsh environment of alpine pastures than high-productive breeds. As a result, Highland cattle were able to gain body weight, even on the nutrient-poor pastures of our study area, where both of the production-oriented breeds lost weight.

Additionally, the low average temperature of 10.0°C (SD: 5.0°C, range: −2.8 to 21.8°C) in the study area during the study time forced cattle to invest thermal energy. Highland cattle may save energy because of their woollier fur, which provides better insulation than the short fur of Original Braunvieh or Angus × Holstein cattle.

Finally, the positive weight gain of Highland cattle may be promoted by more efficient movement and foraging behavior. By selecting plant species more evenly and consequently moving about one quarter less and lying more, Highland cattle save legwork and kinetic energy. Moreover, they have to move significantly less body mass with each step. Thereby, Highland cattle balance the lower nutrient content of their diet.

Technically, the positive weight gain of Highland cattle could result from a higher dry matter intake. This parameter was not measured, but visual observation indicated rather smaller than larger bites and bite rates for Highland cattle. This goes along with Fraser et al. (56), who found a higher weight gain despite smaller dry matter intake for a traditional breed in comparison to a high-productive beef breed.

The findings of this study suggest that anatomical characteristics as well as movement and foraging behavior depend on the level of breeding intensity. The differences among breeds arose during the breeding process, since the underlying mechanisms of artificial selection do not differ from natural selection: Populations adapt to drivers of selection. The more important a criterion is for the reproductive success, the more clearly the population will evolve with respect to this trait (60). By strictly selecting for milk or meat yield, breeders establish strong selective forces that unintentionally override many traits less focused on. Characteristics that are less important for reproductive success (i.e., breeders do not select for them) are subordinated to stronger drivers. Subsequently, if there is no evolutionary pressure for a certain trait, it will alter or disappear unintentionally (61). If, for example, breeders do not select for efficient conversion of fiber-rich fodder, efficiency becomes a less essential driver of reproductive success and subsequently decreases. Instead, cattle adapt to nutrient-rich and concentrated feed.

Implications for Management, Breeding, and Biodiversity

The general tendency of cattle to avoid plant species of low forage quality (14) and the places where such plants are dominant (23) counteracts pasture improvement and maintenance. To reduce the abundance of weeds and shrubs and thereby maximize pasture use, cattle should ideally forage all plants and evenly visit all parts of a paddock. Usually, alpine grasslands are so heterogeneous that cattle almost inevitably use it unevenly (23). Highland cattle, which grazed most evenly among the breeds investigated, were able to exploit even unattractive plants and places.

The breed differences in space use evenness, in impact of slope, and in impact of the distance to water were most evident on pasture 3, which was more heterogeneous and offered poorer forage quality than the two other pastures in the experiment. This observation emphasizes the benefit of undemanding breeds, especially for grasslands that are unsuited for modern agricultural management (62).

It is indisputable that the production output of Highland cattle is low. Under intensive housing conditions, they cannot compete with the growth rate and carcass weight of other breeds (15). Their real advantage is to cope with unfavorable conditions. This is highlighted by the small, yet existent increase in body weight of Highland cattle during the experiment, whereas the other breeds lost weight due to the poor nutritive supply. Though modern breeds have a higher weight gain potential, they cannot reach it on nutrient-poor pastures. Therefore, grazing such areas with high-productive breeds is economically inefficient due to the loss of body weight. In contrast, Highland cattle, which grow less effective and efficient in intensive farming systems, are still able to create a small output under poor conditions, resulting in a positive cost-value ratio (63).

In this experiment, low-productive breeds were represented by Highland cattle, but there are many other low-demanding and low-productive breeds in most European mountain regions. Among these are Tarentaise, Valdostana Castana, Vosgienne, Hinterwaelder, Grauvieh, Murbodner, Galloway, Dexter, and numerous others. Like Highland cattle, these local breeds are adapted to grazing nutrient-poor pastures and thereby, contribute to sustaining semi-natural grasslands unsuitable for high-productive breeds. Using local livestock also provides cultural ecosystem services by maintaining cultural heritage and genetic diversity of livestock. Although it remains to be tested whether other low-productive cattle breeds behave similarly to Highland cattle, this study demonstrates a strong effect of breeds' productivity on numerous traits neglected by output-oriented breeding. This suggests that other low-productive breeds may also be appropriate for grassland conservation.

Breeders of low-productive cattle are proud of the benefits their animals provide, including high robustness, soil protection, reduction in problematic plant species, increased biodiversity, and a general efficiency even in these low-productive systems. Breeders should bear in mind that these qualities are closely related to the low productivity of this breed. Although it is tempting to modify breeding aims toward higher output, our data suggest that if Highland cattle were bred more productively, many of these benefits would be lost, as has been the case with other breeds. On the other hand, breeders of high-productive cattle may consider differences among individuals as a potential to increase production efficiency (9, 10).

In mountainous regions, pasture biodiversity is not only under general pressure of climatic and socio-economic changes (64–66). The structural changes in modern agriculture have also negatively affected low-productive grasslands: In conjunction with poor forage quality, pastures and meadows that are difficult to manage due to steep slope, too-wet or too-dry conditions become unattractive to farmers of high-productive cattle, because these animals cannot exploit their genetic potential under these conditions, as demonstrated by Bovolenta et al. (67). Therefore, the intensity of management decreases, and pastures are eventually abandoned (68, 69). As a consequence, the rich biodiversity of European mountainous pastures suffers, for example, from the continuous spread of shrubs and wood on formerly diverse and open grasslands (17, 70–72). Although biodiversity conservation has begun to receive increasing attention as an important ecosystem service of alpine pastures (73), not even public financial support for mountain farmers is currently able to halt the abandonment of low-productive pastures (74). An appropriate use of these habitats is grazing with undemanding livestock, such as goats, sheep, or low-productive cattle breeds. There is no need for farmers to change their entire livestock, but some low-productive animals can often be added to existing herds without difficulty, as they are undemanding, not only in forage quality, but also in housing conditions. Incorporation of low-productive cattle breeds is, therefore, a key strategy to use low-productive grasslands efficiently and to conserve their biodiversity.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in the online repository Zenodo https://zenodo.org/record/3707638.

ETHICS STATEMENT

This animal study was reviewed and approved by Veterinary Office of Grisons (authorization GR2018_12). Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

CP, JI, JB, TB, and MS conceived the ideas and designed the methodology. CP and MS collected the data, analyzed the data, and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Management Strategies for Lamb Production on Pasture-Based Systems in Subtropical Regions: A Review

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Sheep production on pasture plays an important role in subtropical climates around the world, with great economic and environmental relevance to those regions. However, this production is much lower than its true potential in subtropical regions, largely due to lack of knowledge of how to feed grazing lambs, and mitigate gastrointestinal parasite infections. Due to weather instability and the high growth rate of tropical grasses, it is difficult to adjust the quality and quantity of feed consumed by lambs. In addition, due to warm, wet weather during spring, summer, and autumn, gastrointestinal parasite infection can be intense on subtropical pastures. Thus, the objective of this paper is to summarize 17 years of research in southern regions of Brazil testing alternative management for sheep farmers under these challenging conditions. Our review indicates that ewes play important roles raising their lambs. Besides protecting and providing milk, they leave a better pasture structure for lamb nutrition. The use of creep feeding and creep grazing are additional alternatives to improve lamb growth. However, feeding supplementation with concentrate can deteriorate pasture quality at the end of the summer–autumn season. Gastrointestinal parasitic infections can be reduced with improved lamb nutrition, although L3 larvae of *Haemonchus contortus* can be present at various pasture heights. This indicates that it is difficult to control L3 ingestion solely by manipulating grazing heights. We summarize important technologies for raising lambs on pasture-based systems to make the best of high herbage growth and minimize intense parasitic infections common in subtropical regions. We discuss research results in light of the latest studies from other ecoregions and climates, although there is a lack of similar research in subtropical regions of the world.

Keywords: sheep, concentrate supplement, pasture management, performance, ewe

INTRODUCTION

Sheep production plays an important role in subtropical climates around the world as exemplified by Australia, Brazil, China, South Africa, Spain, and Uruguay. This occurs both at the subsistence and commercial levels for meat and wool. Today, lamb meat production is growing in importance, emphasizing the need to understand its potential economic and environmental impact within production systems around the world. Subtropical pastures are often characterized by two distinct sets of forage species: one that thrives in the warm season and another completely different group that grows in a cool season characterized by frosts and short-duration freezes. This requires different technology for each season whether the warm- and cool-season forage species share the same pasture at different times or grow in distinct paddocks.

To optimize lamb production sustainably, it is necessary to understand key animal/environment dynamics. In subtropical areas, it is possible to raise lambs with either temperate, tropical pastures or both pasture types. This pasture growth is associated to relatively high rainfall in southern Brazil [1,200–2,100 mm; (1)] distributed along the year. This gives subtropical pastures great potential for low-cost lamb production based on pasture. However, due to weather instability, it is difficult to adjust lamb diet quality and quantity (2) and to control gastrointestinal parasite infection (3, 4). Because of high humidity and temperature, highly productive tropical pasture can meet lamb nutritional needs and generate complex canopy structures that need to be properly understood for sheep production. The challenge is to make the best of pasture growth for raising lambs in this environment.

There are key technical issues related to grazing sheep supplementation, sheep gastrointestinal parasite control, and the economic aspects of sheep production in subtropical regions. These have been studied in the southern region of Brazil. Our objective was to summarize 17 years of research carried out by the Sheep and Goat Production and Research Center (LAPOC) at the Universidade Federal do Paraná and by the Sheep Production Teaching and Research Center (CEPOV) at the Universidade Federal do Rio Grande do Sul. Our results will be compared with those of the most recent studies reported in other ecosystems and climates.

GRAZING SHEEP MANAGEMENT AND SUPPLEMENTATION

Weaning and Concentrate Supplementation

Subtropical areas can support both temperate and tropical pastures in overlapping seasons that provide forage year-round, begging the question "why do producers feed concentrates to sheep?" One possible answer is that, despite the lower costs of pasture-based systems, these can carry high risks. These are associated to the fact that flock managers have limited control of feed production and gastrointestinal nematode (GIN) infestations (3). Efforts to produce younger slaughter lambs (3–6 months old with a minimum live weight of 30 kg) when there is a price incentive and energy deficits during periods of greater flock nutrient requirement also contribute to additional pasture-management challenges.

Feed supplementation can affect grazing sheep in different ways, and the understanding of all the complex variables involved is still not completely known. Detailed reviews about the effects of supplementation on grazing sheep were compiled by Dove (5), Clark and Woodward (6), and Kenyon and Webby (7). However, some main effects of supplementation on grazing sheep in subtropical region can be listed: (1) type of supplement (8, 9); (2) level of supplement (10, 11); (3) type of pasture (8); (4) type of animal (12); and (5) animal feeding system (13). One of the most important feed supplement effects is related to the energy/protein ratio of the animal diet. When energy or protein fails to meet animal requirements, ruminants will only respond to the one that is limiting (14). In addition, one supplement compound can affect the digestibility of other compounds (15). Highly degradable carbohydrate can, for example, decrease fiber digestibility. The amount of concentrate supplied to the animal can also modify lamb performance. Aguerre et al. (10), for example, found that supplementing grazing lambs with greater amounts of sorghum grain resulted in higher ruminal fermentation rates that reduced fiber digestibility and total organic matter intake.

In addition to the supplement quantity and quality, the way it is provided to the animal has an important influence on its performance. Supplementing sheep with grain on pasture can promote a more variable rumen pH than if it is given as a total mixed diet, thereby affecting lamb digestion and growth (10). High feeding rates can also increase the passage rate through the rumen, decreasing overall feed digestibility (15). All these effects can also be influenced by animal genetic characteristics. Amino acid requirement of an animal, for example, is related to its performance level (12). Greater genetic lamb performance potentials require more amino acids than that provided by the milk from the mothers.

In the majority of cases, when concentrate supplement is fed to sheep on pasture, less forage is ingested. The animal will often replace forage with concentrate, but the rate of substitution depends on the amount and type of concentrate ingested (11). According to Garcés-Yépez et al. (16), the more the sheep are supplemented with starch, the higher the forage substitution rate becomes. Supplements with less starch and more digestible fiber promote a less substitution rate. However, this rate varies according to the level of supplement provided to the animals. Garcés-Yépez et al. (16) did not find effect on Bermudagrass hay intake when lambs were supplemented with concentrate at a low feeding level (0.4–0.5% of LW); only when they were supplemented at a higher level (0.8–1% of LW) did forage intake decreased. Ideally, supplement should increase forage intake by maximizing forage digestibility and passage rate; otherwise, it becomes a substitute rather than a supplement. To minimize substitution and maximize forage use efficiency, the first step is to identify what the pasture has to offer and what limits forage intake. Supplement content and quantity can then be tailored to

TABLE 1 | Unweaned lamb slaughter weight and age as well as average daily gain (ADG) of finishing systems on summer pasture from subtropical region of Brazil.

Concentrate supplementation	Pasture	Season	Forage (kg/ha)	Canopy (cm)	Leaves (kg/ha)	Leaf:stem	Lamb (kg/ha)	Slaughter weight (kg)	Age at slaughter (d)	ADG (g)	References
Unweaned and pasture finished											
Exclusively pasture	Tifton-85	Oct-Jan	5,828	27.0	2,130	0.66	14.2	32.0	101	281	(13)
Exclusively pasture	Tifton-85	Nov-Mar	3,247	13.3	1,043	0.69	33.0	32.7	136	135	(19, 20)
Unweaned, finished in pasture, creep supplemented											
1% LW DM/d	Tifton-85	Oct-Jan	3,709	23.9	1,275	0.60	9.75	32.0	105	282	(13)
2% LW DM/d	Tifton-85	Nov-Mar	3,554	14.0	1,049	0.65	34.0	37.3	136	275	(19, 20)

DM, dry matter; LW, live weight.

the flock's need such that it serves as a complement to pasture rather than a substitute (17).

Concentrate formulation designed to supplement pasture-based flocks in subtropical regions should therefore seek to supply minerals, energy, and protein to overcome deficiencies in the forage (18). Otherwise, animal performance and pasture utilization may not meet production goals. One of our studies (13), for example, offered concentrate at 1% of the animal liveweight (LW) to lambs nursing on ewes grazing good-quality tropical pasture (10% crude protein and 55% total digestible nutrients) but did not achieve the expected benefits (**Table 1**). We did not find a performance difference between supplemented and control animals, concluding that the nutrients provided by the pasture and the lactating ewes were sufficient to keep lambs at performance levels equivalent to lambs supplemented at 1% LW. Subsequent studies (11, 20, 21) indicated that, in that type of pasture, lamb supplement must be above 2% LW to induce measurable differences vis-á-vis control animals.

Our studies also focused on lamb supplement effect on pasture canopy structure. Fajardo et al. (11) reported that the level of concentrates fed to lambs to meet NRC (22) recommendations had a deleterious effect on pasture canopy structure when fed during summer–autumn. Offering this supplement during lamb finishing favored forage inflorescence and taller plants. They recommended that supplements fed to lambs on upright tropical grass pastures should be avoided during grass inflorescence if seed production is not a priority. Large bulk grazers mixed with or following more selective sheep might also keep upright grass in vegetative growth since less selective species such as bovids or equids are more likely to ingest fibrous inflorescences (23).

A recent publication described the effect of a lamb feed system on Bermudagrass (*Cynodon* spp.) cv. Tifton-85 sward canopy structure. Silva et al. (24) compared four treatments: unweaned lambs with no supplement, unweaned lambs supplemented with concentrate using creep feeding, weaned lambs not supplemented, and weaned lambs supplemented with concentrate. Weaning caused a greater presence of leaves and stems in all sward strata, which increased when concentrate supplementation was fed to the lambs. Independent of supplementation to the lambs, the systems without weaning fostered a sward structure with a greater leaf/stem ratio, showing that the ewes have an important role of leaving a better sward structure to the lambs. This study also showed, similarly to what was found by Fajardo et al. (11) with an upright tropical grass, that the supplementation of weaned lambs on Tifton-85 had a deleterious effect on sward structure. Concentrate supplementation can reduce the leaf/stem ratio due to the reduction of grazing time and the increase in diet selection for leaves. However, these results observed in the subtropical region are not always found in other ecosystems. Bosing et al. (25), for example, studying grazing sheep performance on the semiarid grassland steppe of northeastern Asia, reported that supplement (250 g/day) prolongs pasture use duration. Contrary to what we observed in subtropical areas, sheep supplementation in semiarid areas allows reduction of stocking rate due to improved animal performance, allowing greater animal LW gain and pasture growth.

Besides favoring performance, interactions between supplement and pasture can increase sheep productivity per area. For example, reduced forage consumption per lamb as a result of supplements can increase pasture carrying capacity and productivity (11). This can be more important to small and medium producers that have limited access to land such that intensifying use of what land they do have may result in economy of scale. However, benefits are accrued more for weaned lambs or aging ewes. For example, in our studies in southern Brazil [(13, 26, 27), **Tables 1**, **2**], creep feeding lambs at 1% LW/day did not increase pasture carrying capacity because their daily forage consumption was negligible vis-á-vis the lactating ewes.

Very few studies have investigated the effect of supplementation and lamb weaning on grassland structure in other subtropical or tropical regions. However, Pullin et al. (29) found that weaning alters the lamb feeding behavior. Lambs that remain with their dams spend less time grazing. In addition, Evan et al. (30), working in a different region and forage species, also agreed with our results that it is difficult to maintain sward height after weaning, particularly toward the end of the grazing period. These studies reinforce our conclusion that early weaning of lambs in subtropical pasture should be avoided. In this scenario, creep feeding, and creep grazing turn out to be important management tools for grazing lambs.

Creep Feeding

The response of lamb nutrition to creep feeding can be affected by different factors such as ewe milk production, level and composition of the supplement, and animal genetic

TABLE 2 | Unweaned lamb slaughter weight and age as well as average daily gain (ADG) of finishing systems on Italian ryegrass pasture from subtropical region of Brazil.

Concentrate supplementation	Season	Forage (kg/ha)	Canopy (cm)	Leaves (kg/ha)	Leaf:stem	Lamb (kg/ha)	Slaughter weight (kg)	Age at slaughter (d)	ADG (g)	References
Unweaned and pasture finished										
Exclusively pasture	Aug-Jan	2,815	17.5	807	1.16	8.4	31.7	105	303	(26, 27)
Exclusively pasture	Sept-Dec	4,395	16.6	979	0.69	9.0	34.0	106	204	(28)
Unweaned, finished in pasture, creep supplemented										
1% LW DM/d	Aug-Jan	2,971	16.3	725	1.24	8.8	32.4	106	294	(26, 27)
2% LW DM/d	Sept-Dec	3,863	14.7	795	0.71	8.0	33.0	90	307	(28)
Unweaned, finished in pasture, creep supplemented										
White clover	Sept-Dec	3,923	15.9	838	0.75		33.2	94	274	(28)

DM, dry matter; LW, live weight.

characteristics. Wilson et al. (31) showed that creep feeding did not affect milk production. However, creep feeding allows the lamb greater energy and protein ingestion and better performance (31, 32). In fact, the effect of creep feeding on ewe milk production should be better studied in a more challenging environment where the ewe nutritional level is deficient. Creep feeding is an important management strategy to improve lamb performance when it provides greater lamb nutrient ingestion (19). However, as mentioned before, this greater response needs to complement pasture quality (17); otherwise, the supplement becomes redundant (13). Monteiro et al. (21) observed greater lamb performance when grazing Italian ryegrass mixed with Tifton-85 if the level of a balanced concentrate provided in creep feeding was above 2% LW. This response also depends on animal genetic characteristics. Lambs selected for greater growth respond more to supplementation in creep feeding (33, 34). Lamb genetics is even more important when different kinds of amino acid are provided in creep feeding. There is a market response only when lambs are genetically more dependent on high diet quality (12). A similar study carried out in South Africa (35) showed that balancing limiting aminoacids through the concentration supplement using creep feed can potentially increase lamb performance. However, the author observed that this is economically viable only in more intense production systems due to high feeding costs.

Greater carrying capacity can apparently compensate, to an extent, for lower average daily gain (ADG) of lambs weaned and finished exclusively on pasture compared to unweaned animals [(13, 26, 27), **Tables 1–4**]. Despite differences in stocking rates partially compensating for animal production decline, weaning may not offer efficient economic compensation. Our studies showed that weaning lambs at around 60 days on pastures can increase losses due to GIN infestation, reaching 20% higher mortality compared to unweaned lambs. Despite greater stocking rates, early weaning on pasture may limit final productivity.

Reduced unsupplemented weaned-lamb performance arises from a metabolic profile that reflects inadequate nutrition, namely, low blood glucose and albumin (36). Once this becomes chronic, animals consume insufficient energy and protein to meet their nutritional needs to reach slaughter weights in a timely manner. This indicates that early weaning for finishing lambs on pasture is not a viable tool. In addition, as mentioned before, we observed that ewes leave a better pasture for the lamb to graze (13), with more leaves and fewer stems than when the lambs were weaned by physical separation. Supplementing lambs post early weaning with a concentrate may rectify this situation. Our research confirms that supplementing lambs is likely needed to compensate for nutritional deficiencies and stress resulting from early weaning (**Tables 3**, **4**).

On cool-season annual ryegrass pastures, increasing concentrate supplement from 1% to *ad libitum* (an estimated 3.2% LW/d) increases lamb ADG and therefore decreases slaughter age vis-á-vis unsupplemented animals (21, 37). Those fed *ad libitum* gain 263 g/day, which meant that they reached their 32-kg slaughter weights at 107 days after birth (DAB), 41 days after weaning (**Table 4**). Depending on economic returns, concentrate supplement could be recommended for finishing lambs if they are maintained on those pastures. When lambs are supplemented using creep feeding, the negative effect of the supplement on sward structure is minimized by the presence of ewes that can regulate pasture regrowth.

Numerous studies (38–44) tested the effect of lamb supplementation by creep feeding on animal performance and GIN infection. They all showed that creep feeding can be used to increase the lamb LW rate of gain and reduce GIN parasitic infection. The supplementation of suckling lambs with creep feed can also improve lamb dry feed intake and rumen development, leading to earlier weaning (45). The earlier a lamb has contact with solid feed, the sooner it will be a fully functional ruminant (46). However, as mentioned before, it is important to be careful that the amount of concentrate provided to the animal complements rather than substitutes nutrients that the pasture provides; otherwise, it would not likely be economically sustainable.

Creep Grazing

Enabling nursing lambs to graze pastures ahead of ewes or allowing them exclusive access to forage banks is called creep grazing. The idea is to allow lactating lambs exclusive access to high-quality forage such as young regrowth, legumes, or

TABLE 3 | Weaned lamb (40 or 60 days (d) old) slaughter weight and age as well as average daily gain (ADG) of finishing systems [providing or not balanced [according to NRC (22)] concentrate supplementation of 2% of live weight (LW)] on warm-season pasture from subtropical region of Brazil.

Supplement/ weaned age	Pasture	Season	Forage (kg/ha)	Canopy (cm)	Leaves (kg/ha)	Leaf:stem	Lamb (kg/ha)	Slaughter weight (kg)	Age at slaughter (d)	ADG (g)	References
Exclusively pasture 60 d	Tifton-85	Oct-Jan	5,670	24.3	1,950	0.66	18.3	32.0	131	107	(13)
Exclusively pasture 40 d	Tifton-85	Nov-Mar	4,170	18.1	1,235	0.61	100.0	21.1	136	57	(20)
2% LW 40 d	Tifton-85	Nov-Mar	4,774	20.0	1,340	0.56	134.0	34.3	136	152	(20)

DM, dry matter; LW, live weight.

TABLE 4 | Weaned lamb (40, 42, or 60 days (d) old) slaughter weight and age as well as average daily gain (ADG) of finishing systems (providing or not balanced [according to NRC (22)] concentrate supplementation of 1%, 2% of live weight (LW) or *ad libitum*) on cool-season Italian ryegrass with and without supplements from subtropical region of Brazil.

Supplement/ weaned age	Season	Forage (kg/ha)	Canopy (cm)	Leaves (kg/ha)	Leaf:stem	Lamb (kg/ha)	Slaughter weight (kg)	Age at slaughter (d)	ADG (g)	References
Exclusively pasture 40 d	Aug-Jan	2,900	20.9	1,182	1.08	31.5	31.5	160	115	(26, 27)
Exclusively pasture 42 d	Aug-Jan	3,226	19.3	1,101	0.54	45.3	32.0	198	69	(26, 27)
1% LW 42 d	Aug-Jan	3,794	19.4	1,241	0.50	29.7	32.0	153	106	(26, 27)
2% LW 42 d	Aug-Jan	3,584	19.6	1,153	0.49	36.5	32.0	137	151	(26, 27)
ad libitum 42 d	Aug-Jan	3,589	22.5	1,198	0.51	54.5	32.0	107	263	(26, 27)

DM, dry matter; LW, live weight.

other highly digestible, protein-rich plants that ewes cannot reach. In a trial using the legumes *Medicago sativa* and *Lotus corniculatus*, creep-grazing lambs gained 223 g/day, 38.5% more than unsupplemented control animals and the same as those fed with a supplement (47). In these situations, supplement ideally does not substitute for dam milk but, rather, complements it (48).

In New Zealand, creep grazing with protein-rich forage species such as ryegrass/white clover is used to suppress GIN in lambs (49). Sykes and Coop (50) explained that protein supplied through pasture affects sheep ability to respond to infection and may be a useful tool to minimize dependence on chemical methods of parasite control. Such targeted creep grazing might be useful in suppressing GIN in warmer subtropical climates, such as southern Brazil, during lamb finishing. In research at LAPOC, Salgado et al. (51) evaluated GIN infection and body condition score (BCS) of lambs in different production systems. Unweaned lambs allowed to creep graze (free access to *Trifolium repens*) and creep feeding (2% of LW/day of a balanced concentrate) had the best performance. Lambs weaned at 60 days on pasture with no supplement had the lowest performance (live weight gain, FAMACHA, and BCS). Endoparasite infection control and the nutritional status of the lambs were positively influenced by the production system, mainly when they were not weaned and/or received concentrate supplementation on pasture.

The importance of creep grazing for lamb production worldwide has been recognized for more than 60 years (52). Creep grazing has been mentioned not only as an additional feed (53) but also as a management tool for controlling parasitic infection in lambs (54). However, very few studies have been carried out in subtropical regions with grazing ewes using tropical grass species. Our research indicated that this technique can be very useful not only to improve lamb ADG and parasitic control but also to provide high-quality forage in periods with low pasture availability such as between winter and spring periods in the subtropics [(28), **Table 2**]. That period typically has cool-season forages in decline with slow initial warm-season pasture growth. This period is also associated with the need for good-quality forage because of potential lamb development, a reflection of the autumn breeding period (28).

Our research (28) demonstrated a potential for using creep grazing to finish lambs during periods in which forage quantity and quality do not meet animal requirements. In creep grazing areas, clover herbage mass reached 2,500 kg DM/ha with 20% crude protein and 75% total digestible nutrients, 25 and 10% greater, respectively, than the Bermudagrass pasture in which ewes grazed. The ADG of creep grazing lambs was similar to those lambs fed with 2% LW in concentrate formulated according to NRC (22) and superior to that obtained by unsupplemented lambs (**Table 2**). Creep grazing also reduced the negative seasonal effects of spring forage slump. Despite the demonstrated potential of clovers for creep grazing lambs, this management tool is not utilized in many tropical and subtropical regions around the world (55).

Stivari et al. (56), at LAPOC, observed a similar initial economic response between creep grazing and concentrate supplementation. Although there is an initial cost of setting up additional fencing for creep grazing, lambs will eventually pay more economic dividends over time. This return may vary with each pasture system and the cost of concentrate vis-á-vis fencing, fertilizer, and seed.

Gastrointestinal Parasites

In subtropical regions, sheep often face health challenges while on pasture, including gastrointestinal nematodes (GINs), such as *Haemonchus contortus*, ingested as larvae on forages (57). Humid, warm-climate pastures offer favorable growth conditions for several GIN species that develop and survive to the infective L3 stage (58). Controlling pasture height to limit GIN L3 ingestion by sheep might not control the infection under these subtropical conditions as well as it does under temperate pastures and mixed cattle-sheep grazing (59, 60). In a study developed at CEPOV, Tontini et al. (3) found infective larvae L3 of *Haemonchus* spp. at different heights of tropical upright grass, from soil to the leaf tip, during summer–autumn. Similar results utilizing tropical grass species were also found by different authors (61–64) proving that sward height control has limited action against parasite infection in tropical pastures. Adjusting pasture height may be more important for temperate conditions (65, 66). Santos et al. (64) explained that high rainfall and air temperature favors the migration of L3 from feces to grass blades. In contrast to tropical pasture grown in a subtropical region, Pegoraro et al. (67) assessed the number of L3 GINs in a cool-season pasture of Italian ryegrass at CEPOV in southern Brazil and found that most of the L3 were below 5-cm height, and low sward height resulted in greater L3 GIN intake by sheep.

Rotating sheep through pasture may interrupt GIN reproductive cycles; however, rest intervals needed between grazing periods may be too long to be practical. This can reduce parasitism in a temperate climate but comes with an overall negative effect on animal performance, including less ADG (68) and sometimes no economic advantage (69). Some GIN larvae hatch and develop into infective L3 within days of deposition and can survive for up to 9 weeks in warm conditions but longer in cooler weather (70) and climates (71). Almeida et al. (72), working with *H. contortus* in humid subtropical pastures in southern Brazil, found that 322, 350, 294, and 182 days were required for *Urochloa decumbens* pasture to be L3-free in autumn, winter, spring, and summer, respectively. Such long rest periods, especially in subtropical and tropical regions, will likely result in poor herbage quality due to rapid plant maturation once sheep are rotated back onto pasture. Shorter rests, normally 21 to 28 days, result in better animal nutrition, but most warm-climate studies indicate that rests of 31+ days are required to even begin lowering GIN L3 viability (73, 74) and 182 to 350 days, depending on season, to completely free the pasture of infection (72). Research by Smith et al. (75) indicated that, if rotations are short, continuous grazing may result in lower GIN infection because grazers are allowed to selectively feed farther from feces in pasture.

As already mentioned, several studies have shown that creep feeding and creep grazing can be an important tool to reduce lamb GIN infestation and can be very useful for subtropical regions (51). In addition to high nutrition demand of lambs and the favorable environment for pasture contamination, lactating ewes are also an important source of infective larvae (76, 77). In fact, these tools can potentially help lambs overcome this challenge through the improvement of animal nutrition and immunity (78). Therefore, creep feeding and creep grazing can be one of the best management strategies for reducing the lamb GIN in subtropical regions.

Economic Aspects of Sheep Production Based on Pasture in Subtropical Regions

Economic evaluation of Brazilian lamb finishing systems carried out at LAPOC (79, 80) confirms that lamb finishing based on pastures generally provides greater returns than when feed concentrate is used, especially when lambs are not weaned early. Stivari et al. (56) found that nutrition is the production factor that most influenced lamb-finishing costs on pasture, independent of supplement strategy. In another study at LAPOC, Stivari et al. (81) compared six scenarios to evaluate the economic feasibility of creep grazing or creep feeding finish lambs. They compared forage allowance of 12 or 8% LW DM/day and the percentage of *T. repens* supplement pasture area (30–50% relative to the primary pasture area). The creep grazing finishing system with 8 or 30% of *T. repens* as well as the creep feeding system (concentrate fed at 2% of LW/day/ha) with 8% LW of forage allowance promoted the best short-term economic results.

Research efforts in southern Brazil's subtropical regions have focused on developing techniques to finish lambs on Tifton-85 and *Panicum maximum* cultivar IZ-5 (common name: Aruana grass) summer pasture (**Tables 1**, **3**). Efforts in winter pastures have focused on Italian ryegrass [(26–28, 79, 80), **Tables 2**, **4**]. The results of these studies summarize the importance of pasture system to lambs, whether they are weaned or not. When lambs were still nursing, ADG reached 190 g at 124 DAB on tropical grasses and 226 g at 115 DAB during winter with Italian ryegrass. These were superior ($P \leq 0.05$) to weaned lambs that gained 87 g ADG on the same pasture. Nursing lambs reached target slaughter weight (32 kg LW) at 117 DAB, similar to lambs fed in confinement (21). Considering the favorable results from unweaned lambs, this simple technique has the potential to lower costs while maintaining productivity and animal well-being resulting from lower lamb stress (19). However, in a system without weaning and 8 months between lambing and slaughtering, ewe recovery time may be too short prior to the next breeding period. In this situation, forage quantity and quality become even more important.

CONCLUSIONS

Besides providing milk and reducing weaning stress, grazing ewes can leave a higher-quality pasture canopy structure for lambs. The amount and type of concentrate supplemented to the lambs can also compensate for lower herbage quality, regardless of canopy structure, and can be adjusted according to pasture characteristics. In turn, concentrate supplementation can indirectly deteriorate pasture quality when flocks consume less roughage. Creep feeding and creep grazing in subtropical regions can also be important alternatives for improving lamb growth and GIN parasitic control.

GIN control in sheep, especially lambs, continues to be a challenge in subtropical regions. In these warm, high rainfall climates, resting pastures between grazing cycles to reduce GIN infection may take too long to be economically viable. However, *H. contortus* L3 larvae presence at various pasture canopy heights indicates that it is difficult to control their ingestion by lambs solely by manipulating grazing heights. Instead, deleterious effects of gastrointestinal parasitic reinfection from pasture can be reduced with improved lamb nutrition.

AUTHOR'S NOTE

The studies summarized in this manuscript were developed by Animal Science Graduate Programs of two universities in Brazil: Universidade Federal do Paraná and Universidade Federal do Rio Grande do Sul.

AUTHOR CONTRIBUTIONS

Every author had important contributions on this manuscript. The first CP and the second authors AM are the head of the research projects, being responsible for all parts of the studies. The third author is a researcher TD, and the fourth, fifth, and sixth authors FA, JPM, and LB are Ph.D. students who developed the research review. The seventh author JPM is a professor who contributed to manuscript configuration and publication. All authors contributed to the article and approved the submitted version.

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Ingestive Behavior of Young Lambs on Contrasting Tropical Grass Sward Heights

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The efficiency of grazing ruminant production systems is directly associated to the animals' ingestive behavior, and to structural characteristics of the pastures. The objective of this study was to evaluate the ingestive behavior of young lambs grazing three different heights of Capim Aruana (*Panicum maximum*). The experiment was carried out in two consecutive years, in which 30 tester lambs (4–5 months old) were equally divided into three paddocks (treatments) corresponding to different average sward heights of Aruana grass: (1) Tall-75 cm; (2) Medium-50 cm; and (3) Short-25 cm in a randomized block design. Ingestive behavior assessments were carried out every 28 days through 10-min observations of the main activities of the animals (grazing, ruminating, idling) and biting rate, from sunrise to sunset. In addition, the productive and qualitative characteristics of the pastures were assessed. Despite differences in pasture structure, grazing time (GT) and idling time were similar among treatments ($P = 0.4266$ and $P = 0.2939$, respectively). The shortest ruminating time (RT, $P = 0.0181$) was recorded in the treatment of lowest sward height. Lambs grazing on this treatment also showed 23% more bites per minute ($P = < 0.0001$) than animals in the Tall and Medium treatments. A Decision Tree analysis was performed for GT, identifying in a hierarchical order that the initial weight of the animals and sward height explained 62% ($R^2 = 0.621$) of the variation, representing the variables with the greatest influence on GT. Initial body weight explained 48% of the model. Thus, our research shows that the different sward heights of Capim Aruana mainly alter the lamb's RT and biting rate, and that the animals' initial body weight is a key factor influencing GT, given that this variable makes lambs more susceptible to changes in sward height.

Keywords: decision tree, grazing time, Capim Aruana, height, biting rate

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INTRODUCTION

Pasture production systems represent a significant opportunity for increasing the sustainable production of ruminant animals worldwide. Under this scenario, animal performance depends of sward attributes such the quality and quantity of forage harvested during the grazing process (1). For instance, the ingestive behavior of young lambs kept on tropical pastures may be influenced by the different structures of swards at which they are exposed during grazing.

Sheep are highly selective animals, a trait that differentiates them from other, larger herbivores (2). This characteristic of selectivity inherent to the species is even more important in young lambs, as they progress through complex feeding periods and behavioral transitions. In addition, lambs are positioned at an optimal point on their growth curve, as they display high levels of intake and performance rates during this period (3). Because of these specific characteristics, it is necessary to understand the ingestive behavior of animals during this critical period, as it is one of the aspects that determines performance. In animal production systems, feeding is one of the most limiting factors for obtaining good results in productive performance (4). The way in which the forage is available to the animal is known as forage structure, which is responsible for the amount of nutrients ingested during the grazing process (5). According to Silva et al. (6) the structural characteristics of forage plants directly interfere with the ingestive behavior and performance of grazing animals, which, in turn alter the morphological (height, mass, and density) and physiological (photosynthetic rate and phenological stage) characteristics of the forage canopy, modifying subsequent animal and plant responses to grazing.

Tropical grasses are characterized by their high structure and growth rate (7, 8). These characteristics become a relevant issue when we think about the use of these forages for lamb production. Lambs are relatively small animals and seize food with their lips. In tall pastures with leaves above the animals' heads, lambs need to harvest practically leaf by leaf during the grazing process (5). The maintenance of the pasture structure is an important point to be analyzed when only this category is used in the pastoral system, since the growth rate of these pastures may be greater than the pasture harvesting capacity of young small ruminants. Consequently, there will be an accumulation of the most fibrous components of the sward with decreased nutritional quality of the forage on offer. For these reasons, proper management of the structure (height) is important to allow the best use of tropical grasses by young weaned lambs.

The ingestive behavior of grazers in temperate pastures is already well-known and described as the linear relationship between the decrease in height of the forage canopy and the increase in grazing time (9). Nevertheless, knowledge on such interaction is still scarce for tropical forage species and small ruminants. In order to obtain greater efficiency in the production systems of grazing ruminants, it is essential to know the animals' ingestive behavior and its relationships with forage structure. In addition to forage structure, it is necessary to understand how the nutritional composition of forages interact with their structural characteristics to influence foraging behavior and animal performance. Therefore, the objective of this study was to evaluate the ingestive behavior of young lambs grazing Capim Aruana (*Panicum maximum* cv. IZ-5) of different structures.

MATERIALS AND METHODS

Experimental Proceedings

The experiment was conducted during two consecutive years at the Experimental Agronomic Station of the Universidade Federal do Rio Grande do Sul, located at 46, Eldorado do Sul, Brazil—Latitude 29° 13 '26 "S, Longitude 53° 40 '45" W. The climate is subtropical humid "cfa" according to the Köppen (10) classification. The cfa classification is characterized by hot summers with temperature averages over 22°C in the hottest month and well distributed rains (11). The experiment was carried out for 56 days during the summer in the years 2018 and 2019 (between January and March). Before this experimental period, an adaptation period of 7 days was performed to familiarize animals to their environment.

The treatments were characterized by different aimed structures of Capim Aruana (*Panicum maximum*), represented by different pasture heights: (1) Tall Treatment −75 cm of average height; (2) Medium Treatment −50 cm of average height; and (3) Short Treatment −25 cm of average height. To maintain the different pasture structures, strategic mowing was carried out before each experimental period. The pasture was mowed at 5 cm of residual height in all experimental paddocks, performed at different times before the beginning of the experiment (Tall −4 weeks; Medium −2 weeks; Short −1 week before the beginning of the experiment).

For experimental evaluations, 30 young weaned tester lambs with an average age of 4–5 months (at the beginning of the experiment), were used in each year of the study. Lambs were randomly distributed across groups and pastures, considering the variation of gender (female and castrated male) and weight, resulting in a uniform distribution of animals within each treatment group ($N = 10$ lambs/group). A continuous grazing method was used and all treatments had a 12% herbage allowance [12 kg total dry matter (DM) per 100 kg of animal bodyweight (BW)/day] adjusted in the day 1 of the experiment and every 28 days using the "put and take" technique (12). According to this technique, there were two groups of animals, one called "testers" that grazed continuously and showed the effect of the treatments, and another group name "put-and-take" lambs used only to maintain the sward height and regulate forage allowance. The lambs had access to shade, and water and mineral salt in *ad libitum* amounts. The average initial weight of the animals was similar between treatments (21 kg, $P = 0.9401$). Lambs were weighed every 28 days with a previous 12 h fasting of solids and liquids.

Pasture Assessments

Sward height was checked every 7 days using random sampling, using a 1.5-m sward stick (13), taking measurements on 52 random points for each paddock, measuring the highest point of the leaf from the ground. The forage structure is composed not only by height, but also by density, forage mass and plant stage. Height, however, is a measure of high correlation with the forage structure and easy to measure, allowing for a high number of measurements during weekly intervals.

Evaluations to estimate herbage mass were carried out on day 1 of the experiment and every 28 days thereafter using a 0.25 m^2 frame, totaling six sample points per treatment, three at the average pasture height and three at random. These samples were cut close to the ground, collected and weighed. The samples were homogenized, and two sub-samples were taken, one for determining the percentage of dry matter (DM), and another for botanical separation in leaf blade, stem + sheath, inflorescence, other grasses, other legumes, other species and senescent material. The separation of the plant's morphological components allows for the calculation of the leaf: stem ratio, which was the main variable that characterized the pasture. After botanical separation, all subsamples were placed in a forced air oven at an average temperature of 60°C until constant weight, when samples were weighed on a 0.1-g precision balance.

The daily forage accumulation rate was measured every 28 days, using three grazing exclusion cages per paddock, according to Kinglmann et al. (14). The objective of the evaluation was to measure the daily rate of pasture growth, enabling subsequent calculations of forage supply and adjustment of stocking rate. The daily forage accumulation was estimated by the difference between the sample cut inside the cage in the present period, and the forage mass cut in the previous period outside the cage, divided by the number of days in the period.

Forage samples were collected every 28 days using the grazing simulation technique (15) to assess the nutritional quality of forages. Bromatological analyses of forage samples were made to estimate the contents of dry matter (DM, method n° 930.15), mineral matter (MM, method n° 942.05) and crude protein (CP, method n° 984.13), according to the AOAC methodology (16). The analysis of apparent *in vitro* digestibility of organic matter (DIVMO) was performed according to Tilley and Terry (17). The neutral detergent fiber (NDF) concentration was analyzed according to Van Soest et al. (18), while acid detergent fiber (ADF) and acid detergent lignin (ADL) according to Goering and Van Soest (19). Determinations of insoluble nitrogen in neutral detergent (NIND) and insoluble nitrogen in acid detergent (NIAD) were also carried out according to the methodology described by Licitra et al. (20).

Ingestive Behavior Evaluation

The assessment of ingestive behavior was performed with continuous notes during the day (from sunrise to sunset) every 10 min by trained people using the method described by Jamieson and Hodgson (21). The observations were performed only during daytime because most of the grazing activities of ruminants occur during this period (22–25) and nocturnal observations were not possible due to the difficulties to visualize the animals in tall pastures at night. In addition, the effect of sward height could certainly be visualized during the day. The animals were individually identified with fabric collars, in which each animal in the paddock received a collar with a different color. The activities of grazing, ruminating and idling were recorded individually for each tester animal. These assessments were carried out every 28 days. The temperature and relative humidity of the air were also measured.

The ruminating time (RT) was considered the period when the animal was not grazing but when it was chewing the ruminal bolus. The idling time (IT) represented the period when the animal was neither grazing nor ruminating. Grazing time (GT) was the period where the animal was actively grazing or selecting forage, including the period used for displacement during selection of the diet. Within the 10 min of GT assessments, the biting rate was recorded using the "20 bites method" described by Forbes et al. (26), which counts the time spent by the animal to take 20 bites.

Meteorological Data From the Trial Period

In the first year of the experiment (2018), the average temperature was 23.2°C, 75.6% average relative humidity and 106.1 millimeters of rain during the experimental period. In the second year of the experiment (2019), the average temperature was 24.1°C, 74.8% relative humidity and 47.9 millimeters of rain. In the first behavioral assessment in 2018, the daily average temperature was 22.7°C and in the second, 19.7°C. In the year 2019, the average daily temperature in the first assessment was 25.5°C and in the second 26.3°C.

Statistical Analysis

The experimental design used was randomized blocks, in which each year represented a block. Animals were considered the experimental units for variables related to ingestive behavior, and paddocks were considered the experimental units for pasture variables. Analysis of variance were performed to determine the effects of the treatments using the Mixed procedure in SAS 9.4, and the means were compared by the Tukey test at the 5% significance level. The variables evaluated over time, within each year, were considered as repeated measures. In addition to the analysis of variance, correlation analysis between the animal behavior and pasture variables were performed.

The ANOVA model included as fixed effects block, treatment, period (repeated measures over time within each year) and treatment x period interaction. The data of total GT and total IT were not normal (Shapiro-Wilk; $P \leq 0.05$) and were transformed by log and square root, respectively. The results are presented as means adjusted by the LSMEANS (least square means) procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), ± standard error of the mean. The LSMEANS procedure was used because least square means are less sensitive to missing data (27).

The data were also submitted to multivariate Decision Tree analysis performed by JMP software (version 12, SAS Institute Inc., Cary, NC, USA). This analysis allows to understand a result obtained by investigating the degree of interference that the factors studied may have in a given process of interest. The statistical program generates an equation that explains (through R^2 value) which factors most influence a certain variable like GT. The independent variables included as factors in the analysis were initial body weight, herbage mass (DM/ha), leaf:stem ratio, pasture accumulation rate, leaf/ha, stem/ha, senescence/ha, inflorescence/ha, sward height and biting rate.

RESULTS

Ingestive Behavior

The different structures of the Capim Aruana tropical pasture did not influence GT or IT by young lambs ($P = 0.4266$ and $P = 0.2939$, respectively), with averages for GT of 391.1 ± 15.44 min in the Tall; 389.1 ± 12.3 min in the Medium and 428.1 ± 24.3 min per day in the Short treatment. For the variable IT ($P = 0.2939$)

the averages were 174.3 ± 25.8 min in the Tall; 152.9 ± 16.8 min in the Medium and 144.1 ± 21.7 min per day in the Short treatment. In relation to RT there was a significant (P= 0.0181) difference between treatments, being the longest times for the Medium treatment (174.4 ± 8.6 min/ day), which differed from the Short treatment (143.5 ± 7.2 min/ day). The RT in the Tall treatment (153.0 ± 7.3 min/day) did not differ from the other treatments ($P > 0.05$).

There was an interaction between treatment and period (P = 0.0049) in relation to biting rate, in which the Short treatment had

TABLE 1 | Ingestive behavior of lambs recently weaned in different structures of tropical pasture Capim Aruana (*Panicum maximum)* a with period 1 being the initial instant of forage offered to the animals (summer) and period 2 the end of the pasture cycle (next to autumn).

Variables[a]	**Period**	**Treatments**			***P*-value**[b]		
		Tall	**Medium**	**Short**	**Treat**	**Per**	**Treat*Per**
GT (min)	1	383.4 ± 17.5	383.7 ± 13.4	409.3 ± 38.4	0.4266	0.0941	0.2541
	2	399.4 ± 26.2	394.2 ± 20.7	446.8 ± 30.9			
	Mean	391.1 ± 15.44	389.1 ± 12.3	428.1 ± 24.3			
IT (min)	1	205.8 ± 19.4	172.0 ± 16.4	173.9 ± 36.5	0.2939	0.0003	0.4540
	2	142.9 ± 26.0	133.8 ± 18.0	114.3 ± 22.5			
	Mean	174.3 ± 25.8	152.9 ± 16.8	144.1 ± 21.7			
RT (min)	1	130.3 ± 8.2	160.0 ± 12.8	132.7 ± 9.1	0.0181	<0.0001	0.2995
	2	177.0 ± 9.3	188.0 ± 11.1	154.3 ± 10.9			
	Mean	153.0 ± 7.3AB	174.4 ± 8.6A	143.5 ± 7.2B			
Bite Rate (bites/min)	1	19.8 ± 0.8c	25.6 ± 1.0b	30.0 ± 1.4ab	<0.0001	0.0006	0.0049
	2	26.8 ± 1.5b	25.6 ± 1.0b	32.6 ± 1.2a			
	Mean	23.2 ± 1.0B	25.6 ± 0.7B	31.3 ± 0.8A			

[a] *Variables = GT, grazing time; IT, Idling time; RT, ruminating time.*

[b] *Different capital letters differ on the line for each variable; Different lowercase letters differ from each other in the treatment *period interaction for each variable analyzed.*

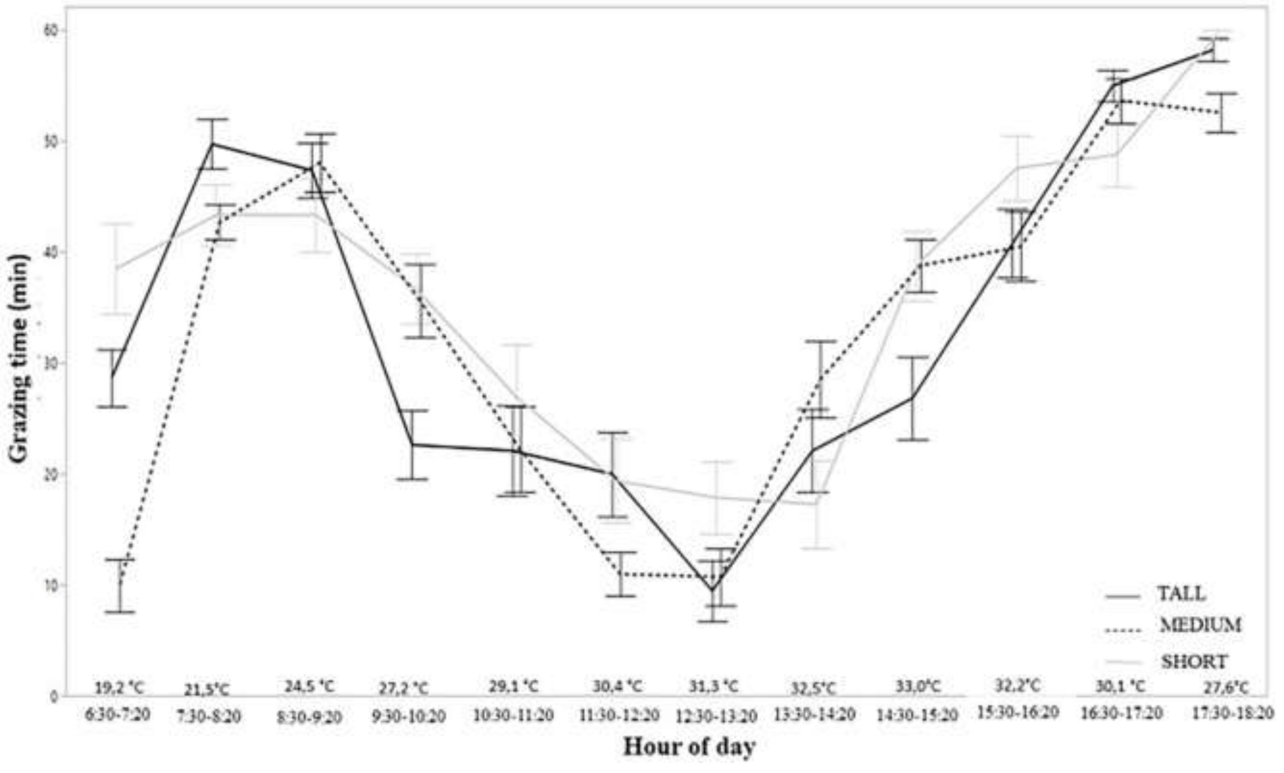


FIGURE 1 | Behavior of grazing activity during the day of freshly weaned lambs in different structures of tropical pasture Capim Aruana (*Panicum maximum*).

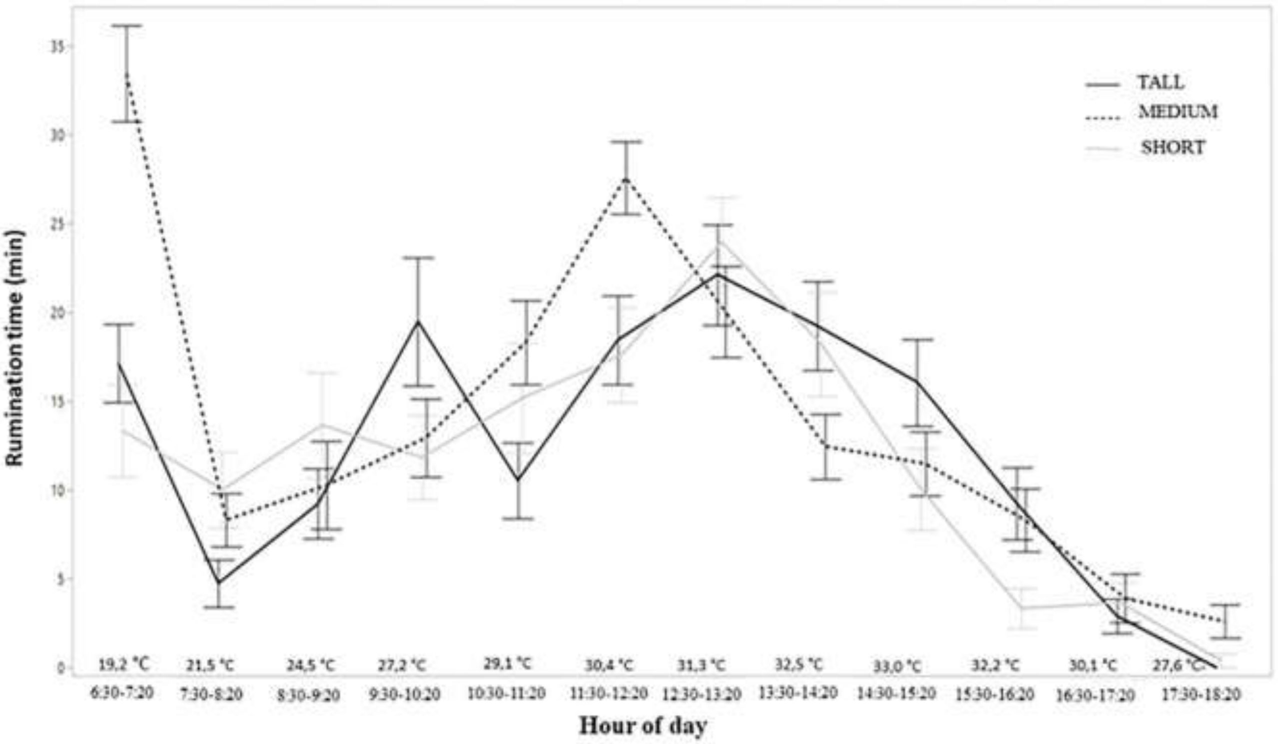


FIGURE 2 | Rumination behavior activity during the day of freshly weaned lambs in different structures of tropical pasture Capim Aruana (*Panicum maximum*).

similar biting rates in both periods, 1 and 2 (30.0 ± 1.4 and 32.6 ± 1.2 bits/min, respectively). Animals under the Medium and Tall treatments showed lower biting rates during both periods, as shown in **Table 1**.

When analyzing the GT of the animals over the different hours of the day, a similar pattern of behavior was observed in all treatments. In general, two grazing peaks occurred throughout the day. There was a peak in the morning with an average duration of 2 h between 07:30 am and close to 09:20 am. During this interval animals grazed more than 80% of the activities recorded. The animals returned to grazing activities after the hottest times of the day, around 02:20 pm, and the amount of time they spent grazing gradually increased, reaching almost 100% of the activities recorded after 05:20 pm (**Figure 1**).

Although the patterns of behavior were similar among treatments, it was possible to identify a different behavior of the Short treatment animals. While at the beginning of the behavior evaluation, at 6:30 am, the animals of this treatment were already in high grazing activity, almost 70% of the time, the animals on the Medium and Tall treatments were slowly starting their grazing activities. This behavior change shows that animals in the Low treatment started their grazing activities earlier in the day than in the other treatments, as shown in **Figure 1**. In addition to this behavior, animals under the Short treatment started to gradually reduce their grazing activity around 9:30 am, while this decline occurred for the other treatments at the same time but in a more pronounced manner. Another difference in behavior for animals in the Short treatment was that most of them did not cease their grazing activity in the hottest hours (11:30 am−02:20 pm), in contrast to animals assigned to taller structures (**Figure 1**).

There was a small difference between the Medium and the other treatments with regards to RT. During the first hour, the percentage of time spent ruminating by animals in the Medium treatment was greater than for the other two treatments, and greater rumination activity was observed by these animals in the hottest periods of the day (**Figure 2**).

Idling behavior across different hours of the day was similar for all treatments. In contrast to GT, idling behavior was less in the early morning and late afternoon, and there was an idling peak between 11:20 am 02:20 pm (**Figure 3**).

Decision Tree Analysis

According to this analysis, it was hierarchically identified that the initial weight of the animals and pasture height explained 62% ($R^2 = 0.621$) of the model, being the variables of greatest influence in the animals GT. The first division of the model showed that the factor of greatest interference in GT was the initial weight of the animals (**Figure 4**), explaining 48% of the variation in the model.

The model estimated that animals with an average initial weight >22.7 kg would have an average GT of 327.1 min and animals weighing <22.7 kg would have an average GT of 470.2 min. For animals of lower weight, the analysis showed that

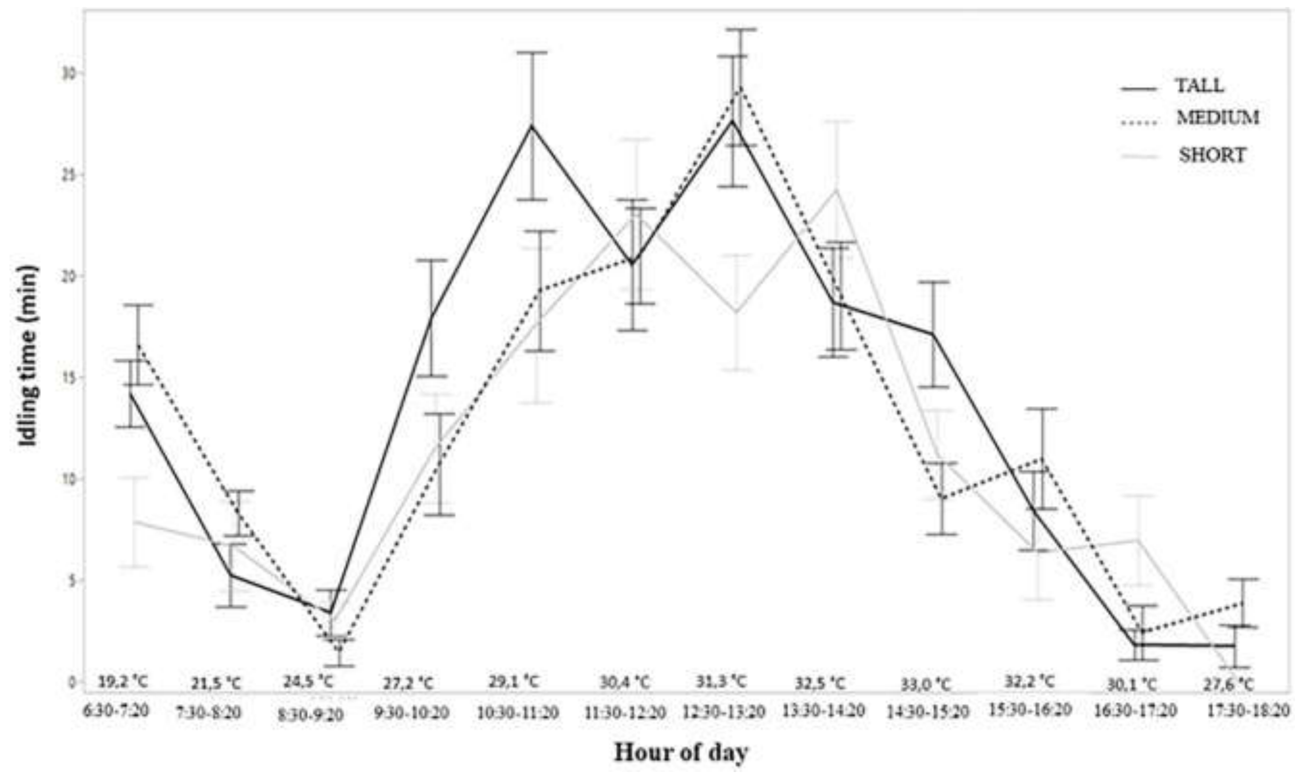


FIGURE 3 | Idling behavior activity during the day of freshly weaned lambs in different structures of tropical pasture Capim Aruana (*Panicum maximum*).

the factor with the greatest influence at explaining the observed variability in GT was forage height. This result demonstrates that the pasture height (structure) is more important for smaller animals.

Pasture

The pasture height showed a negative correlation with biting rate ($r = -0.46$, $P < 0.0001$). Another characteristic of the pasture that showed a significant relationship with biting rate was the number of inflorescences per hectare ($r = -0.51$, $P < 0.0001$). There was a greater amount of inflorescence in the taller swards at the end of the experiment. On average, there was a trend ($P = 0.0967$) for greater amount of inflorescences in the Tall treatment. In fact, a high correlation was observed between sward height and percentage of inflorescence ($r = 0.81$, $P < -0.0001$).

The variables leaf/ha and dead matter/ha showed no significant difference between treatments. The variable herbage mass (DM/ha) differed significantly ($P < 0.0001$) between treatments, being greater for the Tall treatment (**Table 2**). The pasture accumulation rate showed a trend ($P = 0.0513$) for greater values in the Tall than in the Short treatment, and significant correlations with ingestive behavior variables, like IT ($r = 0.63$, $P < 0.0001$) and GT ($r = -0.49$, $P < 0.0001$).

The leaf:stem ratio ($P = 0.0006$) was greater in the Medium and Short treatments than in the Tall treatment. In contrast, the stem/ha variable ($P < 0.0001$) was greater in the Tall treatment, as shown in **Table 2**.

Bromatological Composition of the Diet

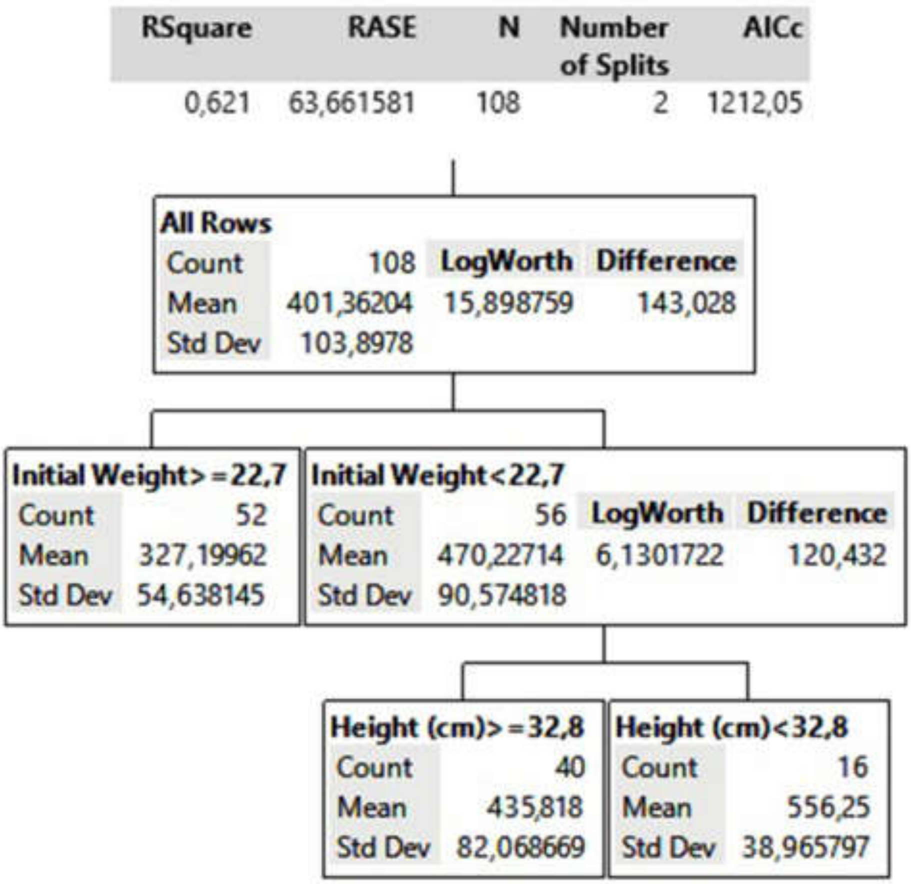
The chemical composition of the diet did not differ among treatments ($P > 0.05$), with similar parameters, as shown in **Table 3**.

DISCUSSION

Ingestive Behavior

This experiment shows that young lamb grazing an upright tropical grass do not vary their GT due to the different sward heights, contrasting with what was discussed by Hodgson (9). This author, reviewing studies with temperate pastures, shows a linear increase in grazing time when the height or mass of the forage decreases. However, according to Sollenberger and Burns (28), this relationship in tropical pastures is not so consistent. Animals may graze taller or shorter tropical grasses for longer periods to compensate the limitations imposed either by pasture height, leaf size, number of stems or amount of herbage mass. However, rumination time in this study was longer in the Medium-height treatment, which demonstrates that factors other than structure can interfere in the GT of young animals grazing tropical pastures.

The leaf:stem ratio and the availability of green leaves in tropical pastures are key characteristics that affect animal ingestive behavior. For instance, Euclides et al. (29) studying *Panicum maximum* and *Brachiaria* spp. in southern Brazil showed that grazing time decreased with increments in the



RSquare	RASE	N	Number of Splits	AICc
0,621	63,661581	108	2	1212,05

FIGURE 4 | Decision Tree model for grazing time behavior variable.

percentage of green leaves and leaf mass. Carvalho et al. (5) explains that the way leaves are presented to animals and how green leaves are apprehended, separately from stems and dead material, are important characteristics that should be considered in tropical pasture management in sheep production systems.

Despite the statistical difference in rumination time between treatments, it is important to consider that over 720 min of daily evaluation, rumination time in the Medium-height treatment was only 30 min greater than in the Short-height treatment. This small difference may then explain the lack of compensation observed for other activities in animals that exhibited shorter rumination times. In addition, these small differences in rumination time may not be physiological, since rumination activity is distributed throughout the day in periods ranging from 2 min to more than 1 h (30). Other activities were similar between treatments, which reinforces the idea that difference for rumination time may be associated with a natural variation in animal behavior that occurs throughout the day.

Biting Rate

The greatest biting rates identified in this study occurred in animals exposed to the Short-height treatment (sward height of 25 cm). Biting rate and intake values by grazing animals are sensitive to variations in the mass and height of the pasture (31). In the case of an erect tropical grass, such as Capim Aruana, the shorter the pasture, the greater the biting rate. This response is consistent with observations in older lambs than those used in this study. Negri et al. (32) working with 120-day-old lambs grazing Capim Aruana (*Panicum maximum*) found that as sward height increased, animals spent more time (seconds) to achieve 20 mouthfuls. Stobbs (33) explains this behavior through the negative relationship between canopy height and density of the dry mass of green leaf blades, compromising the size of the bite due to increments in handling and chewing times. Similarly, Schwartz et al. (34) observed in sheep grazing pearl millet that at high pasture heights animals were forced to graze leaves individually due to leaf length, a behavior that decreased biting rate. Thus, there is a negative relationship between biting rate and height of tropical erect pastures (35). In fact, biting rate proved to be one of the variables that is most responsive to height variation of tropical erect grass.

A negative correlation was found between the rate of biting and the amount of inflorescence/ha in the pastures, whereas a positive correlation was detected between sward height and the

TABLE 2 | Grazing variables in different structures of Capim Aruana (*Panicum maximum)* with period 1 being the initial instant of forage offered to the animals (summer) and period 2 the end of the pasture cycle (near autumn).

Variables[a]	**Period**	**Treatments**			***P*-value**[b]		
		Tall	**Medium**	**Short**	**Treat**	**Per**	**Treat*per**
DM/ha (Kg)	1	4167.8 ± 111.1	2681.7 ± 129.6	1639 ± 37	<0.0001	0.0010	0.8141
	2	5513 ± 143.18	3820.8 ± 85.3	2937 ± 51.4			
	Mean	4840.1 ± 144.7A	3251.3 ± 117.4B	2288 ± 120.6B			
Accumulation Rate (Kg/day)	1	195.1 ± 8.7	104.8 ± 5.3	127.1 ± 20.5	0.0513		
	2	−9.8 ± 0.2	4.8 ± 15.1	32.2 ± 22.2			
	Mean	92.6 ± 17.8	54.8 ± 11.1	79.6 ± 17.1			
Height (cm)	1	79.9 ± 1.2a	44.4 ± 2.7bc	23.8 ± 0.9d	<0.0001	0.7735	0.0120
	2	67.2 ± 0.7ab	50.9 ± 0.4b	25.3 ± 0.6cd			
	Mean	73.5 ± 1.2A	47.7 ± 1.4B	24.5 ± 0.5C			
L:S	1	1.4 ± 0.1	1.4 ± 0	3.7 ± 0.3	0.0006	0.6946	0.2512
	2	0.3 ± 0	1.4 ± 0.1	1.4 ± 0.1			
	Mean	0.8 ± 0.1B	1.4 ± 0A	2.6 ± 0.2A			
Leaf/ha (Kg)	1	1455.6 ± 24.4a	1143.5 ± 66.4ab	805.5 ± 25.7b	0.1438	0.4845	0.0322
	2	1016 ± 15ab	1553.5 ± 120.3a	1223 ± 23.2ab			
	Mean	1235.8 ± 39.7	1348.5 ± 75	1014.3 ± 41.2			
Stem/ha (Kg)	1	1964.1 ± 72.7	1080.1 ± 93.2	564.7 ± 38.8	<0.0001	<0.0001	0.5855
	2	3685.2 ± 191.1	1992.5 ± 24.5	1378.1± 88.1			
	Mean	2824.6 ± 176.9A	1536.3 ± 85.6B	971.4 ± 87B			
Dead Matter/ha (Kg)	1	323.9 ± 12	305.3 ± 28.7	167.5 ± 6.9	0.4263	0.1984	0.0966
	2	517.2 ± 6.7	167.4 ± 9.8	283.9 ± 11			
	Mean	420.6 ± 17.8	236.3 ± 18.4	225.7 ± 12.2			
Inflorescence/ha (Kg)	1	110.8 ± 7.8	17.2 ± 4	3.5 ± 0	0.0967	0.7532	0.8529
	2	39 ± 6.7	50 ± 1.9	0			
	Mean	74.9 ± 7.2	33.6 ± 3.3	0.14 ± 0.04			

[a] *variables = DM/ha, dry matter per hectare; L:S, leaf:stem ratio.*

[b] *Different capital letters differ on the line for each variable; Different lowercase letters differ from each other in the treatment *period interaction for each variable analyzed.*

presence of inflorescence. Likewise, Silva et al. (6) reported that the proportion of leaves in Aruana (*Panicum maximum*) and the presence of inflorescence influenced grazing strategy by lambs. This result shows the importance of avoiding the inflorescence in the pastures in order to facilitate lambs' grazing activities. Thus, the use of a mower or cattle grazing may contribute to manage pastures for grazing young lambs.

TABLE 3 | Bromatological composition of the diet, based on the different structures of Capim Aruana (*Panicum maximum)*.

Variables[a] **(% /kg de MS)**	**Treatments**			***P*-value**
	Tall	**Medium**	**Short**	
Mineral matter	9.8 ± 0.1	11.9 ± 0.2	10.7 ± 0.1	0.1068
NDF	66.5 ± 0.9	69.9 ± 0.7	65.6 ±0.3	0.3856
ADF	35.7 ± 0.3	36.7 ± 0.2	35.2 ± 0.6	0.7472
CP	15.9 ± 0.4	17.2 ± 0.4	17.9 ± 0.2	0.6599
EE	2.2 ± 0	2.5 ± 0	2.6 ± 0	0.0598
NIND	1.6 ± 0	2.3 ± 0	2.3 ± 0	0.4890
NIAD	0.30 ± 0	0.31 ± 0	0.30 ± 0	0.9748
LIGNIN	3.8 ± 0.1	4.6 ± 0	3.9 ± 0	0.2766

[a] *Variables = NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, Crude Protein; EE, ethereal extract; NIND, nitrogen in neutral detergent; NIAD, nitrogen in acid detergent.*

Behavior Throughout the day

When analyzed over the hours of the day, the animals' ingestive behavior showed a natural behavioral pattern (36, 37). There was a peak of grazing in the early morning and late afternoon, and a moderate increase in rumination and idling activities during the late morning and early afternoon. Despite a decrease in GT by lambs during the hottest hours of the day (11:30 am−2:20 pm) the activity, although less frequent, was still observed in this study, contrary to results reported by Starling et al. (38) in tropical conditions. These authors evaluated Corriedale ewes poorly adapted to warm conditions and observed that the animals abruptly stopped their grazing activity at high environmental temperatures. The observation of grazing activity in the hottest hours of the day in our study may be related to climatic conditions that did not trigger high thermal stress and thus allowed grazing to occur at those times. This behavior demonstrates the need for ruminants to be constantly ingesting food (39) and shows that temperature may have a limited effect on the grazing behavior of animals in subtropical conditions.

The Short-height treatment showed that animals were already grazing more intensely than lambs in the other treatments during the early hours of the day. During the first hour of evaluation, animals in the Tall-height treatment grazed on average for 30 min and the Medium-height treatment for 10 min, whereas the Short-height treatment grazed on average for 40 min. Although there was not a significant difference in GT among treatments, the greater availability of leaves seems to prompt animals to graze earlier during the day.

In studies with sheep on bermudagrass pasture, Poli et al. (40) found that grazing was the activity that took up most of the lambs' time in the three production systems (lambs weaned on pasture; unweaned lambs exclusively on pasture; and unweaned lambs supplemented on pasture), consistent with results from the present study.

Decision Tree Behavior

The Decision Tree analysis allowed us to explore the factors that influenced GT. The initial weight of the lambs had a key influence on GT. Lighter lambs grazed for longer periods than heavier lambs. The model highlights the importance of the structure of tropical pasture for animals under lower initial body weights, as height appears as the second factor influencing grazing time. In support of this, Emerenciano Neto et al. (41) report that among various structural characteristics, pasture height was a key variable influencing animals' foraging decisions.

Although there were no significant differences in GT between the different pasture-height treatments, important variability was observed for initial body weights within treatments and between years. Such variation allowed for distinguishing the effect of pasture height on initial body weight. The importance of animal size was also mentioned by Carvalho et al. (5). They explain that young and light lambs can be largely affected by herbage components, mainly due to the difficulty of bite formation by a small mouth area, which in turn influences the animals' grazing capacity.

The longer grazing times by lighter animals can lead to greater energy expenditures, with potential negative effects on performance. Thus, increasing body weights would be an ideal scenario for early weaned lambs entering tropical pastures. For larger lambs, the structure of tropical pasture has less influence on the time invested in grazing, whereas young lighter lambs may benefit from grazing shorter pastures, given that for animals of lower weight forage height had the greatest influence on GT.

These are innovative results that highlight the importance of initial weight for a weaned lamb to enter an erect tropical grass pasture. It is important to wean animals at proper body weights and developmental conditions, so that they can face the challenges imposed by tropical grasses, characterized by their large structure of leaves and stems combined with a fast growth rate.

These results also show the importance of assessing ingestive behavior as a tool to understand the factors that have direct effect on animal productivity. Our study shows important relationships between erect tropical grass pasture and young lamb size, generating innovative management decisions. In fact, there is a need to have a minimal lamb weight to face the challenges promoted by a tropical pasture. In this study the structure of a tropical pasture becomes less of a concern as the animal is heavier than 23 kg.

CONCLUSIONS

Grazing time by young weaned lambs did not differ among different structures of an erect tropical grass sward, suggesting that other factors may influence foraging behavior. Biting rate proved to be the main variable that differed among the gradient of grazing structures presented. Body weight and height of the upright tropical grass pasture had a strong influence on lambs' ingestive behavior. Maximum pasture height and minimum body weight should be considered when young lambs graze an erect tropical grassland.

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 Universidade Federal do Rio Gande dos Sul Animal Care and Use Committee. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

This study was developed by Animal Science Graduate Program at Universidade Federal do Rio Grande do Sul and it was financed by development agencies of the Brazilian Ministry of Education and Ministry of Science and Technology. Every author had important contributions on this manuscript. JS and LI developed the research project and fieldwork. CP is the head of the research project, being responsible for all parts of the study, from the project to the publication. JT was primarily responsible for the statistical analysis and results and discussion of the article. EM and JV contributed to the configuration of the manuscript and writing corrections. All authors contributed to the article and approved the submitted version.

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Meeting Breeding Potential in Organic and Low-Input Dairy Farming

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Low-input (LI) dairy farming, relying heavily on grazing, is increasing in popularity for perceived sustainability, welfare, and milk nutritional quality benefits. However, there is little research into the breed suitability for these systems. The popular Holstein–Friesians are not well-suited to LI production as, to achieve their potential high yields, they require high levels of concentrate intakes and veterinary inputs. Holstein–Friesians were traditionally bred for high milk yields, which often correlate negatively with functional traits, such as fertility and health. This drives the need for alternative breed choices, and UK dairy farmers use several crossbreeding practices. Additionally, classic measures of production efficiency (kilogram feed per liter of milk) are not the sole priority in LI systems, which also aim for improved health, fertility, forage conversion, and milk quality. This study aimed to explore the effect of breeding strategy on LI and organic production in dairy systems, collecting data from 17 farms throughout England and Wales: 7 organic and 10 low-input conventional systems with both purebred and crossbred cows from different breeds. Records from 1,070 cows were collected, including background data, health, fertility, breeding, and parity. Additionally, milk was analyzed on four occasions (autumn 2011 and winter, spring, and summer 2012). Principal components analysis was used to visualize the effect of management, Farm ID, and stage of lactation on LI production. The analysis clustered cows by Farm ID, showing that individual management practice on each farm had the greatest impact on various production traits. Cows were allocated a composite score based on their yield, health records, and milk fatty acid profile, and a linear mixed-effects model indicated ($p < 0.01$) that crossbred New Zealand Friesian cows scored highest, whereas Dairy Shorthorn cows scored the lowest. This paper highlights weaknesses in current breeding programs for LI and organic farms in the UK, in terms of the alignment of breeds with husbandry practices. Additional research is needed to explore any gene by environment interactions to meet the true potential of individual cows and certain breeds under LI and organic management.

Keywords: low-input, organic, dairy farming, milk quality, grazing

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INTRODUCTION

Organic farming in the UK is defined by European Union (EU) regulations (1) and certifying bodies such as The Soil Association (2) and Organic Farmers and Growers (3). However, many farms operate low-input (LI) systems, which are not organic and not formally defined or regulated. LI farming refers to the practice of using fewer inputs than conventional agriculture but not necessarily meeting organic or other quality assurance standards. Motivations toward LI farming include economic, environmental, and social parameters (4). The main criticism of organic and LI farming is that, compared with intensive systems, lower yields require more land to produce the same amount of food, leading to poorer biodiversity if seminatural vegetation is converted to agriculture (5). However, rejecting organic production methods by emphasizing yield productivity ignores opportunities for practices that enhance sustainability; therefore, alternative metrics must assess LI systems. Over the past 60 years, dairy farming has typically focused on making better use of inputs, maximizing profit, relying heavily on high yields and improved feed efficiency (kilogram dry matter intake per liter of milk) [e.g., (6) Milkbench + system]. However, in organic and LI dairying, priorities are different, whereas profit is still essential; the production system involves fewer inputs. Feed efficiency is equally important, but the pathway to achieve this is mainly on reducing external inputs rather than maximizing outputs, a practice that may also benefit herd health (7). Reducing the intensity of production lowers veterinary bills and costs associated with inseminations while using optimal grazing strategies (such as mob-grazing) to enhance soil and sward health, meaning cows consume a richer pasture and produce more nutritious milk (8, 9). A robust method to determine sustainability, accounting for animal health/welfare, nutritional quality, and environmental/social impacts, is needed. Although fully exploring the sustainability of LI dairying is beyond the scope of this study, this paper explores aspects of breeding, production output, health and milk quality of LI, and organic dairy farms.

Traditionally, Holstein/Friesians (HF) has been at the forefront of high yielding dairy production globally. Holsteins are primarily selected for their production traits (milk yield and composition), whereas more traditional Friesians can be selected for functional traits (health and fertility). However, HF cows are not well-suited to LI and organic systems, as they require relatively high levels of both concentrates to achieve maximum yield potential and veterinary inputs (10). Instead, breeds able to maintain health and productivity with LIs are preferred. As a cross, HFs are bred for production traits (higher yield), which are often negatively correlated with functional traits, such as a decline in fertility and health (11). To maximize the potential of both alternative and high-yielding breeds, LI and organic dairy systems have increased their interest in crossbreeding dairy cattle, introducing genetics from more robust breeds (12). Additionally, functional traits are heavily influenced by the local environment and have low heritability (11), making it difficult to select genetic lines to improve health and fertility. For this reason, LI and organic systems benefit from crossing with breeds known to have stronger functional traits.

Organic and LI systems often rely on crossbreeding strategies to optimize their herd health and yield potential. A strong reason for crossbreeding is the resulting heterosis or hybrid vigor in the first generation (F1). Crossbred offspring (including HF crosses) outperformance relative to the parental average is one way to improve functional traits (13) without impacting milk production. However, to extend the benefit beyond the first generation, a carefully designed system is required for rotational crossbreeding: crossing two F1 individuals only expresses half the hybrid vigor, whereas introducing a third breed preserves up to 86% of the heterosis (12). Crossbreeding high-production HF with traditional breeds better suited to LI management (with high forage diets) shows potential. For example, recent studies comparing breeds and crossbreeding regimes in Switzerland and the UK showed more traditional breeds, or crossbreeding with traditional breeds can significantly improve the economic performance and milk quality in LI grazing based dairy systems (10, 14). The indicators from these studies are positive, but further research is needed to identify the key mechanisms required to produce predictable, repeatable, efficient, and effective crossbreeds.

There is very little recognized research into breeding for crossbred cattle in smaller LI and organic dairy systems. Yet, these farms have progressed with crossbreeding for many generations within their herds, each using a different strategy to search for breed combinations that perform within their system (15). Therefore, there is not a clear breed (or crossbreed) that typically outperforms others in LI systems, in the way that HF dominates conventional production. In addition, most scientific research has focused on HF because they account for 95% of the EU dairy cow population (16). UK organic milk was valued at £351 million in 2018, with over 25% of UK households purchasing organic milk, representing 5.1% of retail milk sales (17), highlighting the increased need to develop appropriate crossbreeding schemes for such production chains. Studies from a range of countries argue that, due to genetic × environment (GxE) interactions, optimal genetic progress requires either independent breeding programs or an index (to rank sires against requirement) specific for each farming system (18–23). This approach would directly benefit LI and organic systems.

The complexity of breeding support for LI dairying is not well-established in the UK. In LI and organic dairy systems, the diet is predominantly forage; therefore, it is beneficial to have cows that efficiently convert forage, especially grazing, to milk (24). However, current UK breeding objectives available do not include forage conversion as a desirable trait when calculating economic values of genetic gain. Instead, the Agriculture and Horticulture Development Board (AHDB-UK levy board funded by farmers and growers) breeding index for year-round calving focuses on milk production (34.4%), health (21.8%), fertility (15.3%), and temperament, among other traits (25). The AHDB also has a Spring Calving Index, aimed at herds making use of grazed grass by assigning 71.6% of the weighting to fitness traits, but the dominant individual driver is still production (27.4%), and the link between efficiency (with an emphasis on forage conversion) in LI systems has yet to be fully explored. Typically, LI and organic management that supports animal health and mastitis is the main concern (26), whereas health and fertility remain essential in these systems; the risk of illness (for example, acidosis) is much reduced. Although these UK resources for dairy breeding selection exist, other options seem more appropriate for organic and LI production.

Milk quality has gained a lot of media attention recently, continuing the debate around the role of milk in human diets and the environment (27). Milk fatty acid (FA) profile is strongly influenced by management, and there is a clear difference in the FA profiles of organic and conventional milk (28–30) between the different stages of lactation (31) and seasonally (32, 33). Additionally, FAs can vary as much within- as between-breeds (34, 35), making it harder to isolate breeds that could give an "optimal" FA profile within a specified management system. Some FAs have been studied closely for their effects on human health. The main FAs considered to have a positive effect on human health are alpha-linolenic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), oleic acid (OA), and cis-9 trans-11 conjugated linoleic acid (CLA9). Alpha-linolenic acid is the most abundant omega-3 (n-3) FA and promotes healthy aging and fetal development (36, 37). The long chain n-3 FAs, EPA, DPA, and DHA are anti-inflammatory and reduce the risk of coronary heart disease (CHD) (38). OA can reduce the risk of CHD and promotes stable cellular membranes (39). CLA9 has been shown to lower the risk of CHD and enhance the immune system (40, 41). In contrast, FAs highlighted as undesirable in human nutrition due to their association with increased CHD risk are lauric (C12:0), myristic (C14:0), and, in particular, palmitic (C16:0) acids (39). Also, the most abundant omega-6 (n-6), linoleic acid, is an essential FA in human diets, but if total n-6 is in excess, as prevalent in Western diets, it becomes pro-inflammatory with negative health effects (42). Of greater relevance is the dietary ratio of n-6/n-3, which, when too high (the exact optimal ratio is unknown), may cause inflammations and increase CHD risk (42, 43). Although there is currently no premium in UK linked to milk fat composition, in the USA, CROPP's organic "Grassmilk^{TM}" receives a 15% premium above standard organic milk prices for meeting minimum requirements for the n-6/n-3 ratio, total n-3, and CLA (29). This demonstrates the potential for other sectors and countries to create premium dairy products with an increased concentration of beneficial FAs.

Historic approaches to breeding in dairying have not taken a whole system view, generally resulting in poor health traits and concentrate-dependent cows (16). If robust methods to identify cattle that best suit a particular system are to be developed, there is the potential to improve animal health and welfare, production, nutritional quality, milk FA profile, and efficiency. This paper aims to identify breeds within LI and organic dairy systems that can maintain health and yield while producing milk with a beneficial FA profile. The objectives are to (a) define the variables most relevant to LI and organic farming and observe differences in the management system (individual farms), (b) identify breeds that are similar across the farms and quantify differences, (c) develop a score for LI-production (LI-P) to identify breeds that best suit LI and organic production in terms of production, health, and milk composition with respect to consumer health.

TABLE 1 | Background information on each farm.

Farm ID	Management	No. of cows included	Calving	Breeds and crosses represented[a]
1	Organic	40	Spring	AYR, JE, HF, NZF, SR, SH
2	Organic	42	Year-round	HF, JE, SR
3	Low-input	55	Spring	BS, JE, HF, SR
4	Low-input	52	Spring	NZF, JE, HF
5	Organic	49	Year-round	HF, SR, SH, MRI
6	Low-input	28	Spring	HF, JE, SR
7	Organic	61	Autumn (late)	AYR, HF, SH, SR
8	Low-input	113	Year-round	BF, HF, SR, SH, MRI
9	Low-input	60	Autumn (early)	BF, JE, HF, NZF, SH
10	Organic	55	Autumn (early)	BF, BS, HF, MO, SR
11	Low-input	66	Spring	JE, NZF, BF, HF
12	Low-input	27	Spring	BF, SR, JE
13	Low-input	84	Year-round	AYR, BF, HF, SR, MO, NZF
14	Low-input	76	Spring	BF, JE, NZF, SR, HF, MRI
15	Organic	93	Autumn	AYR, HF, MO, SR, JE
16	Low-input	97	Spring	AYR, JE, HF, NZF
17	Organic	72	Autumn	AYR, HF, SH, XX

[a] *HF, Holstein/Friesian; NZF, New Zealand Friesian; BF, British and unknown Friesian; JE, Jersey; SR, Scandinavian Red; SH, Shorthorn; AY, Ayrshire; MO, Montbelliarde; BS, Brown Swiss; MRI, Meuse Rhine Issel; XX, crossbred with unknown breed composition.*

MATERIALS AND METHODS

Data Collection

Data for this study were collected from 17 dairy farms (7 organic and 10 LI-conventional) throughout England and Wales between November 2011 and October 2012. All herds were a mix of both purebred and different crossbred cows (**Table 1**). Herd sizes ranged from 150 to 550 cows, and a total of 1,070 cows were recorded to encompass a broad range of breeds and crosses from each farm. A one-off questionnaire was completed to gain information on pre-survey health and parity as well as a breeding pedigree for all individual cows (according to the farmers' records). Milk from each cow was sampled over four dates: autumn 2011 (D1), spring (D2), summer (D3), and autumn 2012 (D4). A corresponding questionnaire for each farm and cow was used to record husbandry practices on all sampling dates, including milk yield, disease incidence, health treatments, cow diet, calving intervals, milking, and grazing management. Organic farming standards require concentrate feed to be sourced organically and have strict land management application practices (2), whereas LI follow similar practices but are not certified organic. Organic and LI farms fed similar levels of concentrate per cow, and organic farms typically fed more conserved forage (**Supplementary Table 1**). Access to grazing varied across the year and individual management (**Supplementary Table 2**). All milk samples were analyzed for basic composition, somatic cell count (SCC), and FA profile. All procedures were acceptable to internal ethical review, in accordance with EU Directive 2010/63/EU for animal experiments and approved by the Animal Welfare and Ethical Review Body at Newcastle University.

Milk Analysis

A representative raw milk sample was collected from each cow during milking in the parlor on each sampling date. Milk samples were preserved with Bronopol and kept at ambient temperature during transportation to a commercial National Milk Recording (44) lab. Basic milk composition was analyzed using Milkoscan FT 6000 (Foss Electric, Hillerød, Denmark) (milk fat, protein, urea, and lactose content), and SCC was recorded using a Fossomatic instrument (Foss Electric). The samples were then transported at ambient temperature (10–25°C) to Newcastle University, frozen at −20°C. Bronopol preserves milk for more than 5 days and is effective unless temperatures are consistently high (45); ambient temperature varied by season, but milk was frozen within 4 days of collection. There is some evidence that Bronopol may have a small impact on minor long-chain FAs (46) and protein concentration (47). However, all milk samples in this study were treated the same and are therefore comparable. Milk was defrosted at 4°C, stirred thoroughly to homogenize, and 3–4 ml of milk was transferred in a 7-ml container, frozen at −20°C, and freeze-dried. The lipid was extracted using the method described by Chilliard et al. (48), where 130 µg of lyophilized milk was methylated and esterified. Gas chromatography (Shimadzu, GC-2014, Kyoto, Japan) equipped with a flame ionization detector and by using a Varian CP-SIL 88 fused silica capillary column (100 m × 0.25 mm ID, 0.2 µm film thickness) was used to analyze the FAs. The gas chromatography method has been previously described by Stergiadis et al. (49). Individual FAs were identified against peaks generated by a 52 methyl FA standard, with the area under each peak quantifying the relative proportion of each in the total FAs. An FA methyl ester standard and published chromatograms (50, 51) were used to identify the FAs, and correction factors for short-chain FAs were applied using the method described by Stergiadis et al. (49).

Data Handling

Breed Combinations

The farmers' breeding records categorized all animals. Cows were given a code based on their sire, dam, and predominant breed, for example, a pure-bred Jersey = JE; sire Jersey and dam Ayrshire = JEAYR; sire Jersey × Shorthorn and dam Jersey × Ayrshire = JEX (**Table 1**). The X indicates a majority genetic contribution and/or a back cross. Including the sire and dam breeds for all cows across the study resulted in around 40 different breed combinations of varying population sizes, depending on the sampling date. This ranged from a single representative on one farm (British Friesian × Montbelliarde) to 119 HF individuals across all farms for D2. To rationalize the number of crossbreed combinations in this study, there is no differentiation between the contribution of genetics by parents' sex. For example, both a cross from a Jersey sire and HF dam and from an HF sire and Jersey dam are labeled HFJE.

Data Analysis

Microsoft Excel was used for data handling, whereas all statistical analysis was completed using "R" (52). The background information on the farms and monitored cows is displayed in **Table 1**.

Low-Input-Production and Principal Components Analysis

The initial data collection involved 1,070 cows, but for some farms and/or cows on some sampling dates, there are missing and incomplete records. For the observational statistics, the cows selected had records on any given date for production, health, and FA composition results (explained later). This resulted in 299, 757, 772, and 613 cows on D1, D2, D3, and D4, respectively.

Focusing on the available data, using a combination of farm records and results from milk analysis and the priorities of typical LI practices, the variables selected to define LI-P were split into three main criteria:

1. Production:
    i. Milk yield (L/day).
    ii. Total fat and protein solids (kg/day).
2. Health:
    i. Udder health; SCC ($\times 10^3$ cells/ml milk).
    ii. Treatments, including antibiotics (e.g., for mastitis or metritis) or other (e.g., for lameness, milk fever, or pain/inflammation).
3. Fatty acid profile:
    i. Percentage of total profile with desirable FAs (n-3, OA, CLA9, EPA+ DPA+ DHA).
    ii. Percentage of total profile with FAs often consumed in excess and undesirable (C12:0, C14:0, C16:0, n-6, and n-6/n-3 ratio).

The elements of LI-P had different units (FAs were proportional, yield: liters/cow/day, SCC: $\times 10^3$ cells/ml milk, etc.); thus, the data was standardized (normalization to mean of zero and standard deviation of one) (53) to give each element of LI-P the same weight. Principal components analysis (PCA) in the package "vegan" (54) was used to aid visualization of the effects of Farm ID (2–17) on LI-P. Two sets of graphs were produced from the PCA. First, graphs (**Figure 1**) in which points represent samples/records from cows (at each farm, one graph for each the four dates), where the closer two points are to each other in PCA ordination space, the more similar their characteristics (in terms of production, health, and milk FA profile). The points in these graphs were color-coded by farm identity to aid interpretation. Second, PCA graphs of these characteristics (**Figure 2**), in which points close together, indicate co-occurrence on similar farms or farming systems. This second set of PCA graphs were also broken down by date. In other words, the characteristics that are grouped together in **Figure 2** can be associated with cows and/or farms that occupy similar ordination space in **Figure 1**.

Descriptive Statistical Analysis

For the descriptive statistical analysis, additional inclusion criteria were considered: on any sampling date, records existing

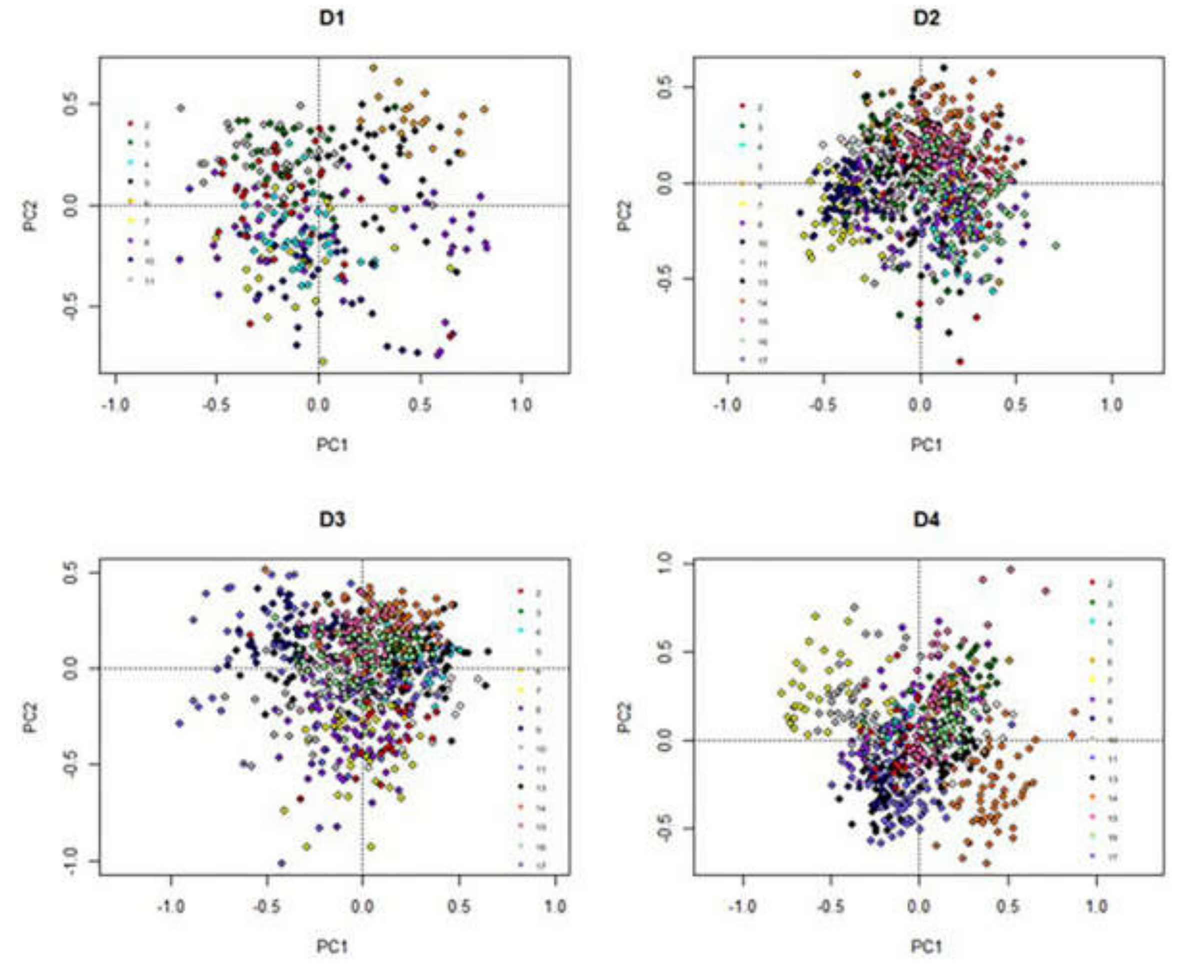

FIGURE 1 | Principal components analysis based on low-input-production, highlighted by Farm ID. C12:0 = Lauric Acid, C14:0 = Myristic Acid, C16:0 = Palmitic Acid, CLA.9 = Conjugated linoleic acid (C18:2, c9t11 isomer), n3 = omega-3, n6 = omega-6, n6n3 = omega-6/ omega-3 ratio, EPA + DPA + DHA = EPA = Eicosapentaenoic Acid + DPA = Docosapentaenoic Acid + DHA = Docosahexaenoic Acid, SCC = Somatic Cell Count, Treatments = Health Treatments.

for at least six cows of the same breed (combination) from at least three different farms. These criteria resulted in the most breed combinations and ensured comparison between breeds rather than individual farm management style. After these additional inclusion criteria had been applied, there were eight breeds for comparison: Ayrshire cross (AYRX, $n = 100$), HF (HF, $n = 325$), HF × Jersey (HFJE, $n = 184$), HF × Scandinavian Red (HFSR, $n = 274$), Jersey cross (JEX, $n = 121$), New Zealand Friesian cross (NZFX, $n = 90$), Dairy Shorthorn (SH, $n = 80$), and Scandinavian Red cross (SRX, $n = 140$). The number of cows represented by each breed from each farm is available in **Supplementary Table 3**.

The "R" package "nlme" (55) was used to model "Breed" against the variables described for the LI-P, with Season and Farm ID as random factors. The linear mixed-effects model accounts for variation explained by the fixed effects (Breed) and random effects (Season and Farm ID). As farms were observed across the four sampling dates, these related measures would violate the independence assumptions made by a linear model, hence, the use of "Farm ID" and "Season" as random factors. Days in milk did not differ between the breeds (F-statistic = 1.50, $p = 0.165$), allowing the breed to be compared without differentiating or adjusting for the stage of lactation. On each date all cows from the same farm were fed the same ration, not as individuals (**Supplementary Table 1**). The feed data did not meet the assumptions of the model; therefore, mean and standard deviations are given, but a p-value is not provided. Observationally, there was no big difference in the amount of concentrate fed between the breeds, but there was a notable difference in the amount of conserved forage-fed between breeds (**Table 2**). Concentrate feeding is thought to have the biggest impact on the FA profile (56); therefore, no corrections were made to the data before analysis.

Traditionally, *post-hoc* Tukey honest significant difference tests are used for multiple comparisons of levels within a factor. However, due to the complexity of this data set with multiple levels of comparison (8), some with few replicates, controlling the familywise error rate even by this approach would risk numerous type 1 errors (false-positives) and would be misleading (57–59).

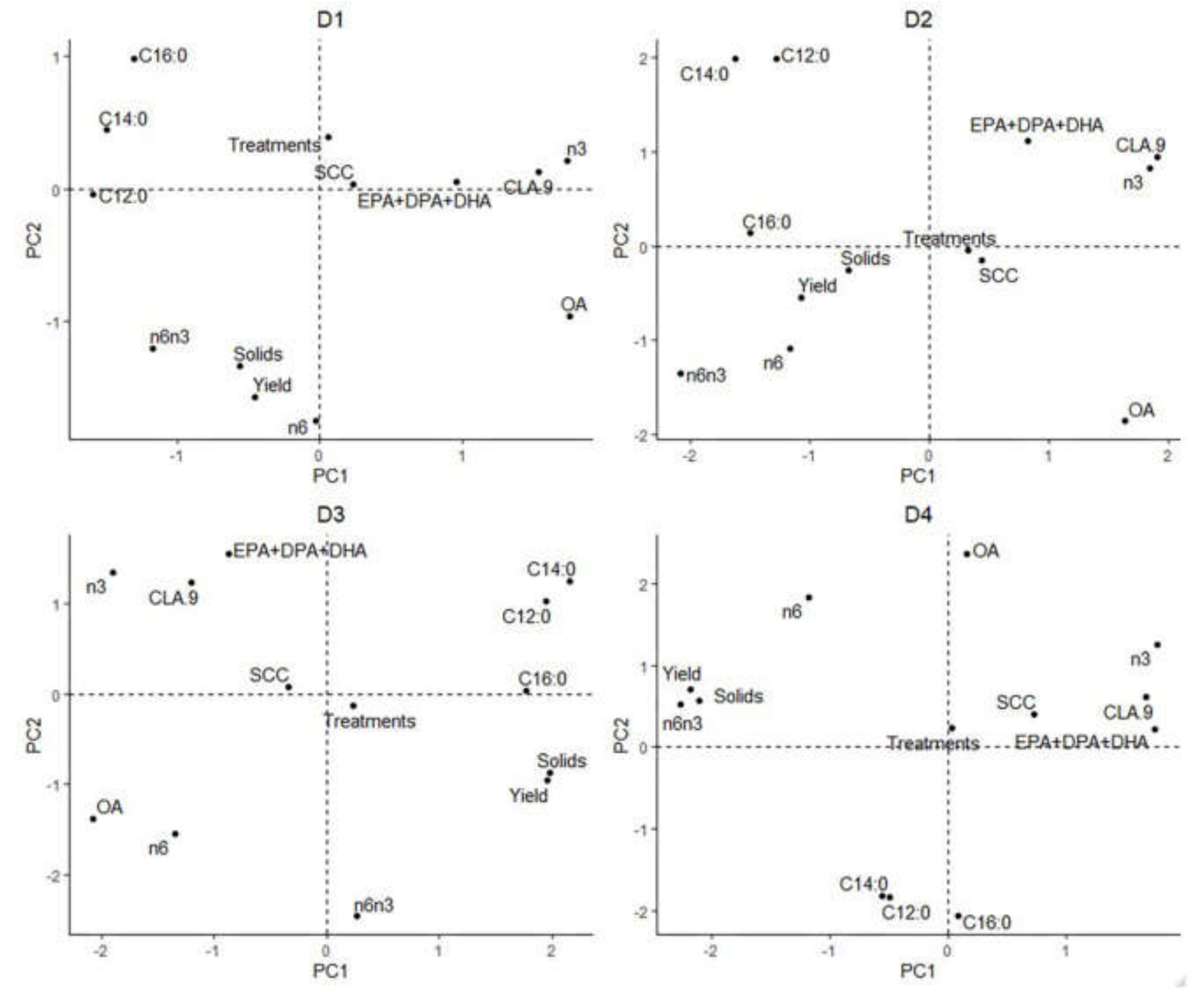

FIGURE 2 | Principal components analysis displaying characteristics of low-input-production.

Low-Input Production Score

To create a universal score for each record, common units are required. Using the variables selected for LI-P, scores were created for each cow record to assess the best performing breed. Milk yield, total fat and protein solids, SCC, and proportions of desirable and undesirable FAs were (higher rankings indicate more beneficial qualities) scored as described next.

1. Production records [milk yield (L/day) and total fat and protein solids (kg)] were allocated into five groups of equal observations, rated 1–5 with 5 the highest and 1 the lowest. Scores were combined to make a total production score, out of 10.
2. SCC ($\times 10^3$ cells/ml milk) was allocated into five groups of equal observations rated 1–5 with 5 the lowest and 1 the highest. For veterinary treatments, cows were given a 1 if they received no treatments and 0 if they had been given antibiotics or an alternative (e.g., for mastitis or metritis or other, e.g., for lameness, milk fever or pain/inflammation) at least once since the previous collection date, which was added to the SCC category resulting in a total health score, out of 6.
3. For desirable FAs (OA, CLA9, n-3, and EPA + DPA + DHA), concentrations were ranked and allocated to five equal groups with a score of 5 was given to the highest and 1 to the lowest group, whereas undesirable FA (often consumed in excess) (C12:0, C14:0, C16:0, n-6, and n-6/n-3 ratio) scores were reversed, 5 to the lowest group. FA categories were combined to create an FA score, out of 45.

These individual assessments were then used to calculate a single score (out of one) for each cow record using two alternative approaches. The score weightings are based organic and LI values, the AHDB Spring and Autumn calving indices (60)

TABLE 2 | Effect of breed on components of low-input-production: production (milk yield and total fat and protein solids), health (health treatments and SCC), and nutritionally relevant FA in milk (expressed as a percentage of the entire FA profile).

	AYRX[a]	HF	HFJE	HFSR	JEX	NZFX	SH	SRX	Sig[b]
n	100	325	184	274	121	90	80	140	
Days in milk	154 ± 94.25	182 ± 96.89	157 ± 102.23	161 ± 94.26	134 ± 85.21	138 ± 96.88	153 ± 106.91	151 ± 93.30	ns
Concentrate feed (kg/day)	3.0 ± 1.54	3.3 ± 2.15	3.6 ± 1.68	4.5 ± 2.45	3.1 ± 2.49	3.5 ± 3.00	3.3 ± 1.49	3.1 ± 2.55	NA
Conserved forage (kg/day)	3.7 ± 4.91	6.1 ± 5.25	6.5 ± 5.65	5.6 ± 5.48	3.1 ± 3.80	1.7 ± 2.94	8.2 ± 4.78	3.2 ± 4.09	NA
Production									
Yield (L/day)	20.2 ± 7.33	21.2 ± 8.67	21.8 ± 8.89	21.9 ± 9.50	17.9 ± 7.47	20.1 ± 7.08	17.8 ± 8.81	19.7 ± 7.68	***
Solids (fat and protein) (kg/day)	1.7 ± 0.799	1.6 ± 0.618	1.8 ± 0.704	1.7 ± 0.627	1.7 ± 0.652	1.7 ± 0.555	1.5 ± 0.763	1.6 ± 0.516	***
Health									
SCC ($\times 10^3$ cells/ml milk)	243 ± 454.2	234 ± 584.5	248 ± 782.1	293 ± 885.4	247 ± 664.1	232 ± 944.9	261 ± 688.2	170 ± 400.8	ns
Treatments	0.41 ± 0.818	0.34 ± 0.713	0.35 ± 0.670	0.24 ± 0.549	0.12 ± 0.369	0.08 ± 0.343	0.09 ± 0.284	0.18 ± 0.527	**
Median SCC ($\times 10^3$ cells/ml milk)	78.5	78.0	70.0	73.0	84.0	56.5	89.0	73.0	
FA profile									
C12:0[c]	3.2 ± 0.797	3.3 ± 0.807	4.0 ± 0.918	3.6 ± 1.003	3.9 ± 0.832	3.4 ± 0.822	3.7 ± 0.844	3.8 ± 0.771	**
C14:0	10.9 ± 1.82	11.4 ± 1.53	12.2 ± 1.69	11.8 ± 1.56	11.6 ± 1.45	10.7 ± 1.74	11.6 ± 1.49	11.9 ± 1.40	**
C16:0	29.6 ± 4.79	32.5 ± 4.92	32.9 ± 6.23	31.4 ± 3.99	31.4 ± 6.32	29.6 ± 5.10	29.9 ± 3.93	31.3 ± 5.68	***
n-6	1.6 ± 0.300	1.7 ± 0.532	1.6 ± 0.419	1.6 ± 0.462	1.4 ± 0.434	1.6 ± 0.437	2.1 ± 0.454	1.4 ± 0.444	**
n-6/n-3	1.0 ± 0.309	1.4 ± 0.655	1.4 ± 0.441	1.3 ± 0.485	1.1 ± 0.526	1.3 ± 0.765	1.9 ± 0.887	1.1 ± 0.514	*
OA	20.3 ± 3.87	18.8 ± 3.87	16.6 ± 4.02	19.5 ± 4.01	17.8 ± 4.45	20.2 ± 4.22	20.2 ± 3.46	18.6 ± 4.20	**
CLA9	0.99 ± 0.418	0.88 ± 0.507	0.67 ± 0.451	0.79 ± 0.416	0.93 ± 0.491	1.03 ± 0.454	0.74 ± 0.602	0.91 ± 0.417	ns
EPA + DPA + DHA	0.23 ± 0.074	0.20 ± 0.073	0.19 ± 0.056	0.19 ± 0.046	0.23 ± 0.070	0.22 ± 0.085	0.20 ± 0.083	0.21 ± 0.056	**
n-3	1.7 ± 0.460	1.4 ± 0.513	1.3 ± 0.450	1.3 ± 0.295	1.4 ± 0.412	1.5 ± 0.504	1.4 ± 0.650	1.4 ± 0.295	***

[a] *AYRX, Ayrshire cross; HF, Holstein/Friesian; HFJE, Holstein/Friesian × Jersey; JEX, Jersey cross; NZFX, New Zealand Friesian cross; SH, Shorthorn; SRX, Scandinavian Red cross.*

[b] *P-values < 0.05. ***$P < 0.001$, **$P < 0.01$; *$P < 0.05$, t: $P < 0.1$, ns: $P > 0.1$.*

[c] *C12:0, Lauric Acid; C14:0, Myristic Acid; C16:0, Palmitic Acid; n-6, omega-6; n-6/n-3, omega-6/omega-3 ratio; OA, Oleic Acid; CLA9, Conjugated linoleic acid (C18:2, c9t11 isomer); EPA + DPA + DHA, eicosapentaenoic acid + docosapentaenoic acid + docosahexaenoic Acid; n-3, omega-3.*

Mean ± standard deviation (SD) and ANOVA p-values.

and the premium offered for FA quality by Organic Valley's Grassmilk® (29).

- Weighted health score: the scores were weighted at 30% production, 50% health, and 20% FA.
- Weighted production score: 60% production, 30% health, and 10% FA.

For example:

Weighted health score = 30% * (production score/10) + 50% * (health score/ 6) + 20% * (FA score/45).

RESULTS

Low-Input-Production and Principal Components Analysis

The PCA result is displayed in **Figures 1**, **2**. On D1, 46% of the total variance was explained by PC1 (29%) and PC2 (17%). On D2, 43% of the variance was explained by PC1 (25%) and PC2 (18%). On D3, 51% of the variance was explained by PC1 (31%) and PC2 (20%). On D4, 55% of the variance was explained by PC1 (29%) and PC2 (26%).

The individual farm had major influences on LI-P, especially on D4 (autumn 2012) (**Figure 1**), where cows from the same farm are clearly clustered together. Farm 7 cows are tightly clustered in the negative PC1 axis and positive PC2 axis, whereas Farm 14 cows are clustered in the negative PC1 and PC2 axis. The beneficial FAs n-3 and CLA9 and EPA + DPA + DHA generally occurred close together in PCA ordination space, whereas the detrimental saturated FAs C12:0, C14:0, and C16:0 are together in the opposite axes quadrants on all four sampling dates, D1–D4 (**Figure 2**).

Interpretation of **Figure 1** is aided by cross-referencing with **Figure 2** to superimpose the latter onto **Figure 1**. For example, on date D1, many cows from Farm 8 are associated with high levels of CLA9 and OA in milk. In contrast, Farms 2, 3, and 11 have higher saturated FA: C12:0, C14:0, and C16:0 concentrations. However, D2 cows from Farms 6, 15, and 16 are associated with the beneficial FA EPA + DPA + DHA and CLA9 and Farm 17 with n-6 and a high ratio of n-6/n-3. Across all four sampling dates, Farm 7 (yellow) stands out for producing milk with elevated n-6 content and n-6/n-3, although no farm is consistently associated with beneficial FA in milk.

Effect of Breed on Low-Input-Production

The mean values for the components of LI-P for the eight most common breeds and crosses are shown in **Table 2**. Averaging data (over four dates) from multiple farms with similar breed combinations indicated that the individual parameters used to define LI-P did significantly differ between breeds, although, again, there was no difference in the stage of lactation between the breeds in this data set. The highest yielding breed was the HF (21.2 L) and the HF crosses (HFJE: 21.8 L and HFSR: 21.9 L), and

TABLE 3 | Effect of breed on health score and production score ± standard deviation.

	NZFX[a]	AYRX	HFJE	SRX	HFSR	JEX	HF	SH	Sig[b]
n	90	100	184	140	274	121	325	80	
Health[c] score	0.60[d] ± 0.136	0.60 ± 0.167	0.58 ± 0.164	0.58 ± 0.143	0.57 ± 0.163	0.57 ± 0.167	0.57 ± 0.165	0.50 ± 0.133	*
Rank	1	2	4	3	5	7	6	8	
Production score	0.61 ± 0.169	0.60 ± 0.202	0.61 ± 0.194	0.59 ± 0.170	0.59 ± 0.198	0.59 ± 0.199	0.57 ± 0.198	0.50 ± 0.197	**
Rank	1	3	2	4	5	6	7	8	

[a] *AYRX, Ayrshire cross; HF, Holstein/Friesian; HFJE, Holstein/Friesian × Jersey; JEX, Jersey cross; NZFX, New Zealand Friesian cross; SH, Shorthorn; SRX, Scandinavian Red cross.*

[b] *P-values < 0.05. **P < 0.01; *P < 0.05.*

[c] *Maximum possible score = 1.*

[d] *Where mean values are equal the lower standard deviation dictates the rank.*

D1, autumn 2011; D2, spring; D3, summer; D4, autumn 2012.

HFJE had the highest fat and protein solids (1.8 kg). However, HF and the crosses had the lowest concentrations of long-chain n-3 FAs [EPA + DPA + DHA [HF: 0.20%, HFJE: 0.19%, and HFSR: 0.19%]], and HFJE and HFSR had the lowest total n-3 (both 1.3%). Additionally, HFJE had the highest concentrations of C12:0 (4%), C14:0 (11.2%), and C16:0 (32.9%). AYRX had the lowest concentration of C12:0 (3.2%), C16:0 (29.6%), and n-6/n-3 (1.0) and also had the highest concentration of OA (20.3%), CLA9 (0.99%—not significant), EPA + DPA + DHA (0.23%), and n-3 (1.7%). SH had the lowest average daily yield (17.8 L) and solids (1.5 kg), a high average cell count (261 × 10^3 cells/ml milk), the highest concentration of n-6 (2.1%) and n-6/n-3 (1.9) and had a low concentration of EPA + DPA + DHA (0.20%), n-3 (1.40%), and CLA9 (0.74%).

There was no difference in the SCC between breeds, but 12% of SCC recordings from individual cows were above the EU standard, ranging from 400,000 to 9,000,000 cells/ml milk. This resulted in SCC having a very wide standard deviation; therefore, the median values were included in **Table 2** (as well as mean values) for a more representative SCC status. The median cell counts for each breed are below 90,000 cells/ml milk. Most health treatments were given to the AYRX (0.41), whereas the NZFX (0.08) and SH (0.09) received the least.

Low-Input-Production Score

The two LI-P scores for each breed combination are presented in **Table 3**. The NZFX was the highest-scoring breed, ranking first under both the weighted health and production scenarios, whereas SH was the lowest-scoring breed ranking last in both scenarios. The largest change in the LI-P score with the different weightings was HFJE, which scored fourth in the health score, but second, emphasizing production.

DISCUSSION

The data collected for this paper provides valuable information from commercial farms of direct practical application for farmers, in an area lacking in the scientific literature. As a study monitoring on-farm activities, many variables are not controlled, but the statistical model mitigates some of these effects. The data collected is of sufficient quality and range to provide invaluable insights into LI-P systems in the UK. This includes the effects of breed combinations on LI-P and determining how and why breeds are suited to different farms. Although this paper does not draw definitive conclusions, it explores the current status of dairy breeding strategies and highlights how farmer's decision-making should direct future LI (cross) breeding research.

Low-Input Production

The influence of farm management (e.g., breed, diet, calving date, and nutrition) on milk composition, yield, and animal health has been well documented (28, 29, 61, 62). These effects are seen in the PCA analyses (**Figure 1**), where each farm system clusters (apart from D1, with fewer records). Most organic cows were autumn calving, and many LI were spring calving (**Table 1**). Due to this collinearity, it would be statistically difficult to identify if management (organic vs. LI) or stage in lactation affected LI-P. Additionally, the collinearity violates the assumptions of most statistical models on the independent influence of factors; it would therefore be incorrect to separate these in an attempt to identify whether the management or lactation stage has the strongest influence on LI-P. It is clear, nevertheless, from **Figure 1** that LI-P is very closely associated with individual farms. The specific aims and preferences of individual farmers result in decisions about suitable breeds for that particular system, and as these management decisions are unique to each farm, the effect of breed on LI-P is multifaceted.

Feeding

Although the scoring system aimed to identify breeds well-suited to LI farming, there were differences in supplementary feeding between breeds, which could influence findings. The amount of concentrate feed offered was fairly consistent across breeds (from 3.0 to 4.5 kg per head per day), although conserved forage offered was more variable, ranging from 1.7 to 8.6 kg per head per day. Increasing fresh forage in the diet influences milk fat composition, raising CLA9 and omega-3 (29, 63), and if we assume fresh forage consumption is indirectly proportional to the amounts of other feeds offered (32), we could expect the ranking of the breeds to follow a similar pattern—driven by the positive influence of milk fat composition to these composite scores. However, although this holds for the best and worst ranked breeds under both scores [NZFX ranked first on both scores, had the lowest supplementary feeding, the highest concentration of CLA9 [1.03%], and the second highest concentration of n-3 [1.5%] and SH, eighth on both scores, had the highest level of supplementation offered], the ranking of all other breeds does not follow combined supplementary feeding rates. The AYRX outranked both SRX and JEX in health and production scores, whereas HFJE outranked JEX in both scores and SRX in production score; yet, both AYRX and HFJE received more supplementary feed than JEX and SRX. Despite receiving higher levels of supplementary feed than JEX and SRX, milk from AYRX cows had the highest concentration of n-3 (1.7%) and second-highest CLA9 (0.99%) among all the breeds. At the other end of the health and production ranking, JEX cows were judged seventh and sixth yet were offered the second-lowest level of supplementary feeding, hence expected to have a relatively high grazing intake. Despite the evidence that feeds management has the greatest impact on the FA profile (29, 61), this study sampled milk from a wide variety of farms and breeds where the effect of diet was possibly minimized, potentially displaying differences between the breed.

Animal Health

SCC is an indication of udder health, cow welfare, and milk quality. Generally, if SCC is below 100,000 cells/ml milk, the cow is considered healthy, whereas above 200,000 cells/ml milk, the cow is likely to have at least one mastitic quarter, and, although some cows naturally have higher SCC, above 400,000 cells/ml milk is deemed unfit for human consumption by the EU (64). During the study, only 19% of high SCC (>400,000 cells/ml milk) cows received a health treatment (veterinary or other). Under EU organic guidelines, cows are expected to resist infection through effective management (65), suggesting that the farmers in this study were more likely to allow cows to build immunity to fight infection rather than treat with antibiotics. Interestingly, HF and HF crosses were responsible for 41% of the high cell counts, whereas only 4% of cows with SCC over 400,000 cells/ml milk were the best performing breed (NZFX), providing evidence that NZ genetics have effective health traits. Additionally, this portion of animals with high cell counts highlights the need and potential benefit of breeding for improved health traits, especially in organic production systems, when prophylactic treatment is not an option. A recent report found antibiotic use in livestock decreased 40% from 2013 to 2017 (66), but there is still pressure on dairy industries to reduce antibiotic use due to antimicrobial resistance, which already impacts human and animal health (67).

Breeding Objectives

The effect of forage diets on milk FA profile has been well-researched (29, 46, 61), but forage conversion by diverse breeds in LI systems has not. Most of the research into forage conversion has predominantly focused on HF (68, 69). Other studies have suggested that the JE × HF cross is better suited to a pasture-based system (70–72) but only compared with HF. As a generalization, HFs were bred for their production traits rather than milk composition or health traits (73). This was reflected in this study, as cows with HF genetics had the highest yield (21.2–21.9 L/day), and HFJE had the most protein and fat solids (1.8 kg/day), but SCC was highest for HFSR (294,000 cells/ml milk) and HFJE (third highest: 248,000 cells/ml milk). Although HFs are important in the UK, and their crosses have worked well in some grazing based systems, further research into forage conversion in more diverse breeds is needed to improve LI and organic dairy systems. Although cattle diets might be the dominant factor controlling milk FA profiles, there is also evidence that heritability affects milk fat composition both within and between breeds (20, 34). This suggests a combination of feeding forage and selective breeding may optimize FA composition for consumer health. Despite breeding bodies and milk purchasers prioritizing milk fat and protein content, there is currently no premium to reward fat composition in the UK. Organic Valley's "Grassmilk^{TM}" (USA) receives a 15% premium above organic prices for n-3, CLA9 content, and n-6/n-3 ratio (74). This demonstrates a market for optimizing milk fat composition and thus creates a marketing opportunity for UK milk.

An alternative benchmark for LI dairy is the New Zealand National Breeding Objectives, in which grazing is emphasized and priority placed on forage conversion, the yield of milk components (protein and fat %), health, and fertility (75). Based on the importance of forage in NZ dairying, it is unsurprising that the NZ Friesian cross outperformed all other breeds in this study, ranking first in both performance scores (**Table 3**). Although the breed is an important component of management, diet is the strongest factor that influences FA composition in milk (46), whereas high intakes of forage in the diet increase milk n-3 concentrations and reduce n-6/n-3 ratio (28, 29). The contribution of milk FA profile to the LI-P score identifies a breed's ability to graze and use grass efficiently; therefore, the concentrations of n-3, CLA9, or n-6/n-3 ratio in milk could be used to predict how well forage is converted to milk.

Effect of Breed on Low-Input-Production

The results of this study confirm that although management on individual farms affects LI-P, the breed also plays an important role. Despite ranking last under both scenarios (**Table 3**), shorthorns are well known for their positive temperament, high fertility, and efficiency in converting forage to milk (76), which are all metrics important for LI dairying although not formally analyzed in this study. In terms of desirable milk–fat composition, AYRX had the most desirable FA profile. However, AYRX yielded less milk (20.2 L/day) than the more productive HF crosses (21.2–21.9 L/day) and came fourth for SCC (243,000 cells/ml milk). Despite this, the AYRX ranked second in health and third in the production score. Ayrshires are commonly used in organic systems because of their ease of management, forage to milk conversion, and overall health and longevity (77). The Jersey crosses did not rank well (rank = seventh weighted health and sixth in production score), but the Jersey has many desirable traits for organic and LI systems (78). The Ayrshire, Shorthorn, and Jersey have merits beyond the scope of this study to measure; additionally, the low UK population of these breeds offers less scope for selection than the more popular HF.

Scandinavian Reds have a reputation for good udder health (79), and the SRX had the lowest average SCC (170,000 cells/ml milk); however, the HFSR had the highest average SCC (294,000 cells/ml milk), but interestingly, the median of both SR crosses was the same (73,000 cells/ml milk). This suggests that farms with a high mastitis challenge might cross HF with SR due to their reputation and breeding history, potentially instead of changing management to reduce infection risk.

The breeds in this study are generally popular and well-suited to organic and LI farming. Despite this, many of the desirable traits for organic and LI dairying were not measured in this study (forage conversion, fertility, temperament, ease of calving, etc.). It is easy to pick and choose the characteristics that could make a breed look "better" or "worse;" it can be subjective, but farmers make their decisions based on their priorities and what works best for their specific system, and despite the low score for LI-P, many of these breeds are all essential for LI and organic dairying.

Heterosis

Another important factor to consider in a crossbreeding program is heterosis and the effects of back-crossing, as demonstrated from the breeding approach used on these organic and LI farms. All farms had at least three core breeds (**Table 1**), most of which get crossed and back-crossed. In this study, of the 1,070 cows selected from 17 farms, 40% were F1 (first generation crosses), 40% were F2 or subsequent generations, and only 20% were purebred. This confirms that in these LI and organic systems, cross-breeding is essential to develop robust, productive cows. As discussed, much of the published research is centered on HF crosses, which, as demonstrated by this study, are not representative of LI and organic management practices on the UK farms studied. Additionally, maximizing the benefits of hybrid vigor can be complicated and unpredictable, but challenging organic conditions often make heterosis worthwhile (80). Partially due to the emphasis on specific breeds, such as HF, there is little readily available, independent advice for farmers with alternative breeds, regarding heterosis. Further studies are needed using a diverse range of breeds to fully understand this effect and the benefits it offers (81), but as demonstrated by the predominance of crossbreeding in this study, the industry is ahead of the science—farmers are investigating the effects for themselves.

Genotype by Environment Interaction

The genotype by environment interaction (GxE) is key to distinguishing between intensive and LI or organic breeding programs. Nauta et al. (18) first explored the GxE differences between organic and conventional dairying and reported heritabilities of SCC and production traits that warrant a re-ranking of dairy bulls for organic systems. The abundance of cross-breeding in this study indicates that farmers are learning about how (cross) breeds interact with their environments, potentially observing heterosis and GxE independently, suggesting that for LI and organic breeding objectives to be successful, the science will have to align with farming practices. Rodriguez-Bermudez et al. (23) conclude that by breeding for intensive systems, organic cows will not meet their potential due to the impact of GxE interactions on performance. To improve efficiency in LI/organic dairying, genotypes must be well-adapted to their systems, which has less emphasis on production but a greater focus on fertility and resilience (81). Keeping the GxE interaction in mind when developing and evaluating breeding programs is essential to allow livestock to meet their potential—regardless of the system that they are kept in.

To conclude, this paper highlights weaknesses in current UK breeding programs for LI and organic dairying due to limited past research on forage conversion to healthy milk and a bias toward HFs. The lack of robust scientific evidence necessary to advance breeding systems has resulted in the science-base often being behind best farming practices. Evidence from this study indicates that New Zealand Friesian and Ayrshire genetics could suit some LI/organic farms. Thorough further research is needed to explore the GxE and forage intake and conversion to meet the true potential of cows under these management systems. The ideal scenario would be for farmers to access an interactive flow chart to guide them through breed selection based on inputs, constraints, and priorities within their system, resulting in an indexing system unique to each farm.

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 all procedures were acceptable to internal ethical review, in accordance with EU Directive 2010/63/EU for animal experiments and approved by the Animal Welfare and Ethical Review Body at Newcastle University. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

HD conducted the data analysis, writing, and formatting of the manuscript. EC and SS collected the data and managed all the laboratory work. RS provided statistical advice and editing. CL, GB, and SS developed the experimental design, editing, style, and formatting. All authors contributed to manuscript revision, read, 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.2020.544149/full#supplementary-material

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Local Small Ruminant Grazing in the Monti Foy Area (Italy): The Relationship Between Grassland Biodiversity Maintenance and Added-Value Dairy Products

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The literature indicates that grazing small ruminants, when adequately managed, contributes to grassland biodiversity maintenance. On the other hand, milk and cheese from grazing animals show higher nutritional and aromatic quality than those from stall-fed animals. The relationship between the two issues has rarely been addressed. This article provides information for a discussion of this relationship. First, two case studies are reported. Local breeds of small ruminants fed by grazing on pastures within the Special Area of Conservation "Monti Foy" in the Northwestern Basilicata region (Italy), with a stocking rate of 4.0 LU ha^{-1} $year^{-1}$, showed the best effectiveness for the maintenance of grassland botanical biodiversity. Milk and cheese from pasture-fed goats showed higher contents of beneficial fatty acids, phenols, and vitamins A and E; higher degree of antioxidant protection; and richer volatile compound profiles, in particular for terpenes content. Finally, some recommendations for the management of grazing systems in similar mountain areas are offered, including a viable approach for land managers to preserve the grassland biodiversity of pastures and provide high-quality products that are valuable both for their nutritional quality and for their contribution to the economic sustainability of mountain communities.

Keywords: grazing, mountain, local breed, small ruminant, dairy product quality, biodiversity maintenance

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INTRODUCTION

The grazing system has been an important component of the Mediterranean environment for millennia; thus, it represents a valid tool for managing and preserving that environment (1–3). In the Mediterranean environment, various ecosystems coexist, herbaceous, bushy, and woody, and are not always in balance; however, they are prone to rapid recovery and are thus considered very resilient (4, 5).

Rangeland management is generally difficult due to the complexity of the ecosystems, with great diversity in plant communities, soils, and grazing practices (6, 7). Several authors have pointed out the importance of a correct livestock management on overgrazed or undergrazed areas, in order to preserve or increase the floristic richness and the nutritional value of grassland (8–10) and forage and to improve the animal productive performances (milk yield) (11).

Good management of extensive silvopastoral systems could play an important role in the delivery of many ecosystem services, as was recently exhaustively stated by the UK National Ecosystem Assessment (12). In mountain areas characterized by forests/shrubs and meadows, well-managed pastoral activity could be considered a tool for landscape preservation, fire prevention (13), and grassland biodiversity maintenance, contributing to the overall economic benefit of mountain communities.

Grazing behavior is another key factor in specific landscape and pasture biodiversity determinism (14). Grazing behavior has important consequences; in addition to contributing to animal nutrition, it affects the specific characteristics, features, and quality of animal products (milk and dairy products) (15–17). When local breeds are reared in an adequately managed and rational grazing system, they are successful in preserving grassland biodiversity. When they browse the apices and flowers of plants that may be unpalatable for cosmopolitan breeds, local breeds limit the diffusion of various unpalatable and weed species and maintain the floristic balance, thus enhancing the nutritional value of pastures (18).

To protect pastoral areas, the European Union has developed a series of measures (EC Reg. No. 796/04 and subsequent amendments). In particular, Standard 4.6 ("Minimum Livestock Stocking Rate and/or Appropriate Regimes") aims to "ensure a minimum level of maintenance and avoid the deterioration of habitats" and to protect pastures, especially through avoiding grassland degradation in certain ecologically significant areas [Annex IV of Council Regulation (EC) No. 1782/2003].

In the central area of the Basilicata region, which is mostly mountainous, there is a deep-seated tradition of dairy products from small ruminants reared in extensive and semiextensive systems, expressing the interaction among the environment, animals, and human practices (19). The Special Area of Conservation "Monti Foy" is interesting in terms of biodiversity maintenance. However, the misuse of pasture resources can affect the balance of the entire system (20). The mountainous area is characterized by a semiextensive livestock system, with local breeds being reared at pasture, resulting in overgrazing situations in summer, at a mean stocking rate of 6 LU ha^{-1} $year^{-1}$, and undergrazing in other seasons. This grazing system, in addition to the expansion of plants indicators of pasture degradation such as thistles (*Cirsium arvense*, *Carduus* spp.), asphodels (*Asphodelus ramosus*), ferns (*Pteridium aquilinum*), and brambles (*Rubus fruticosus*), has led to the worsening of the grassland composition.

The diet of grazing animals, especially sheep and goats, varies according to the season due to the plant species available for grazing, the plants' phenological stage, climate conditions, and feeding behavior (plants and aerial parts browsed by animals) (21). This diversity affects the content of volatile compounds in milk and cheese, particularly the presence and abundance of molecules that affect flavor and aroma (22, 23). These volatile compounds are found in greater amounts in milk and dairy products when the animals are fed at pasture, particularly when they browse dicotyledons (15, 24–27). In addition, several studies have shown that ruminant products from grazing systems show variation in the content of beneficial compounds, such as particular classes of fatty acids (FAs), phenols, and vitamins A and E, and a higher degree of antioxidant protection (DAP), and that these contents are higher overall than in products from housed animals. In particular, the increase in FAs of healthy interest in milk occurs already 3 days after the abrupt transition from indoor to pasture diet (28). Furthermore, these products are perceived more positively by consumers because of their richer sensory profile (29, 30).

Vast areas of rangelands across the world are being grazed with increasing intensity. The interactions between livestock production and grassland biodiversity and conservation are debated (1); however, their connections with the quality of animal products have been less focused so far. The main aim of this work is to provide information for a discussion, based on published scientific studies, on (a) grassland biodiversity and conservation, (b) mountain dairy product quality, and (c) interactions between them in a specific mountain area. The discussion aims to lead toward a hypothesis for a revaluation of the traditional management system of the mountain agrosilvopastoral production chain, which is able to produce high-quality food and maintain and enhance grassland biodiversity.

CASE STUDY 1: GRAZING SYSTEM, GRASSLAND BIODIVERSITY, AND CONSERVATION

At the experimental farm (1,230 m a.s.l.) of the CREA–Research Center for Animal Production and Aquaculture in the municipality of Potenza (southern Italy), several studies have been carried out on the relationships between the grazing behaviors of local breeds and pasture biodiversity. The farm is included in the mountain Special Area of Conservation "Monti Foy" (40° 37′ N, 15° 42′ E) (defined by EU Habitats Directive 92/43/EEC), which is included in the list of Sites of Community Importance in the Mediterranean biogeographical region (IT9210215). In this area, the semiextensive livestock system is based mainly on local breeds (Garganica and Capra di Potenza goat breeds and Gentile di Puglia and Merino-derived sheep breeds). In the routine management of the experimental farm, sheep were fed at pasture with 2.2 LU ha^{-1} $year^{-1}$ stocking rate, whereas goats were reared at 2.1 LU ha^{-1} $year^{-1}$ stocking rate in separate fields.

A recent study (20) aimed to evaluate the effect of different stocking rates on the botanical parameters of natural pastures. Dry and pregnant Gentile di Puglia sheep were assigned to the permanent natural pasture previously grazed by goats for over 25 years, with an average potential yield of 5 t ha^{-1} $year^{-1}$ (rich pasture). Ewes were allotted to three groups and assigned to three plots, characterized by Natura 2000 habitat 6210 seminatural dry grasslands *Festuco-Brometalia* (plot 1) and Natura 2000 habitat 6510 *Lowland hay meadows* (plots 2 and 3), with stocking rates of 0.2 LU ha^{-1} $year^{-1}$ (plot 1), 4.0 LU ha^{-1} $year^{-1}$ (plot 2), and 6.0 LU ha^{-1} $year^{-1}$ (plot 3), the two limits indicated by the EU Standard 4.6 and an overgrazing situation (20). The animals grazed 8 h per day from early May to late September, sheltered overnight, and received pasture hay *ad libitum* as dietary supplementation to the grazing intake. The hay was produced from an area in the same farm, out of the three plots, characterized by seminatural dry grasslands *Festuco-Brometalia*. In the plots, visual assessment was carried out on seven functional groups: grasses, legumes, other species, palatable vs. unpalatable plants, thorny species, shrub species, and bare soil (expressed as percentage of coverage). The study on grazing behavior and the effect on grassland composition, combined with the results of the degradation of vegetation and biodiversity, revealed the limits of the monospecies flock mostly in the undergrazed plot (0.2 LU ha^{-1} $year^{-1}$). Plot 1 showed a decrease in palatable species (from 98 to 85%) and a proportional increase in unpalatable and thorny species. Thorny species (*Carduus* sp.) increased from rare to >20%, with *Crataegus monogyna* (hawthorn) and *Ononis spinosa* increasing up to 20–25% in comparison to the level under the previous grazing management system (grazing goats with a stocking rate of 2.1 LU ha^{-1} $year^{-1}$). Plot 2 showed the best effectiveness for the maintenance of the grassland botanical composition, with palatable species (30% each for grasses, legumes, and others) unvarying at 90%, thorny (thistles) species at <5% and unpalatable (ferns) species at 5%. In plot 3, a severe drop of the palatable species was observed in summer, as well as increase in bare soil (from 0 to 30%) and increase in/appearance of thistles/asphodels. Afterward, the area was interested by a great fire (summer 2017) during the 6th year of grazing by solely sheep; the extension of the event was explained also with the missing pruning of the bushes, usually done by grazing goats, and the abundance of dry grass in the undergrazed areas (unpublished data).

CASE STUDY 2: QUALITY OF DAIRY PRODUCTS FROM GRAZING SYSTEM

Studies were conducted at the CREA experimental farm to evaluate the effect of feeding at pasture compared with other feeding treatments on volatile organic compounds (VOCs), FAs, α-tocopherol, retinol, and DPA in goat milk and cheese. VOC content was assessed by multiple dynamic headspace extraction and gas chromatography (GC)–mass spectrometry (31). FA separation and quantification were carried out using a GC, as reported by Di Trana et al. (32), and fat-soluble vitamins and DPA were assessed according to Pizzoferrato et al. (33). Local Mediterranean Red breed goats were used. A first study evaluated the VOC content and profile in the milk of goats fed (a) at pasture (grazing), (b) pasture hay harvested from the same grazing area, and (c) mixed hay (alfalfa, perennial rye grass, and orchard grass). The difference observed among the three diets could be linked to the contributions of the different plant species measured in the diet (**Table 1**). Milk from the grazing goats showed significantly higher monoterpene and sesquiterpene content than milk from the goats fed on pasture hay and mixed hay. The contribution of forbs (38%) might explain the result.

A second study was carried out in the same area to examine the effect of pasture vs. indoor feeding systems during winter, spring, and summer on α-tocopherol and retinol, FA content, and DPA in goat milk and cheese. Two homogeneous groups were used: goats grazing 8 h per day on native herbaceous pasture (G) and goats housed and fed *ad libitum* with hay harvested from the same native pasture (H), both supplemented with concentrate feed (600 g/head per day at 13% CP). The results showed that the qualitative profiles of milk and cheese were very different between the G and H groups throughout the seasons (**Figures 1A,B**). Tocopherol and retinol increased in milk by 61.3 and 20.0% in the G and H groups, respectively. The same trend was observed for DAP; this index was 61.6% higher in milk from grazing goats than in milk from the housed goats fed hay. Highly significant differences between the milk fat quality of the G and H groups were detected. In fact, conjugated linoleic acid (CLA) and ω-3 FA content were higher in milk from goats grazing on native pasture than in milk from housed goats (**Figure 1A**). The cheese quality almost completely reflected the milk quality. Cheese produced from the G group goat milk was richer in sesquiterpenes, tocopherol, and retinol than cheese produced from the H group milk; similarly, the DAP index was higher in cheese from the G group than in cheese from the H group (**Figure 1B**). The results confirmed that feeding on a grazing basis conferred higher total quality on milk and cheese than the housing feeding system throughout the whole grazing season.

TABLE 1 | Monoterpenes and sesquiterpenes content (mean ± SEM) in milk from three feeding systems [from (34)].

Feeding treatment	Grazing	Pasture hay	Mixed hay	
Plants category in the diet (%)				
Grasses	40	36	40	
Legumes	22	33	50	
Forbs	38	31	10	
Milk VOC (ng/L)				
Monoterpenes	$2,031.0^a \pm 429$	$1,374.0^a \pm 226$	$718.0^b \pm 154$	$P < 0.05$
Sesquiterpenes	$4,480.0^a \pm 626$	$2,334.0^b \pm 324$	$610.0^c \pm 152$	$P < 0.05$

[a,b,c]*Letters mean significant difference among means. The significance of the diet botanical composition (plants category) was not detected.*

DISCUSSION

Feeding Management and Grassland Biodiversity and Conservation

The results of the stocking rate case study 1. Sepe et al. (20) are in agreement with Petz et al. (36), who identified three livestock stocking rate categories at pasture, indicated by the authors as "grazing intensities": low (0.0–0.4 LU ha^{-1} $year^{-1}$), moderate (0.4–0.6 LU ha^{-1} $year^{-1}$), and high (0.6–1.0 LU ha^{-1} $year^{-1}$) grazing intensities, calculated as the ratio between biomass grazed and biomass available for grazing. The results showed that, on average, only 4.2% of the biomass produced annually was consumed by livestock. Erosion prevention was 10% lower in areas with high grazing intensity than in areas with low grazing intensity. Therefore, the authors found lower

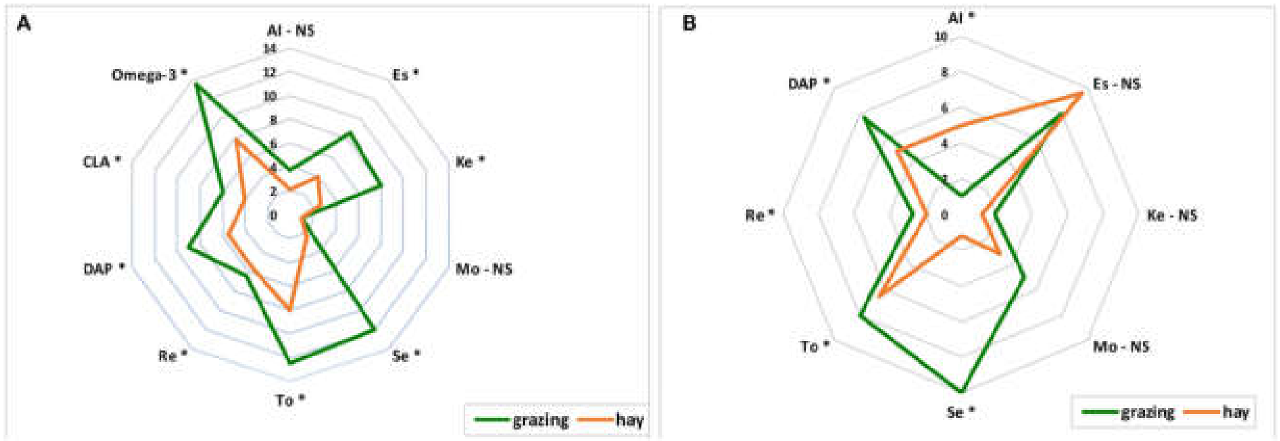

FIGURE 1 | Qualitative profile of goat milk **(A)** and cheese **(B)** from grazing and hay feeding system [from (35)]. AI, alcohols; Es, esters; Ke, ketones; Mo, monoterpenes; Se, sesquiterpenes; To, tocopherol; Re, retinol; DAP, degree of antioxidant protection; CLA, conjugated linoleic acid. $^{*}P < 0.05$; NS = not significant.

biodiversity values, lower capacity for erosion prevention, and unsustainable forage utilization in high-grazing-intensity areas. The case study 1 results reported in this article agreed with Petz et al. (36) that grazing systems, when adequately managed, can contribute to the maintenance of botanical biodiversity. The results reported here supported by the aforementioned studies on goat grazing behavior (17, 21), together with the elements of the traditional management system in that area, led the authors to grazing practice recommendations that include the use of local-breed sheep and goats because they are capable of exploiting natural resources in a sustainable manner that protects the environment [as emerged from previous studies reviewed by (18)]. The authors advised a stocking rate of 4.0 LU ha^{-1} year^{-1} to avoid limit situations (undergrazing or overgrazing) in the case of rich pasture and to contribute to the maintenance of grassland biodiversity and conservation, the main reason for which dairy products from mountain systems show high-quality standard, as discussed in the following subsection.

Quality of Milk and Cheese From Pasture-Based Diets

Overall, goat products from grazed herbage revealed higher-quality values, for example, in monounsaturated FA and polyunsaturated FA (PUFA), which are beneficial for human nutrition, and higher total consumer acceptability of cheeses (37). A study on goats grazing on native pasture compared to stall-fed goats revealed an increase in the CLA and ω-3 contents achieved in the milk of goats fed at pasture (32). Moreover, the docosahexaenoic acid and eicosapentaenoic acid content reached interesting levels in the milk fat of grazing goats that may be linked to the content of precursors in the diet, such as long-chain omega-3 PUFA. These results agree with Decandia et al. (38), who found higher CLA and VOC content, particularly ketones and aldehydes, in the milk of goats browsing a Mediterranean lentisk-based shrubland than in the milk of housed goats. Diminishing amounts of fresh grass percentages in the diet of Camosciata goats led to significant decreases of vaccenic, rumenic, and α-linolenic acids in milk, thus determining a worsening of the health value of milk fat associated with an increase in the percentages of hypercholesterolemic saturated FAs (39). A sudden transition of dairy Valdostana goats from winter indoor to pasture-based diets significantly affected the concentrations of FA in milk already 3 days after the diet change. In milk short- and medium-chain FA rapidly decreased after transition, whereas the sum of CLA isomers and omega-3 FAs markedly increased (28). A study conducted in Northern Europe confirmed that the milk from grazing goats had significantly higher fat, protein, and total nonfat solids than the milk from goats kept indoors (40). Grazing caused significantly higher concentrations of vitamin A and D$_3$ than in the milk from goats fed hay. For goats on grass diet, the rumenic acid and $n{-}3$ FA contents of the milk increased significantly. Additionally, the $n{-}6/n{-}3$ ratio in the milk from goats fed grass was significantly lower than that in the milk from goats fed indoor.

Several investigations have reported that the diet ingested by goats influenced milk and cheese polyphenol content. An increase in the total polyphenol content in goat milk and cheese was obtained from grazing animals compared with stall-fed goats (41). These results are in agreement with Cabiddu et al. (42) and Chávez-Servín et al. (43), who observed a feeding system effect (free-range grazing and indoor-fed animals) on phenolic compounds and antioxidant capacity in goat milk, whey, and cheese.

A large study has highlighted the predominant effect of pasture-based diets compared to rations based on hay on the content of fat-soluble carotenoids and vitamins in milk and cheese (44). Pasture-based rations were associated with higher levels of xanthophyll, retinol, α-tocopherol, and total antioxidant capacity (TAC) in cheese than hay-based rations, whereas in milk and cheese a higher percentage of concentrates in the herd diet led to lower xanthophyll and α-tocopherol contents (15, 37, 44, 45).

Regarding VOC content and profile, goats fed with fresh and different meadow species transmit different characteristics to Caciotta cheese that are also perceivable on a sensorial level (22). Seasonal variations in the availability and quality of grazing grass influence the quantitative and qualitative content of VOC compounds in cheese obtained from grazing goats (31, 42, 46). Some volatile compounds, e.g., terpenes, can be used as biomarkers because they can be transferred from herbage to milk and contribute a characteristic flavor to the cheese. Terpenoids and FAs were found to be valuable as chemical fingerprint for the characterization of the dairy cows' feeding regimen (47). Indeed, the authors suggested that coupling terpenoids and FAs information could be suitable for tracing Asiago d'Allevo PDO cheeses produced during the early and late summer grazing and the autumn/winter indoor seasons.

The odor profiles of milk and cheeses were explained in a study where milk and cheese showed significant differences over three seasons, especially in ketones, alcohols, and ester compounds (46). The detection of sesquiterpenes could be extremely useful in distinguishing whether a cheese has been produced with milk from animals fed on pasture or with the total mixed ration system (48). In this context, the traceability of products obtained from grazing animals compared to stall-fed animals represents an ongoing current objective. Future directions converge toward the development of a tool or procedure based on scientific parameters that in synthesis shows indications of the origin of the product and its healthy quality.

Pizzoferrato et al. (33) developed the DAP index, calculated as the molar ratio between an antioxidant compound and a selected oxidation target. It evaluated goat cheese resistance to oxidative reactions. It is noteworthy that DAP values in goat products were 10-fold higher in grazing goats than in stall-fed goats. The DAP index was able to distinguish dairy products when the grazed herbage in the goats' diet exceeded 15%. These results agree with Delgadillo-Puga et al. (49) and Cabiddu et al. (42), who found an increase in PUFA, DAP, and phenol content in the milk of goats reared in shrubland compared to stall-fed goats.

Recently, a new index, the General Health Index of Cheese (GHIC), was developed by Giorgio et al. (50); this index combines in a single value the contributions of several components to cheese quality. It takes into account different indicators of products obtained from animals fed with fresh forage or at pasture: polyphenols, CLA isomers, PUFA, omega-3 FA, and TAC. In addition to CLA, PUFA, and omega-3, which are already known to be health-promoting compounds, polyphenols and total antioxidant capacity were used in GHIC calculations because of health researchers' increasing interest in these compounds. The GHIC index, which combines the positive components found in cheese, seems to distinguish cheeses obtained from different fresh forages.

Dairy products from the grazing system, compared to those from the indoor-fed supplementation strategy, carry a real added value because of their impact on human health because of their higher content of beneficial metabolites (30), as well as the hedonistic and sociological aspects.

The authors refer to the role of small ruminant grazing in the framework of the Millennium Assessment (51). There, the relationship between feeding at pasture and biodiversity is included in the provisioning of habitat services because grazing facilitates the life cycles of animals and plants, prevents the occurrence of less valuable ecological states through the encroachment of bush and/or invasive species, and conserves wildlife and protected areas in coevolved landscapes. In the most important cluster of habitat services, grazing systems support the maintenance of species life cycles and the connection of habitats. The Millennium Assessment showed that "with appropriate actions, it is possible to reverse the degradation of many ecosystem services over the next 50 years, but the changes in policy and practice required are substantial and not currently underway."

CONCLUSIONS

The livestock system based on grazing local breeds can provide benefit to both the environment and the mountain population, given the habitat service that it provides. Two case studies were presented in this article with the aim of presenting two issues concerning the mountain system that are usually considered separately. Combining the outcomes of the aforementioned studies, the authors recommend a management system that revalues the traditional approach. This system, which has traditionally proven to be more sustainable and respectful of the mountain environment, consists of (i) mixed flocks of local breeds of small ruminants, sheep, and goats, in variable percentages (up to 80% sheep and 20% goats); (ii) grazing system with stocking rates ranging from 2.1 to 4.0 LU ha^{-1} $year^{-1}$; (iii) supplementation of diet, during lactation, with native pasture hay and concentrated feed. This management system, in comparison with sheep-only herds, allows high-quality dairy products even in summer, when sheep are in a dry stage (physiological stage after lactation). The transferability of this system to other, similar Mediterranean areas would be limited only by the yield of the pasture. On less rich pastures, the recommended stocking rate would be reasonably lower, i.e., 0.2 LU ha^{-1} $year^{-1}$.

In the mountain livestock system of Monti Foy, the management system recommended in the present article would contribute over time to grassland biodiversity preservation, in addition to preventing fire. In addition, milk and cheese from the grazing system are richer than those from the housed animals feeding system, mainly owing to the higher content of healthy compounds, as well as the hedonistic characteristics. When the relationship between grassland biodiversity maintenance and this quality is taken into account, these products appear worthy of being valued and sold at higher prices, which is a viable way to reward farmers who sustain the struggle to live and produce in mountain areas and encourage them to continue their work and not give up in these tough but incomparable production systems. Finally, the mountain management system recommended in the present article, inspired by the traditional system, offers an approach for mountain area land managers, a viable way to produce high-quality food together with preserving the system.

As a new perspective, further research could aim to find new markers/indicators of the high quality of the products from local breeds in grazing system and more strictly relate them to the mountain system. This request often comes from the stakeholders (farmers/cheesemakers). To this end, a multidisciplinary study may be a viable approach, involving countries in the Mediterranean area with similar mountain systems, to address the complex relation among grassland biodiversity, livestock breeding, and livestock products. The evaluation of those markers would concur with the development of an economic model that can recognize and assign the added value, thus supporting and protecting production systems that would otherwise be less competitive and less economically sustainable.

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.

AUTHOR CONTRIBUTIONS

SC for conceptualization, resources, review, final review, and supervision. MM for analysis of resources, writing—original draft. AD for writing and final review. LS for conceptualization, resources, writing, review, final review, and editing. All authors contributed to the article and approved the submitted version.

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Early-Life Dam-Calf Contact and Grazing Experience Influence Post-Weaning Behavior and Herbage Selection of Dairy Calves in the Short Term

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Rearing dairy calves with their mothers could teach them how to graze, optimizing grass use, and improving their welfare and performance. We tested the short-term effects of dam-calf contact experience on grazing and social behavior of weaned calves, monitored over seven days for their first post-weaning grazing experience. "Dam" (D) calves were reared and grazed with their mothers until weaning. "Mixed" calves (M) were separated from their mothers after 4 ± 0.5 weeks, they experienced dam-calf contact, but not grazing. "Standard" (S) calves had never experienced either dam-calf contact (separated at birth) or grazing. Each group grazed an equivalent pasture plot offering heterogeneous herbage. Scan sampling of calves' activities was performed every 5 min, 6 h per day, on Days 0, 1, 2, 3, and 7. Daily, the time when calves started grazing after introduction to pasture, and the number and duration of their grazing cycles were measured. Daily activities were differentiated into ingestion, rumination, and idling. The proportion of time that calves spent grouped with other individuals or isolated, and standing or lying were recorded. When grazing, their bites were characterized by botanical family group, height of the selected bite and vegetation status. Individual average daily gains from the 2-week periods before and after grazing were calculated, and were equivalent between groups (313 ± 71 g/d). On Day 0, D-calves started grazing immediately (1 ± 4.1 min), unlike M- and S-calves (39 ± 4.1 and 23 ± 4.1 min), and D-calves grazed patches of dry grass 21.7 times less than M-calves and 16.9 times less than S-calves. Dry herbage patch preference and grazing start time differences disappeared on Day 1. Calves spent the same time ingesting and idling, but M-calves spent on average 1.6 times less ruminating than D- or S-calves. The D-calves showed grazing behavior similar to that of adult cows, selecting grasses throughout pasture utilization, although legumes and forbs were present in the grazed layer. On the contrary, M- and S-calves did not express any specific preference. The S-calves spent more time isolated but had more positive reciprocal interactions than the calves in the other groups.

Keywords: grazing behavior, dairy calves, grazing experience, dam-calf contact, post-weaning, social interactions, first grazing

INTRODUCTION

Maximizing production while reducing costs and labor are the main aims of modern dairy systems. This trend often results in an intensification of farming practices, which weakens societal acceptance of dairy production systems (1). Consumers are taking ever greater interest in how their food is produced, and are increasingly aware of environmental issues and animal welfare (2, 3). In dairy production, the most common welfare concerns are the separation of calves from their dams (4) and restricted access to pasture for animals in intensive systems (5). Pasture for dairy cattle offers several advantages for animal welfare and health, such as expression of natural behavior and possible reduction of lameness and claw disorders (6–8) or increased movement with positive effects on longevity (9). Grazing systems also reduce management and feeding costs for the farmer (10, 11). In commercial dairy farms, calves are usually separated from their dams close to birth, and rarely experience grazing during their early lives (12). In France, 60 % of dairy farms use seasonal batch calving during autumn and winter, in order to turn out animals to pasture in the following spring (13). Then, calves and heifers usually graze from spring to autumn, before their first year of age, but only 2% of dairy farms turn out calves to pasture before 6 months of age (13). This strategy allows the synchronization of the peak of herbage growth and the peak of lactation of dairy cows, with fresh herbage covering a large part of their nutritional requirements (14). At the same time, calves have also grown and matured sufficiently and are able to be moved to pasture.

Le Cozler et al. (13) reported that only 4% of farmers keep calves with their dam at later than 24 h, but this practice is increasingly used. Michaud et al. (15) investigated farms using a suckling practice in France (Massif Central, East and West of France), and found that 62 farms out of 102 kept calves with their dam or with a foster cow between 1 and 60 days of age. The presence of the dam in the early stages of a calf's life can have positive effects on its social interactions, feeding behavior, and growth (16–18). The dam is the primary social model and plays an important role in the acquisition of foraging behavior and feed selection (19, 20). Pullin et al. (20) found that lambs grazing with their dam spent more time foraging, were more active, developed long-term feed preferences and learned aversion to toxic feed more effectively than lambs grazing alone. Young animals learn by emulation of social models or by trial and error, although in most cases this last is less efficient (21). Calves usually are neophobic: they tend to choose feed and places they already know, so that individual learning in a new environment takes more time than learning by social models (22, 23). Lopes et al. (24) observed that heifers with early grazing experience, compared to inexperienced heifers, affected grazing behavior and milk production only in the first days on pasture, but showed that the animals would generally adapt to a new environment and a novel feed easily, especially during their first year of life. Dairy calves that have learned to graze with their dam might therefore more efficiently recognize herbage quality and select specific patches when turned out to pasture after weaning, compared to calves that never grazed before. However, it is unclear whether this advantage holds only in the first grazing day or is more persistent.

In the present study, the following hypotheses were tested, comparing three groups of calves with contrasting rearing experience on their first grazing days after weaning. We expected calves that had experienced dam-calf contact and grazing in their early life to show grazing and probably social behavior that was different from that of inexperienced calves, and more typical of adult dairy cows. The longer dam-calf contact lasted (a few weeks or until weaning), the greater would be the expected differences in calves' social behavior. The present study also evaluated the persistence of the expected differences in grazing or social behavior in the short term after weaning.

MATERIALS AND METHODS

Experimental Design

The experiment was performed in 2019 at the INRAE experimental farm of Marcenat (DOI: https://doi.org/10.15454/1.5572318050509348E12), located in the Massif Central (45°15′N, 2°55′E; 1150 m a.s.l.). All animal-related procedures were carried out in accordance with the guidelines for animal research of the French Ministry of Agriculture and all other applicable national and European regulations for experimentation with animals (https://www.recherche-animale.org/sites/default/files/charte_nationale_portant_sur_l_ethique_de_l_experimentation_animale_243579.pdf). The experiment started February 12. The early grazing period started July 22 and ended July 29. Three breed-balanced groups of eight dairy calves (Holstein and Montbéliarde) with different experience backgrounds were compared (**Table 1**): a group of "Standard" calves (S) that had been separated at birth from their dam and had never experienced grazing, a group of "Dam" calves (D) that had been reared and grazed with their dam until weaning, and a group of "Mixed" calves (M) that had been separated from their dam at 4 ± 0.5 weeks of age and had never experienced grazing. All calves were weaned at age of 10.9 ± 1.1 weeks. Before weaning, D-calves were housed separately from their dams at night and had free access to the dam cowshed during the day. Starting from May 5, when the calves were 4.6 ± 3.2 weeks old, the day cowshed access was replaced by free access to pasture with dams. The M-calves, until age 4.0 ± 0.5 weeks, were reared in the same way, except that they had no access to pasture. From this age until weaning, they were reared like S-calves, i.e., in separate housing and fed bulk milk with an automatic milk dispenser. D- and M-calves were reunited with their dams after morning milking at 9:00 a.m. and separated before evening milking at 3:30 p.m. At weaning, all calves were moved to a new pen, with one pen for each group to prevent mixing. In this pen, calves ingested 0.5 kg/d/calf of hay and 2.0 kg/d/calf of concentrate (Startivo, Centraliment, 15006 Aurillac). Hay was distributed in the evening with no refusal left in the morning. Concentrate was distributed half in the morning and half in the evening, until the end of the study. After the last weaning, all calves spent at least six days indoors all together to allow the latest weaned calves to adapt to the new conditions. At the beginning of the grazing period (week 15), D-, M- and S-calves were, respectively, 14.9 ± 3.2, 16.1 ± 2.8 and 15.3 ± 3.6 weeks old and weighed 131 ± 18.3 kg, 123 ± 17.4 kg, and

TABLE 1 | Feeding plan (milk, concentrate, and hay) of the three groups of calves (Standard, Dam, Mixed) during the first 15 weeks of age.

Group	Week	1	2	3	4	5	6	7	8	9	10	11..	15
Standard	Milk[1] (kg/d)	6.0	7.0	9.0	10.0	10.0	10.0	9.0	7.0	5.0	3.0	Weaning	Start grazing
	Concentrate[2] (kg/d)	0	0	0.2	0.4	0.6	0.9	1.2	1.5	1.8	2.0	2.0	2.0
	Hay[3]	0	*ad libitum*									*ad libitum*	0.5
Dam	Suckling period	24 h/24 h	Between morning and evening milkings (=during the day)									Weaning	Start grazing
	Concentrate (kg/d)	0	*ad libitum*									2.0	2.0
	Hay	0	*ad libitum*									*ad libitum*	0.5
	Pasture with dams	/		/	*During the day*							/	
Mixed	Suckling period	24 h/24 h	During the day		10.0	10.0	10.0	9.0	7.0	5.0	3.0	Weaning	Start grazing
	Concentrate (kg/d)	0	*ad libitum*		0.4	0.6	0.9	1.2	1.5	1.8	2.0	2.0	2.0
	Hay	0	*ad libitum*		*ad libitum*							*ad libitum*	0.5

[1] *bulk milk distributed individually by automatic feeder.*

[2] *first age concentrate distributed individually by automatic feeder (Standard group and Mixed group after separation from the dam) or in collective bucket (Dam group and Mixed group before separation from the dam).*

[3] *permanent grassland hay (first cut) distributed in a rack.*

TABLE 2 | Characteristics of vegetation offered on the experimental plots (mean ± standard deviation).

Plot characteristics	Dam	Mixed	Standard
Patch type (%) and description			
Dry (≥70% dead material)	15.5 ± 4.1	16.0 ± 2.1	13.3 ± 2.5
Green (< 70% dead material)	84.5 ± 7.2	84.0 ± 4.1	86.7 ± 6.0
Grasses (≥ 70% grasses)	65.1 ± 9.7	69.1 ± 6.6	64.2 ± 7.3
Legumes (≥ 30% legumes)	17.1 ± 4.2	13.6 ± 2.0	13.3 ± 3.4
Forbs (≥ 30% forbs)	17.8 ± 3.6	17.3 ± 1.4	22.5 ±4.8
Tall (≥ 25 cm)	51.2 ± 7.8	48.1 ± 4.5	53.3 ± 7.6
Intermediate (7 cm ≤ x < 25 cm)	33.3 ± 5.7	35.8 ± 4.1	32.5 ± 5.1
Short (< 7 cm)	15.5 ± 5.2	16.0 ± 2.6	14.2 ± 3.1
Composition and nutritional value			
Dry matter *(g/kg),*	32.5 ± 3.3	28.3 ± 5.9	31.6 ± 2.5
Organic matter digestibility *(g/kg DM)*	67.2 ± 2.8	67.0 ± 3.8	66.0 ± 1.7
NDF *(g/kg DM)*	53.9 ± 4.4	53.8 ± 1.2	53.9 ± 1.8
ADF *(g/kg DM)*	27.6 ± 1.6	28.2 ± 1.0	28.5 ±1.9
Crude protein *(g/kg DM)*	12.0 ± 1.6	12.2 ± 2.7	10.6 ±1.8

128 ± 23.5 kg respectively, on average. They had been weaned for 30 ± 22, 33 ± 20 and 33 ± 24 days, respectively. Calves were turned out to pasture on July 22 (Day 0), from 9:00 a.m. to 5:00 p.m.

Characteristics of the Experimental Plot

Calves grazed a permanent pasture divided into three equivalent neighboring 0.15 ha plots. No close visual contact was allowed between the three groups of animals, by fencing the plots so that they were at least 15 m apart. To encourage grazing selection for all three groups of calves, the whole plot was strip-mown 28 days before the grazing period started. At Day 0, plots were thereby composed of alternate 3 m strips dominated by mature vegetation and vegetative regrowth. The botanical composition of the whole pasture was determined using the vertical point-quadrat method from Daget and Poissonet (25). The pasture was dominated by *Lolium perenne* (39.0%), *Agrostis tenuis* (15.0%), and *Trifolium repens* (13.5%). *Rumex obtusifolius* was also present (3.1%). At the beginning of the experiment, three 10 cm × 3 m grass samples were collected on each plot, perpendicularly to the mown and unmown strips, equally harvesting the same length from both. They were oven-dried at 60°C for 72 h and analyzed for proximate composition as described by Coppa et al. (26) (**Table 2**).

Observations and Behavior Measurements

Calves were weighed once a week, and individual average daily gain (ADG) from the 2-week periods before and after the grazing period started was calculated.

Individual daily activities and behavior were observed by scan sampling at 5-min intervals (27, 28) on the day the calves encountered the pasture for the first time (Day 0), the next three days (Day 1, Day 2, Day 3) and one week later (Day 7), focusing on the first days as most of the differences were expected here (24). On each plot, four calves were randomly assigned to two observers for 6 h per day (9:00–12:00 a.m. and 2:00–5:00 p.m.). For observations, calves were always identified by the same numbers painted on their back. At the end of the afternoon, the calves went back indoors for the night where they were fed with hay and concentrate (Startivo, Centraliment, 15006 Aurillac). Observers, randomly assigned to a group of calves, changed experimental group between each morning and afternoon. Each day, the time taken by calves to start grazing was measured. A calf was considered to have started grazing if it was observed taking a bite in at least three out of four successive observations (29), following the flowchart in **Figure 1**. From the time the calf started grazing, the grazing cycle lasted until it showed more than three other successive activities (i.e., it stopped grazing for at least 15 min), according to Manzocchi et al. (30). The duration of a grazing cycle and the number of grazing cycles, as just described, were calculated following the flowchart in **Figure 2**.

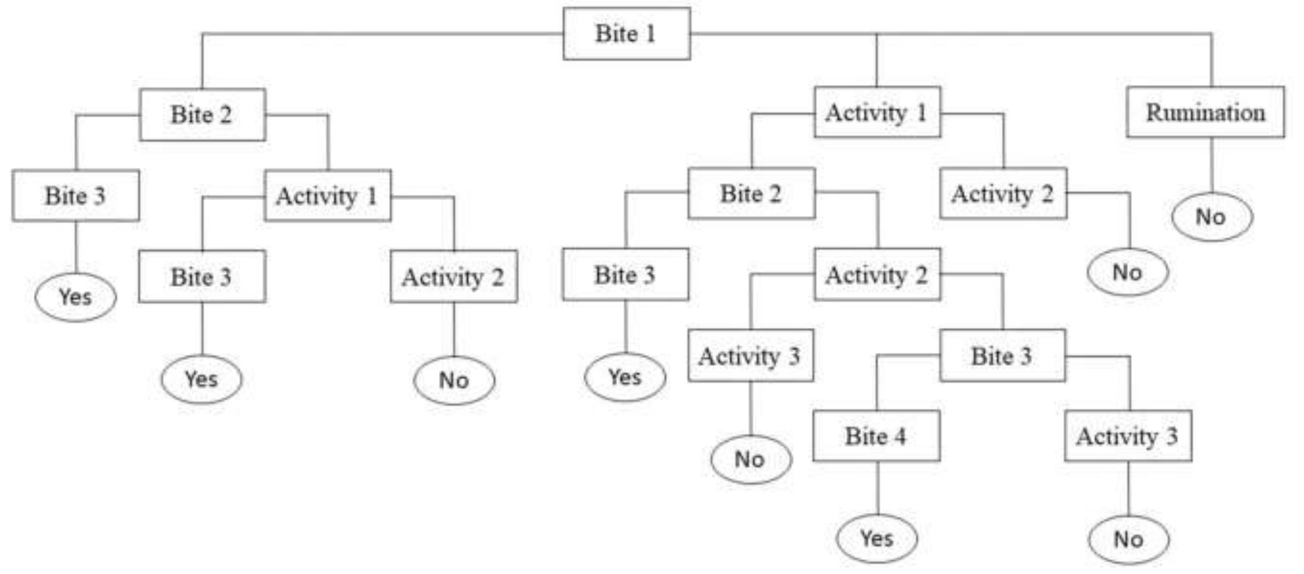

FIGURE 1 | Flowchart of the conceptual scheme used during observations to tell whether calves started grazing at Bite 1 (Yes = at least three bites over four observations; No = flowchart restart to the next observation).

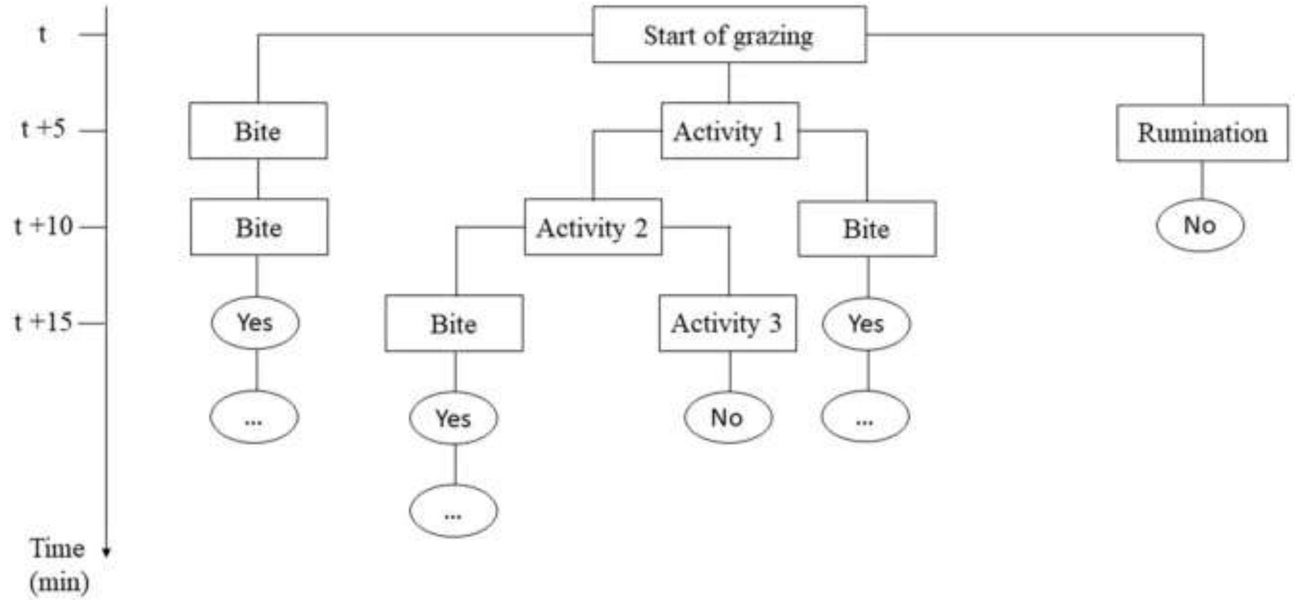

FIGURE 2 | Flowchart of the conceptual scheme used during observations to tell whether a grazing cycle was established (Yes or No) and measure its duration.

Daily activities were then differentiated into three groups: ingestion (grazing and drinking water), rumination, and idling. The latter comprised four subcategories: resting (observation, sleep, self-grooming), positive interactions (licking, sniffing, head play), negative interactions (head-butting, pushing, fighting) and *ad hoc* activities (walking, exploring, stereotypies, vocalizing) (**Table 3**). The daily proportion of ingestion, rumination and idling time was calculated as a percentage of the total daily observations. The daily proportion of resting time, socializing time and *ad hoc* activities was calculated as a percentage of the idling activities. Each time one of the activities was recorded, observers also indicated whether the calf was grouped with other calves or isolated, i.e., at least 3 m away from other calves, and whether it was standing or lying. The daily proportions of time spent grouped and standing were calculated over the total number of observations of the day. When calves

TABLE 3 | Description of daily activities recorded by scan-sampling differentiated in four subcategories.

Daily activity	Subcategory	Behavior type	Description
Ingestion			*Grazing and drinking water*
Rumination			*Ruminating*
Idling	Resting	Observation	*Standing or lying, without sleeping*
		Sleep	*Sleeping*
		Self-grooming	*Self-licking, rubbing, defecating and urinating*
	Positive interaction	Licking	*Licking another calf's head or body*
		Sniffing	*Sniffing another calf's head or body*
		Head play	*Rubbing the head against the head of another calf*
	Negative interaction	Head-butting	*Pushing the head against the head of another calf*
		Pushing	*Pushing the head against the body of another calf*
		Fighting	*Two calves pushing each other*
	Ad hoc activities	Walking	*Walking*
		Exploring	*Sniffing the floor, sniffing/ licking objects, discovering the environment*
		Stereotypies	*Cross-suckling, tongue rolling and repeatedly sniffing/licking objects*
		Vocalizing	*Mooing punctually and/or repeated*

were grazing, their bites were characterized by botanical group (grasses, legumes and forbs), the height of the selected bite (tall, intermediate, short vegetation) and the vegetation status ("dry" or "green"), according to Koczura et al. (31). Briefly, patches were characterized according to the visually estimated proportion of dry senescent herbage, of botanical family groups and of their height (26, 32). A patch was coded as "dry" if the dry senescent vegetation represented more than 70 % of the bite, as "green" if it was $< 70\%$; as dominated by "grasses" if the bite contained more than 70% of grasses, by legumes or forbs if they represented more than 30%; tall if herbage height was ≥ 25 cm, and small if it was ≤ 7 cm, as detailed in **Table 2**. Observers were able to get close to calves due to their adaptation to human presence achieved during the pre-weaning experiment. When calves ingested forbs, observers reported whether or not they selected *Rumex* thanks to a binary variable (1 = the calf tried to eat *Rumex* at least one time in the observation day). The daily proportion of vegetation type ingested by calves was calculated as a percentage of observations comprising the vegetation type compared to the total number of grazing observations of the day.

The weather was exceptionally hot on the afternoons of Day 2 and Day 3. The average maximum daily temperature during these afternoons was 31.2°C, whereas between 2000 and 2019, the average maximum temperature in July was 21.6°C (INRAE CLIMATIK 2.1.5, Marcenat weather station). Behavior observations at pasture were therefore made throughout the day on Day 0, Day 1, and Day 7, but only in the morning on Day 2 and Day 3. The daily ingestion, rumination and idling activities, together with the number and duration of grazing cycles, were accordingly calculated only for Day 0, Day 1, and Day 7, as the morning alone was not considered representative of the ingestion and rumination cycles of a whole day. On the other hand, the characterization of grazed bites and time needed to start grazing were calculated on mornings only for all days, the numbers of bites observed during the morning being considered sufficient and representative to express preference, as differences between morning and afternoon on those days were equivalent.

Statistical Analysis

Daily activities and grazing cycles were analyzed with a repeated MIXED model on SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Group (Dam, Mixed or Standard), day (only 0, 1, or 7) and their interaction were included as fixed effects. The individual calf was considered as the subject of repetition, with day being the repeated factor. We used a compound symmetry covariance structure. Time to start grazing and herbage selection were analyzed with the same model, except that the day effect included all days. Average daily gain was analyzed with a similar repeated model, which included group, period (before or after pasture) and their interaction as fixed effects, calf as subject, and period as repeated factor. In this last model, the number of days since each calf had been weaned was used as a covariate. The effect of the age and BW of calves were tested as covariates as well, but were found to be non-significant, and so were not finally included in the model. For all data, normality of residuals was checked using the Shapiro-Wilk test. The frequency of times calves tried at least 1 time to include *Rumex* in their bites was compared between groups using a Chi^2 test. Significance was set at $p < 0.05$.

RESULTS

ADG Before and After Grazing

During the 2 weeks before start of grazing, the ADG of D-, M- and S-calves did not differ significantly ($p = 0.177$), at 285, 355, and 480 g/d, respectively. During the following 2 weeks it increased by 313 g/d on average for all the groups.

TABLE 4 | Effect of early dam-calf contact and grazing experience on post-weaning daily activities and grazing cycles of dairy calves (Day 0, 1 and 7 after start of grazing).

Item	Dam	Mixed	Standard	SEM	Group	Day	Group × day
Daily activities (% of daily total observations)							
Ingestion time	55.8	58.3	58.2	1.88	ns	**	†
Rumination time	11.0^{a}	6.9^{b}	10.2^{a}	1.01	*	*	ns
Idling time	33.2	34.8	31.7	1.88	ns	***	ns
Grazing cycles (by day)							
Duration (min)	57.8	55.9	55.6	4.97	ns	†	ns
Number	3.1	3.5	3.2	0.21	ns	*	ns
Idling activities (% of daily idling observations)							
Resting time[1]	64.6^{a}	55.4^{b}	56.5^{b}	2.55	*	***	ns
Ad hoc activities[2]	30.2^{b}	37.3^{a}	35.4^{ab}	1.89	*	***	ns
Positive interactions[3]	1.6^{b}	0.7^{b}	3.3^{a}	0.51	**	ns	ns
Negative interactions[4]	3.7	6.5	4.8	1.07	ns	**	ns
Proportion of time (% of daily observations) spent:							
Lying	15.8^{b}	9.8^{c}	20.5^{a}	1.60	***	***	***
Isolated	22.3^{b}	19.1^{b}	31.6^{a}	2.07	***	**	***

$^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $^{\dagger}p < 0.10$; ns $p \geq 0.10$.

$^{a-c}$Means within a variable with different superscript letters differ at $p < 0.05$.

[1] *Resting time: observation, sleeping, self-grooming.*

[2] *Ad hoc activities: walking, exploring, stereotypies, vocalizing.*

[3] *Positive interaction: licking, sniffing, head play.*

[4] *Negative interaction: head-butting, pushing, fighting.*

Effect of Early Dam-Calf Contact and Grazing Experience on Calves' Daily Activities

Once turned out to pasture, the daily activities of the three groups of weaned dairy calves did not strongly differ (interaction groups × day non-significant). Overall, during Days 0, 1, and 7, calves in the three groups spent almost the same time ingesting ($p = 0.081$, on Day 1 M-calves tended to spend less time ingesting than D- and S-calves) and the same time idling (**Table 4**), but M-calves spent on average less time ruminating than D- and S-calves (1.54 times less). During idling activities, D-calves spent more time resting than M- or S-calves (1.16 and 1.14 times more, respectively), and M-calves spent more time in *ad hoc* activities than D-calves (1.24 times more). The S-calves had more positive social interactions than the calves in the other two groups. On Day 0 and Day 1, S-calves spent more time lying than D- or M-calves (**Figure 3I**). On Day 0 and Day 1 they spent more time isolated than calves in the other two groups (**Figure 3II**).

Effect of Early Dam-Calf Contact and Grazing Experience on Calves' Herbage Selection

When moved to pasture, D-calves started grazing immediately (**Table 5**), whereas it took S-calves 23 ± 4.1 min to actively start to graze. The M-calves needed a further 20 min. On Day 0, the herbage selection was different between groups: D-calves grazed "dry" patches 21.71 times less than M-calves and 16.90 times less than S-calves. On Day 0, no differences between groups were observed for botanical composition and height, except for forbs: on that day, S-calves grazed 13.73 times more forbs than M-calves and 3.89 times more than D-calves. On Day 1, Day 3, and Day 7 all three groups of calves started grazing 5 ± 2.8 min after arriving on pasture, whereas M- and S-calves started grazing 15 ± 0.7 min after D-calves on Day 2. From Day 1, M- and S-calves reduced their proportion of "dry" patches to meet that of D-calves, with no longer any significant differences between groups. Overall, we found that the proportion of tall vegetation in the bites decreased from Day 1 to Day 7 and conversely that the proportion of short vegetation increased in the bites from Day 1 to Day 7. On Day 2 and Day 3, M-calves showed a higher proportion of intermediate vegetation than D- and S-calves (2.07 and 3.03 times more, on average). D-calves continuously maintained stable the proportion of grasses in their bites throughout the plot utilization, while M- and S-calves decreased their proportion over time (0.75 times less from Day 0 to Day 7, on average), increasing in parallel those of legumes and forbs (7.57 and 4.46 more times on average, respectively). On Day 0, none of D-calves grazed *Rumex,* on the contrary to M- and S-calves (4 and 6 calves, respectively) (**Figure 4**). This difference disappeared in the following days, already on Day 1.

DISCUSSION

Effect of Early Dam-Calf Contact and Grazing Experience on Calf Grazing Behavior

To our knowledge, only a few published studies have focused on dairy calf grazing behavior, and this is the first time that the effects of an early dam-calf contact have been investigated on calf grazing behavior, directly after weaning. As expected, the

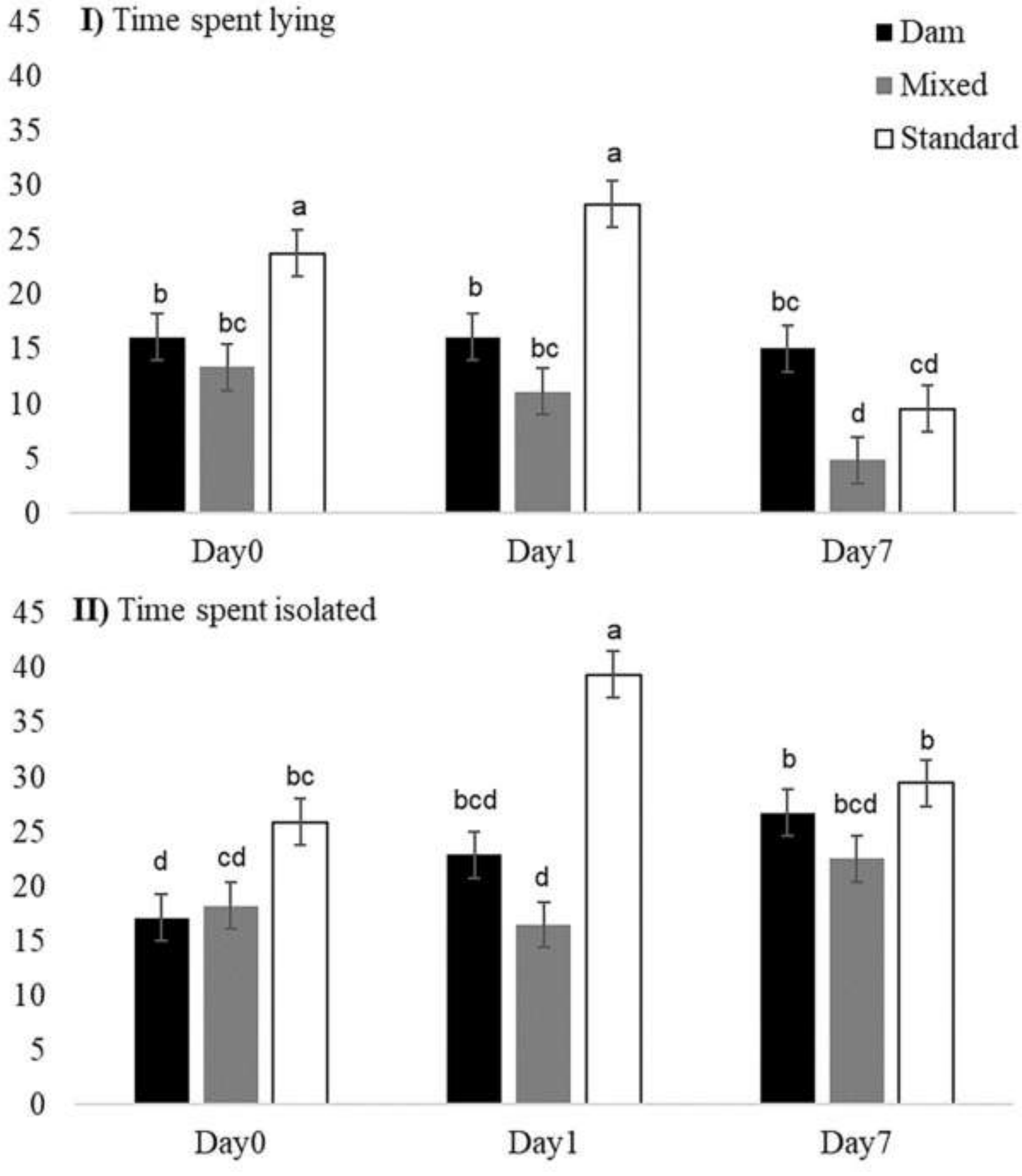

FIGURE 3 | Effect of early dam-calf contact and grazing experience on **(I)** daily time spent lying (%) and **(II)** isolated (%) by calves on Day 0, Day 1, and Day 7 at pasture. Bars are standard errors. $^{a-d}$Means within a variable with different superscript letters differ at $p < 0.05$.

main differences in calf grazing behavior were mostly observed on the first day on pasture (Day 0): D-calves started grazing immediately when moved to pasture, whereas S- and M-calves started 23 and 43 min later, respectively. In several studies, it is reported that inexperienced heifers need a few hours (12) or a few days (24) to start grazing. This lag occurs even when animals are put on pasture with experienced heifers. In our study, the three groups were separated to prevent visual contact between experienced and non-experienced animals. Calves that had experienced pasture with dams in their early life then immediately remembered how to graze, unlike calves from the other groups. This is consistent with findings of Lopes et al. (24), who observed that heifers that had once experienced pasture instantly remembered how to graze the following year. The M- and S-calves took slightly longer to start to graze, probably because they had no social model or experienced individuals

TABLE 5 | Effect of early dam-calf contact and grazing experience on time to start grazing after introduction to pasture and characteristics of selected bites by dairy calves.

Item	Group	Day					SEM	Group	Day	Group × day
		0	1	2	3	7				
Time to start grazing (min)	Dam	1^{e}	6^{e}	3^{e}	2^{e}	4^{e}	4.13	***	***	***
	Mixed	39^{a}	4^{e}	18^{bcd}	11^{cde}	7^{de}				
	Standard	23^{b}	4^{e}	19^{bc}	6^{e}	2^{e}				
Herbage selection (% of ingestion observations)										
Green	Dam	97.9^{a}	100^{a}	97.2^{a}	100^{a}	98.1^{a}	0.03	***	***	***
	Mixed	54.5^{c}	98.0^{a}	99.2^{a}	98.5^{a}	95.2^{a}				
	Standard	64.5^{b}	94.6^{a}	92.8^{a}	98.3^{a}	99.5^{a}				
Dry	Dam	2.1^{c}	0^{c}	2.8^{c}	0^{c}	1.9^{c}	0.03	***	***	***
	Mixed	45.6^{a}	2.0^{c}	0.8^{c}	1.5^{c}	4.8^{c}				
	Standard	35.5^{b}	5.4^{c}	7.2^{c}	1.7^{c}	0.5^{c}				
Grasses	Dam	86.4^{abc}	75.6^{cd}	81.3^{abc}	91.9^{a}	87.1^{abc}	0.04	***	***	***
	Mixed	87.8^{ab}	86.8^{abc}	59.5^{ef}	52.7^{f}	66.6^{de}				
	Standard	77.8^{bcd}	84.3^{abc}	61.8^{ef}	53.1^{f}	58.3^{ef}				
Legumes	Dam	8.3^{def}	15.6^{bcd}	11.3^{cdef}	4.9^{ef}	7.1^{def}	0.03	***	***	***
	Mixed	10.7^{def}	11.1^{def}	39.6^{a}	40.5^{a}	21.6^{b}				
	Standard	1.60^{f}	4.0^{f}	14.9^{bcd}	13.4^{bcde}	21.0^{bc}				
Forbs	Dam	5.3^{cde}	8.8^{cd}	7.3^{cde}	3.2^{de}	5.9^{cde}	0.03	***	**	***
	Mixed	1.5^{de}	2.1^{de}	0.8^{e}	6.8^{cde}	11.9^{c}				
	Standard	20.6^{b}	11.8^{c}	23.3^{b}	33.5^{a}	20.7^{b}				
Tall	Dam	84.3^{ab}	61.7^{efg}	68.0^{cdef}	76.8^{abcd}	50.2^{g}	0.06	ns	***	***
	Mixed	73.8^{abcdef}	83.5^{abc}	61.3^{defg}	49.9^{g}	52.2^{g}				
	Standard	76.1^{bcde}	89.2^{a}	77.7^{abc}	77.5^{abc}	58.6^{fg}				
Intermediate	Dam	7.0^{g}	19.1^{cde}	18.1^{cdef}	11.9^{efg}	32.3^{ab}	0.04	***	***	*
	Mixed	14.0^{defg}	11.9^{efg}	34.8^{ab}	26.4^{bc}	41.5^{a}				
	Standard	7.1^{fg}	6.8^{fg}	8.2^{efg}	14.5^{defg}	23.8^{bcd}				
Short	Dam	6.7^{cd}	10.4^{bcd}	7.8^{cd}	7.1^{cd}	15.6^{ab}	0.03	ns	**	***
	Mixed	7.4^{bcd}	1.7^{d}	3.9^{d}	22.0^{a}	6.3^{cd}				
	Standard	2.9^{d}	3.4^{d}	13.4^{abc}	5.9^{d}	13.5^{abc}				

$^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $+ p < 0.10$; ns $p \geq 0.10$.

$^{a-g}$Means within a variable with different superscript letters differ at $p < 0.05$.

to emulate (33, 34). Also, inexperienced heifers spend more time exploring and tasting grass than ingesting it, compared to experienced heifers (33), which was numerically the case for our M- and S-calves here before they started grazing, even though exploring time was not long enough to statistically analyze it (data not shown). After starting grazing (on Day 0), M- and S-calves selected mainly "dry" patches, unlike D-calves, which directly grazed only "green" ones. This suggests that inexperienced calves could be neophobic (22): they were probably reluctant to try novel feed, and without a social model, were inclined to choose feed they already knew, or with similar characteristics to hay. Nevertheless, these differences were no longer seen in the following days, showing that calves can soon learn how to graze and cope with novelty.

Once they started grazing, all the calves followed the same pattern from Day 0 to Day 7: they first selected tall vegetation and then intermediate and short herbage as pasture utilization progressed. This is consistent with the selection of vegetation by stratum by experienced grazing cows under rotational grazing (35): once the upper layer is grazed, the height of the patch decreases, moving down to the lower layers (26). However, the botanical selection of the vegetation seemed different between groups: M- and S-calves ingested mainly grasses during the first days, as grasses are almost exclusive in the top layer, and then increasingly legumes and forbs [present in the intermediate and low layers, because of their smaller size; (34)]. On the contrary, D-calves constantly selected grasses until the seventh day of observation, whatever the height of the layer present on the plot. This suggests that inexperienced calves did not select vegetation according to its botanical composition, but rather ingested species according to their presence in the topmost layer as they utilized the plot. Calves that had experienced pasture with dams seem to have learnt to graze like adult cows, which are known to select grasses even on biodiverse pasture (26, 35). Furthermore, M- and S-calves tried to ingest *Rumex*, especially during the first day at pasture, while D-calves rarely approached it. *Rumex*

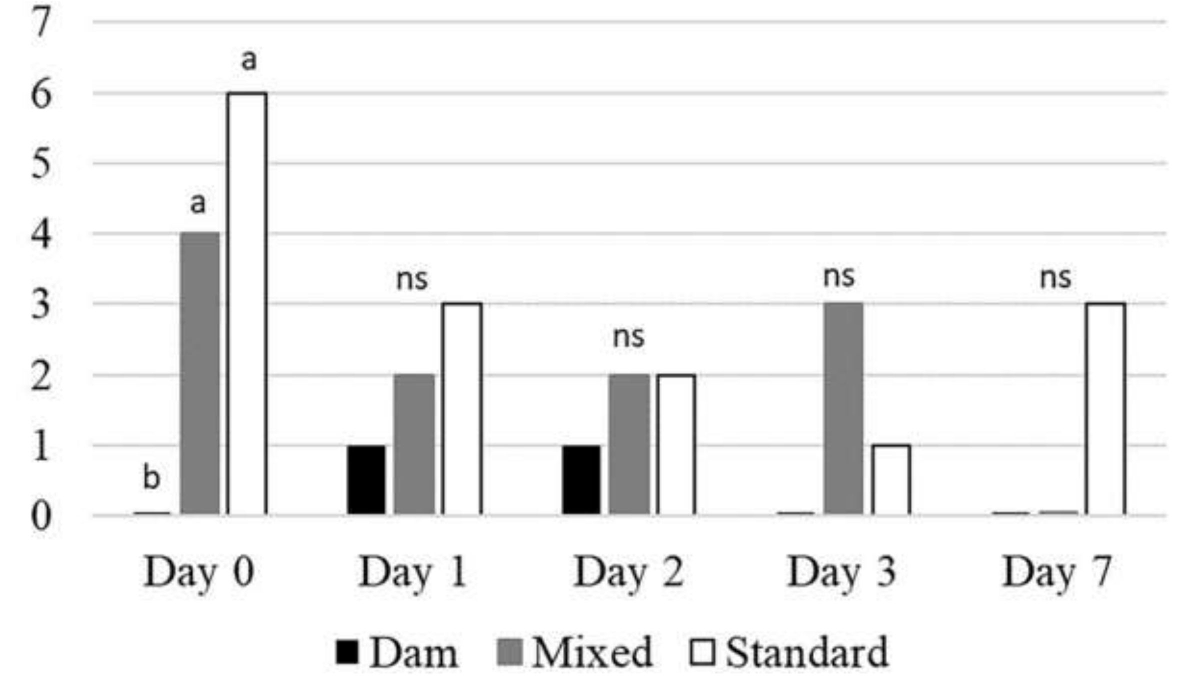

FIGURE 4 | Overall number of calves that tasted *Rumex* each day ($n = 8$ calves × 3 groups). $^{a-b}$Means with different superscript differ at $p < 0.05$, Khi² test; *ns*, not significant.

is one of the main oxalate-producing plants: oxalate can cause poisoning in livestock if present in 10% or more of the dry weight of the plant (36). It is therefore important that cattle learn how to avoid it. This suggests again that calves that have grazed with their dams learnt to choose or avoid some plants (20), while inexperienced calves learnt by trial and error (21). Even though calves that did not experience grazing showed different grazing behavior than D-calves on the first day at pasture, their behavior evolved very quickly (less than a week) into behavior similar to adult cows. This implies that dam-calf contact close to birth has little impact on longer term grazing behavior.

Having experienced dam contact and/or pasture affected the time to start grazing and herbage selection behavior of dairy calves, but did not influence their daily ingestion time or the duration and number of their grazing cycles. All the groups of calves, regardless of their different previous experience, had the same grazing rhythm throughout the trial: this confirms that inexperienced animals exhibit similar grazing times to experienced animals, as found by Lopes et al. (24) and Hessle et al. (19). While idling, M-calves behaved differently from D-calves in *ad hoc* activities (i.e., walking, exploring, stereotypies and vocalizing). This was consistent with the finding of Arrazola et al. (33) highlighting that inexperienced calves spent more time walking and exploring compared to experienced calves, that spent more time inactive. Besides, M-calves spent less time lying than the calves in the other groups. Wilcox et al. (37) demonstrated that standing behavior could indicate a stress condition of the calves, especially in case of chronic stress. Even if we did not directly measure stress of the calves, it could not be excluded that repeating stress factors over time by splitting separation and weaning could have induced a stressful behavior for M-calves. We also found that M-calves spent less time ruminating than D-calves, while the latter spent more time resting. As rumination time is proportional to forage intake, this result suggests that although the ingestion time was similar between groups, M-calves may have ingested less forage than D-calves, as found by Arrazola et al. (33). However, the calves' daily forage intake was not monitored in the present study. A different digestibility of dry senescent and vegetative or tall and short patches (leaf to stem ratio) could also have affected rumination time, but the day by day differences among groups in patches characteristics are not consistent with the trend observed in rumination time. Furthermore, no differences in ADG between groups were observed before and after calves started grazing, even though in the literature inexperienced grazers were found to be nutritionally disadvantaged because of modest foraging behavior that could affect their live weight gains (19). This suggests that the calf daily forage intake was not different between groups. We cannot therefore confirm that the foraging skills of inexperienced calves were inferior, but we can assert that they were not typical of an adult cow.

Effect of Early Dam-Calf Contact and Grazing Experience on Calves' Social Behavior

To the best of our knowledge, this is the first study to investigate dam-calf contact effects after weaning. Valníčková et al. (38) did not find any effect of dam-calf contact on social interactions or play behavior during colostrum feeding. Le Neindre and Sourd

(39) found that heifers reared with foster cows dominated more than heifers reared without cow contact. We thus expected that calves reared with their dams would be more sociable or have more dominant behaviors than artificially reared ones, but we found no differences in negative interactions (i.e., dominance behaviors, such as head-butting, pushing, or fighting) between groups at pasture. Nevertheless, we observed that S-calves had more positive interactions with their companions (particularly licking) than did calves in the other two groups. Pinheiro Machado et al. (40) found that licking behavior between grazing dairy cows was not a random choice but showed a companion's preference for socio-positive interactions. Furthermore, they observed that licking was more persistent in long-established social groups. This could suggest that D- and M-calves may have created bonds rather with dams than with other calves, compared to S-calves, but this point requires further investigation. Besides, the higher proportion of time spent isolated by S-calves, compared to D- and M-calves, could suggest that they exhibit less gregarious behavior. It is however difficult to interpret, because of a lack of literature on this topic.

CONCLUSION

Early life experience with dam and/or pasture influenced calves' foraging skills in the short term after weaning, especially on the first grazing day. Calves that had already experienced pasture with their dams immediately started to graze the day they were turned out to pasture in groups after being weaned. They instantly selected "green" patches of vegetation while grazing, unlike calves that had been housed indoors the whole time, which ingested predominantly senescent herbage on their first day. Daily ingestion time and duration and number of grazing cycles were not affected by previous experience. Nevertheless, botanical selection throughout pasture utilization and rejection of toxic plants (*Rumex*) showed that young calves could already exhibit post-weaning grazing behavior similar to that of adult cows when put on pasture early with their dam. This study provides evidence that separation of dairy calves from their dams close to birth has little impact on grazing behavior, as they grazed similarly to adult cows already in the short term (less than a week after being introduced to pasture). We spotted some differences in social behavior between the calves that experienced dam-calf contact and those that did not, but these differences are not easy to interpret and should be investigated in future studies. Further investigation is also needed to evaluate whether an early grazing experience with their dams could provide positive effects on behavior in the long term and performance in the future lactating careers of these calves.

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 the experiment was performed at Marcenat, INRAE experimental farm (Certificate of Authorization to Experiment on Living Animals No. D 15-114-01). No ethical approval was required because defined severity level was 0.

AUTHOR CONTRIBUTIONS

AN, MK, MC, MB, DP, and BM contributed to the conception and design of the study. AN, MK, MC, DP, and BM participated in the collection of behavioral observations data. AN and MK did the data curation and treatment. MK performed the statistical analysis. AN and MK wrote the first draft of the manuscript. MK and MC supervised the experiment and the writing of the manuscript. All authors contributed to manuscript revision, and all read and approved the submitted version.

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How Long Before a Second Defoliation of Actively Growing Grass Plants in the Desert Grassland?

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In the Desert Grassland, second and subsequent defoliations on perennial grasses during the active growing season can have substantial impacts on grass recovery and reproduction following herbivory. Land managers implement tactics to avoid multiple defoliations on plants by way of rotational grazing, reduced stocking rates, and/or reduced time spent within a given pasture. We explored frequency and rate of defoliation by cattle on perennial bunchgrasses within an 11-day grazing period in three pastures including distance to water (300 and 600 m) and plant height to determine their influence on animal diet selection. Results indicate that 32% of all marked plants were defoliated by cattle and only 5% of defoliated plants were defoliated a second time by day 10 of the grazing period. Defoliation patterns in the studied pastures did not differ between two distances from water, or in relation to plant height. However, at the second defoliation cattle grazed plants that were shorter than at the first defoliation suggesting a selection for high quality re-growth over larger forage on offer. The results of this study show that a 10-day grazing period during the growing season of the Desert Grassland is an effective strategy to avoid second defoliations on individual perennial grass plants while maintaining sufficient forage for use during the dormant winter grazing season.

Keywords: stocking rate, adaptive management, regrowth rate, distance to water, grazing (rangelands)

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INTRODUCTION

Avoiding a second defoliation on individual perennial grasses by livestock during the short 6–8-week growing season of the Desert Grassland has substantial benefits for sustaining and maintaining the condition of the available forage resource for future use (1–3). However, this type of management places limits on livestock production by forgoing the opportunity to graze high-quality summer biomass and particularly the high-quality regrowth of defoliated plants (4, 5). Understanding the rate at which grass plants receive second defoliations provides a basis for optimizing these tradeoffs for land managers (4, 6).

Second defoliations on a grass plant typically occur within 6–14 days of the grazing period, and before all plants within a pasture have been defoliated for the first time (5, 7–10). Livestock preference for re-growth has been related to the increased nutritional value of new regrowth and the removal of the less favored standing-dead biomass from previous growing seasons during the first defoliation (7, 11–14). The time between first and second defoliations is a function of the rate and amount of regrowth, and optimal grazing theory suggests that regrowth should approach a size equivalent to a full bite by the grazer (5). Moreover, a better understanding of the likelihood of repeated defoliation occurrence after cattle enter a pasture is needed since adaptive grazing management (15) aims to prevent multiple defoliations on perennial grasses by way of limiting grazing periods during the active growing season.

By applying these tenets to grazing management, questions are raised about how practical issues such as stocking rate and distance to water might influence the rate of first and second defoliations. These are relevant concerns in the Desert Grassland where typical pasture sizes commonly exceed 500–1,000 ha, and distance to water commonly exceeds 2 km.

Increased defoliation near water is typical because actual stocking rate (animals ha^{-1} unit $time^{-1}$) increases as animals concentrate time spent in the center of the piosphere (16–22). However, there is some evidence that rates of first and second defoliations are not affected at modest (400 m) distances to water if livestock spend <12 days in a pasture (12). And even at low stocking rates, the interval between initial defoliations and successive defoliations have occurred anywhere from 6 to 10 days after the first defoliation (5, 7, 10).

To explore these relationships in the Desert Grassland, we monitored rates of first and second defoliations on perennial grasses as livestock moved into and stayed 11 days in three pastures during the summer growing season. Our monitoring of plant height provided opportunities to evaluate if first defoliations occurred on larger plants, and if plants that were defoliated a second time were smaller than when they were initially defoliated. Four livestock waters across the three pastures provided the opportunity to evaluate whether these patterns differed with distance to water. The primary objectives of this study were to (1) quantify the frequency of initial and repeat defoliation events on perennial grass plants, (2) determine the days to second defoliation after cattle enter the pasture, (3) examine if the height of plants at the second defoliation is equal to height at first defoliation, and (4) determine if distance from water affects the frequency and rate of first and second defoliations by cattle. The results of this study will provide land managers recommendations for timing of moving livestock between pastures in arid and semi-arid grazinglands to reduce the frequency of repeated grazing.

MATERIALS AND METHODS

Study Area

This study was conducted in summer 2013 on the 21,500 ha Santa Rita Experimental Range (SRER) located at the northwestern base of the Santa Rita Mountains, ∼50 km south of Tucson, Arizona, USA (31°50′31"N, 110°51′36"W). The SRER was established in 1902 and is among the oldest continuously operating rangeland research facilities in the world. Much of the long-term historical, ecological, and biological databases are available in digital form (23) on the SRER website (https://cals.arizona.edu/srer/).

The SRER ranges from 900 to 1,400 m in elevation and consists of gently sloping alluvial fans and some steep stony foothills and isolated buttes (24). Average annual temperature is 16°C with several nights of freezing temperatures in the winter and temperatures regularly exceeding 35°C in the summer. Rainfall is bimodal in distribution between winter (November-March) and summer (July-September) seasons, with average annual precipitation increasing with the elevation gradient from 275 to 450 mm (25).

Vegetation is characterized by desert grasslands dominated by short trees, primarily *Prosopis velutina* Wooton, shrubs, cacti, and other succulents, perennial grasses, and other herbaceous species. Perennial grass species include native species *Digitaria californica* (Benth.) Henr., *Muhlenbergia porteri* Scribn. ex Beal, *Aristida* spp. and *Bouteloua* spp. species, *Heteropogon contortus* (L.) Beauv. ex Roemer & J.A. Schultes, and *Setaria macrostachya* Kunth and non-native *Eragrostis lehmanniana* Nees (26).

The SRER has been continuously grazed by cattle since 1916 but until more recently, updates have been made to the livestock grazing management system to incorporate aspects of adaptive grazing principles by introducing a rotational grazing schema across the range. Currently, two herds of ∼500 and 80 animals, respectively, move through the SRER's 38 pastures throughout the year. Livestock grazing management follows adaptive grazing principles to establish expected dormant season grazing capacity based on summer forage production, and summer grazing periods, 10-days in duration, based on avoiding the re-grazing of plants in the summer growing season (see Current Livestock Management Plan and Updates at https://cals.arizona.edu/srer/ and Comparison of Planned Livestock Use and Actual Use Since 2006 at https://cals.arizona.edu/srer/data.html).

Experimental Design

During the 2013 short summer growing season (July to early September), a total of 800 marked perennial bunchgrass plants were randomly selected within three pastures of the SRER to quantify the timing and count of defoliation events during grazing (**Table 1**). At four livestock water sources across three pastures (one pasture with two waters), twenty 5 × 10 m plots were established; 10 plots at 300 m and 10 plots at 600 m from each water source. These distances from water were selected to avoid the expected greater use closer to water and to better represent use across the large pastures (800–1,900 ha). Water sources were set as replicates in the study and distance to water was included as an explanatory factor in the experimental design to assess its relationship with defoliation timing and frequency. Within each plot, 10 grass plants were randomly selected, for a total of 200 plants per water source. The five most common grass species in this 800-plant population were *M. porteri*, *D. californica*, *E. lehmanniana*, *S. macrostachya,* and *Aristida* spp. To facilitate relocation, each macroplot was georeferenced and the individual plants were marked with a 40-penny framing nail driven into the soil and with a small amount of pink flagging attached to the nail head. Previous studies on defoliation patterns indicated that this method of plant marking does not affect animal selectivity during grazing (9, 12, 27).

To quantify the defoliation events during grazing, the height of all selected plants was measured at days 0, 1, 5, 10, and 11 of the days that livestock were in each pasture. Plant height

TABLE 1 | Characteristics and management of the three pastures studied on the Santa Rita Experimental Range in summer 2013.

Pastures and grazing periods					Herd size	Cumulative stocking rate of animal grazing days per hectare during grazing experiment (ADH)				
Pasture name	**Size (ha)**	**Grazing period**	**Grazing days**	**Water sources**	**Animal Units (AU)**	**Day 1**	**Day 5**	**Day 10**	**Day 11**	**Day 15**
5S	1,902	06 July−16 July	11	2	494	0.26	1.30	2.60	2.86	–
5M	1,395	17 July−31 July	15	1	407	0.29	1.46	2.91	3.21	4.38
5N	819	28 July−11 August	15	1	432	0.53	2.64	5.27	5.80	7.91

was measured from the ground to the blade height of the tallest leaf (8, 9, 28). Reduced height between periods and the presence of tiller or leaf utilizations were used to indicate defoliation and categorize plants as undefoliated, defoliated, or re-defoliated. The timeframe and the frequency of observations were set based on studies suggesting that at low stocking rates the interval between initial defoliations and successive defoliations on individual plants is between 10 and 12 days, with the majority of second defoliations occurring from 6 to 10 days after their first defoliation (5, 7, 10). We quantified defoliation at 15 days of grazing for only two water sources (5M and 5N) because logistical issues prevented measurements at the other water sources. Because the sample size was only two waters, we limited our data presentation to simple mean and standard error of percent of plants defoliated once and twice over the 15 days.

We report grazing intensity during the short grazing periods (11–15 days) as both herd size during the start and end of the grazing period in each pasture, as well as the cumulative number of animal grazing days per hectare (ADH, **Table 1**). Cumulative ADH shows how grazing intensity increases for each day the herd continues to graze in a pasture, suggesting that the chances of defoliation on any plant increases through the duration of the grazing period.

Precipitation in July and August 2013 were drier than the long-term (1971–2019) average across the three pastures. The averages for July, August, and Jul-Aug for the combined values for the three closest (within or <200 m from the pastures) long-term rain gauges (gauges NW, DESST, and PAST3; see Precipitation at https://cals.arizona.edu/srer/data.html) were 69, 56, and 125 mm. Values recorded in July, August, and Jul-Aug 2013 and percent of long-term average were 39 mm (57%), 43 mm (76%), and 82 mm (65%), respectively. On 05 July 2013, there was a 30 mm rainfall event to start the summer growing season (see gauges 3 and 4 at https://www.tucson.ars.ag.gov/dap/DataCatalogueOld.htm).

Data Treatment

The following data were summarized for each plant measured during the study: (i) height of the plants at days 0, 1, 5, 10, and 11, (ii) status of defoliation occurrence (defoliated vs. undefoliated); (iii) grazing day of each defoliation event (1, 5, 10, or 11); (iv) number of defoliations, (v) number of days between defoliations (1, 4, 5, 6, 9, or 10); and (vi) distance from water source (300 vs. 600 m).

For each grazing day (1, 5, 10, and 11), we calculated the percentage of plants undefoliated, defoliated once, and defoliated more than one time among total number of marked plants, as well as the percentage of plants grazed for the first or the second time among total number of defoliated plants. Using water sources as replicates, data from all plots at the same distance (300 or 600 m) from the same water source in each pasture were pooled together and count data were transformed to percentages for each replicate.

To assess the frequency of repeat defoliation events, we selected all plants defoliated two times and calculated the difference in days between the first and the second defoliation. For each resulting interval (i.e., 1, 4, 5, 6, 9, or 10 days), we calculated the relative frequencies of repeat defoliation given the number of plants available for second defoliation within that interval (i.e., plants already defoliated once).

Statistical Analysis

The percentages of plants defoliated for the first or the second time at each grazing day were analyzed using Linear Mixed-Effects Models (LMMs) with a temporal correlation structure to account for the days of grazing as a time variable (29, 30). We focused separately on the occurrence of first and second defoliation events (i.e., response variables) to assess their variations over time and space. In both models, grazing days and distance to water were considered as explanatory variables, and the experimental units [4 replicates of water source, which avoids pseudo-replication, (31)] were set as random factors. The interaction between water distance and grazing days was also included in the models. Parameter estimation was based on the Maximum Likelihood (ML).

Before analysis, model assumptions were tested as suggested by Zuur et al. (30) on raw and transformed data. Logarithmic, square root, logit, and arcsine-square root transformations were tested. Although the model results did not differ, the logit transformation $\log\{[(\text{percentages}/100) + 0.01]/[(1 - (\text{percentages}/100)) + 0.01]\}$ (32) was selected for both first and second defoliation data as it better satisfied modeling assumptions and models showed the lowest Akaike Information Criterion [AIC; (33)] values. Finally, pairwise comparisons across factor groups were conducted applying the Bonferroni adjustment.

To assess the influence of plant height on the selection for defoliation, we first compared the height of the plants at the first defoliation with the height of the ungrazed plants. The analysis

TABLE 2 | Results of the Linear Mixed-Effects Models (LMMs) showing the effects of distance from water sources, days of grazing, and their interactions on the occurrence of first and second defoliation events during grazing in three pastures.

Factors	First defoliation				Second defoliation			
	(AIC = 74.607)				(AIC = 61.532)			
	df1	df2	F ratio	*p*-value	df1	df2	F ratio	*p*-value
Distance from water	1	6	0.163	0.700	1	6	0.064	0.809
Days of grazing	3	18	40.018	<0.001	2	12	10.672	0.002
Distance from water x Days of grazing	3	18	0.434	0.731	2	12	0.068	0.935

AIC, Akaike Information Criterion.

used a Two-Way ANOVA including defoliation (defoliated vs. undefoliated) and distance to water as fixed factors. A second analysis compared the heights of the same plants at the first and the second defoliation using a Mixed-Design Two-Way Repeated Measures ANOVA (Split-Plot ANOVA). Distance from water was included in the analysis and set as a between-subjects factor, while the count of defoliation events (first vs. second defoliation) and the interaction between time and water distance were set as within-subjects factors. Plants at the same distance from the same water sources were the subjects for the repeated statement. Because data were normally distributed and variances among groups were homogeneous (as tested with Shapiro-Wilk's and Levene tests, respectively), both Two-Way ANOVA and Split-Plot ANOVA were conducted on the raw data.

All statistical analyses were carried out on R statistical software 4.0.2 (34). The LMMs were conducted with the "lme" function (package: nlme) (35), while the Two-Way ANOVA and the Split-Plot Anova with the "aov" function (package: stats) (36).

RESULTS

Through 11 days of grazing, cattle defoliated 32% of the marked plants (255/800). Approximately 26% of plants were defoliated once (209/800), 43 plants (5%) were defoliated twice, and 3 plants were defoliated three times. Very few plants were first defoliated on day 1 ($n = 10$ of possible 800), but more than half of those ($n = 7$) were defoliated a second time by day 11. Many plants received the first defoliation from day 2 to 5 ($n = 100$), but only 36 (36%) of those were defoliated a second time by day 11. After 5 days of grazing, 13% of plants were defoliated one time and <1% of plants two times. After 10 days of grazing, 25% of plants were defoliated one time and 5% two times. Three defoliations on the same plant were observed only on day 11.

The percentage of plants defoliated for the first or the second time changed over the 11 days but was not related to distance from water source (**Table 2**). Specifically, the percentage of plants defoliated for the first time increased significantly ($p < 0.001$) between days 1, 5, and 10, while no changes were observed on day 11 (**Figure 1A**). Similar trends were observed of increasing frequencies of second defoliation events over time, but no relationship with distance from water source (**Table 2**). No plants were defoliated a second time on day 1, but the percentages of second defoliations varied significantly ($p < 0.01$) from day 5 to day 10 (**Figure 1B**).

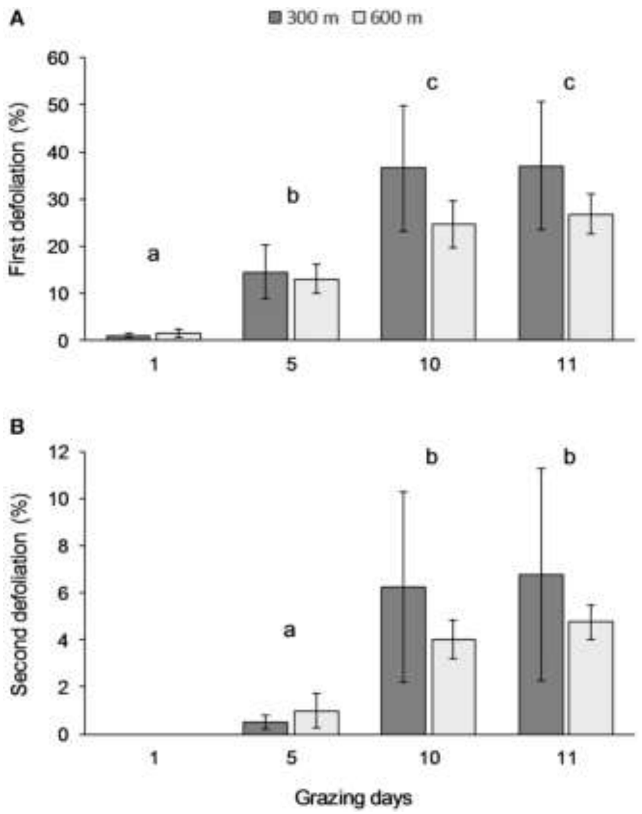

FIGURE 1 | Average percentages of plants defoliated for the first **(A)** and the second **(B)** time at grazing days 1, 5, 10, and 11, respectively at 300 and 600 m from water sources. Error bars represent Standard Error of the Means. Lowercase letters indicate significant differences among days of grazing (**A**: $p < 0.001$; **B**: $p < 0.01$).

For the two waters where defoliation was measured after 15 days of grazing, there was a trend of increased percent of plants grazed for the first and second time between day 11 and 15 at both distances from water (**Table 3**). Large variation in defoliation rates between the two water sources created high standard error values, but the trends suggest that the rate of second defoliations could exceed 15% of plants at 300 m from water.

The average height of the plants (± Standard Error of the Means) at the beginning of the grazing period (day 0) was 6.86 cm (± 0.16) in pasture 5S, 17.42 (± 0.54) in pasture 5M, and 15.01 (± 0.46) in pasture 5N, respectively. The height of the plants defoliated for the first time at all days did not differ from the height of the ungrazed plants on those days (**Table 4**). However, the height of plants defoliated a second time was shorter than those same plants at time of first defoliation, and that difference was not related to distance from water source (**Table 5**). The Split-Plot ANOVA on plant heights confirmed that distance from water did not affect the timing of second defoliation events.

TABLE 3 | Average percentage of plants defoliated for the first and second time through 15 days of grazing at two distances from two water sources (5M and 5N).

Distance from water	First defoliation (%)					Second defoliation (%)			
	Days of grazing					Days of grazing			
	1	5	10	11	15	5	10	11	15
300	1.0 (0)	22.0 (9.0)	48.5 (24.5)	49.0 (25.0)	57.5 (30.5)	1.0 (0)	11.5 (6.5)	12.5 (7.5)	15.0 (9.0)
600	1.0 (0)	8.0 (1.0)	19.5 (7.5)	23.0 (7.0)	37.0 (18.0)	1.0 (0)	3.0 (1.0)	4.5 (1.5)	8.0 (4.0)

Standard Error of the Means reported in parentheses.

TABLE 4 | Average height of the plants undefoliated and defoliated for the first time per grazing day.

Days of grazing	Plant height (cm)	
	Undefoliated	First defoliation
1	11.5 (1.8)	11.6 (4.8)
5	11.6 (1.5)	13.4 (1.2)
10	12.7 (1.5)	12.8 (1.6)
11	13.3 (1.4)	12.3 (1.3)

Standard Error of the Means reported in parentheses.

TABLE 5 | Results of the Split-Plot ANOVA comparing the heights of the same plants at the first and the second defoliation and at 300 and 600 m from water (Average Heights with Standard Error of the Means reported in parentheses).

Factors		Plant height (cm)
Defoliation	*p*-value	0.037
	First defoliation	14.3 (1.6)
	Second defoliation	11.9 (1.0)
Distance from water	*p*-value	0.386
	300 m	11.9 (1.1)
	600 m	14.3 (1.5)
Distance from water x Defoliation	*p*-value	0.558

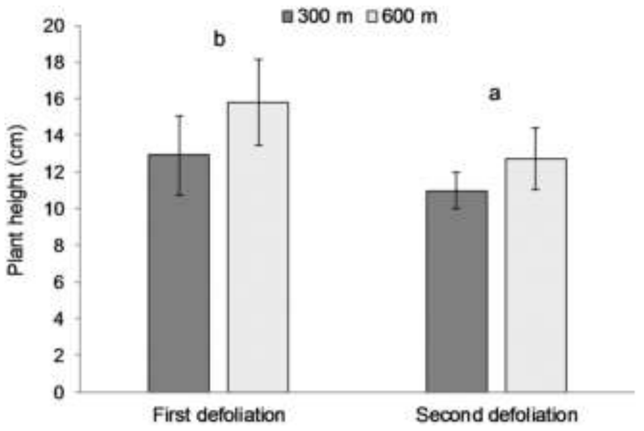

FIGURE 2 | Average heights of the same plants at the time of the first and second defoliation, respectively, at 300 and 600 m from water sources. Error bars represent Standard Error of the Means. Lowercase letters indicate significant ($p < 0.05$) differences among defoliation time.

Nevertheless, plant heights at the first and second defoliations differed significantly ($p < 0.05$) (**Figure 2**).

DISCUSSION

Without a doubt, avoiding a second defoliation during the short summer growing season will result in lost opportunities for livestock to graze the highest quality forage during the growing season. Our study indicates that by the 11th day of grazing, ∼5% of all plants had been defoliated twice, while only 30% of plants had been defoliated at least once. As a result, the cessation of grazing on day 11 will leave the majority of available perennial grass plants undefoliated and, unsurprisingly, these findings are very similar to previous work (7–9, 11, 37). A benefit of foregoing more use of summer growing season biomass is that the remaining biomass is then available for grazing as dormant winter season forage, during which plants are less susceptible to declines in vigor. However, the available mass in the dormant winter season is of lower forage quality than during the summer growing season (3, 5).

As we expected, at the four water sources, there was a substantial increase in the rate of second defoliations around day 10 of grazing with 5% of plants receiving a second defoliation by day 10 and did not decrease on day 11 (8, 9). Our observations at all four water sources during this study stopped at day 11 in order to more effectively evaluate the effects of water on second defoliations during the 10-day grazing period on the SRER. However, observations through day 15 at only two of the water sources provide an opportunity for informed speculation that rates of second defoliations will increase to as much as 15% or as little as 8%, at 300 and 600 m distance from water, respectively. These results suggest that the minimum length of a grazing period to avoid second defoliations should not be shorter than 10 days, and second defoliation rates after 15 days could be about 1 in 8 plants (15%) at 300 m from water. Shorter grazing periods may result in implementation difficulties in large pastures, maintaining animal performance, especially if cows are supporting young calves. As for longer grazing periods up to 15 days, there is no strong evidence that the rate of second defoliations would increase greatly if implementation problems were to delay a planned move to new pastures for a few days after day 10 of grazing.

Unexpectedly, plant height was not different between defoliated and undefoliated plants, suggesting that the forage mass on offer was not critical in the selection of rapidly growing plants during the short grazing period (37). However, plants defoliated a second time were shorter (11 cm) than when first defoliated (14 cm), suggesting that 11 cm of re-growth forage provides enough incentive with higher forage quality for cattle to re-visit previously grazed plants. In addition, it is widely thought that residual stems in bunchgrasses discourage grazing and cause livestock to preferentially select for taller plants with less old-growth material and longer leaf lengths (7, 12–14). The relatively dry conditions in July-August 2013 may have slowed the rate of regrowth following the first defoliation, and therefore delayed the time to second defoliation. However, the large amount of precipitation (30 mm) on 05 July provided very wet conditions to start the summer growing season.

Although distance from water is known to affect cattle distribution and grazing intensity (17, 21, 38), in our study, plant defoliation rates after 11 days did not differ between the 300 and 600 m distances from water. Of course, we would expect defoliations rates to be greater at 100 m than 300 m from water, but we focused at 300 and 600 m because they account for a much greater proportion of the pasture area (1.5–3% for 300 m and 6–13% for 600 m), than the <1% of the pastures at 100 m distance. The absence of a detectable difference in defoliation rates between 300 and 600 m may be due to a combination of environmental and management factors including that adapted cattle have been observed to travel on average 1.6 km from a water source while foraging (5) and move further from water especially when forage is limited or unattractive (39). Additionally, stocking rates are known to affect animal movements and grazing intensity. According to Bailey and Brown (5), under low stocking rates, cattle can travel further from water sources, while at high stocking rates they defoliate more intensively closer to water. We indeed observed higher variability among second defoliation events at 300 m (6.75 ± 4.52) than 600 m (4.75 ± 0.75) distance from water that can be attributed to the different stocking rates among pastures. However, a non-significant trend of greater defoliation rates at 300 than 600 m (71 and 29%, respectively) in the smallest pasture (5N) with the highest stocking rate suggests that even in short grazing periods, greater defoliations could occur closer to water if stocking rates are high (5).

The findings of this study have implications for land management in arid systems when goals of both conservative grazing and animal performance are important. For example, since 2006 management on the SRER has applied a 10-day limit to grazing use to avoid second defoliations in the short summer growing season (see Current Livestock Management Plan and Updates at https://cals.arizona.edu/srer/ and Comparison of Planned Livestock Use and Actual Use Since 2006 at https://cals.arizona.edu/srer/data.html). This management is applied with the purpose of sustaining plant vigor and to provide enough carry-over biomass to support grazing in the winter dormant season. The 10-day grazing period starts after receiving 1.25 cm of precipitation in July and ends when no new growth or flowering occurs typically in mid-September. The 10-day limit is applied to all pastures, whether they are small <200 ha or large >2,000 ha.

In practice, adhering to the 10-day limit is challenging for a variety of reasons including fence or water failures and when young calves slow the cow movement to new pastures. These delays have resulted in 14 or more days of grazing use in the summer months. Based on evidence from only two water sources, the rate of second defoliations could increase to an average of 15% at 300 m from water after 15 grazing days.

Further, the provision of large amounts of undefoliated and only once-defoliated plants for dormant season grazing has benefited the management at the SRER. Amount of dormant season biomass is determined in September and October for all pastures, and stocking rates are set to limit biomass utilization to 40% (see data on Grass Utilization by Livestock Since 2010 at https://cals.arizona.edu/srer/data.html) before the herd moves to the next pasture. In the end, the SRER has an adaptive grazing management program that (1) uses rainfall patterns each year to set the start and end of the conservative summer grazing season, (2) largely avoids second defoliations of plants in the short summer growing season by limiting grazing to 10 days, and (3) establishes the number of allowed animal grazing days/ha (varies from 3 to 18) to maintain utilization <40% based on the amount of summer biomass produced in each pasture.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

SN provided primary authorship and leadership of this manuscript. TL designed and implemented the field experiment. AG conducted all data analyses and assisted with preparing the written manuscript. MM and GR served as Primary investigators for this study with contributions including assistance with project design, funding, statistical analysis design, and manuscript preparation. MN assisted with experimental design and implementation and provided manuscript review. All authors contributed to the article and approved the submitted version.

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Little Difference in Milk Fatty Acid and Terpene Composition Among Three Contrasting Dairy Breeds When Grazing a Biodiverse Mountain Pasture

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In the mountains, autochthonous and robust breeds are often used to valorize biodiverse grasslands. Along with their lower nutrient requirements, compared to specialized dairy breeds, they are expected to be better adapted to complex environments and valorize grasslands into dairy products of high quality. Therefore, the aim of the present study was to investigate the grazing selection of three contrasting dairy breeds on a biodiverse mountain pasture, and its consequences on milk fatty acid (FA) profile and prevalence of individual terpenes. A dual-purpose breed from the Italian Alps, the Valdostana Red Pied (Va), was compared to Montbéliardes (Mo), more specialized in milk production, and the highly specialized Holsteins (Ho). Diet selection was measured by scan-sampling, calculating selectivity indexes, and collecting simulated bites during two consecutive days in June (end of first grazing cycle) and July (second grazing cycle). Milk samples were collected at each milking during these experimental periods. Yield of milk and its fat and protein contents were measured. Milk FA and terpenes were analyzed by gas chromatographic methods. We tested the effects of breed, period and their interaction in a repeated mixed model, and calculated Pearson's correlations between behavioral data and milk FA as well as terpenes. The Va grazed less mature vegetation than Ho, but this difference was not sufficient to lead to a major breed effect on milk FA profile and prevalence of terpenes. However, the proportion of α-linolenic acid (C18:3 n-3) was always higher in the milk fat of Va than Ho (Mo were intermediary), but this without any correlation to grazing selection. This could be a consequence from a different metabolism concerning ruminal biohydrogenation, but must be further investigated. Finally, we confirmed previous studies that highlighted a link between milk quality and cows' grazing behavior, but here without differences among breeds. All cows adapted their behavior to the herbage evolution during the season, leading to higher proportions of unsaturated FA in July than June milks. Our study suggests that under mountain grazing conditions (biodiverse pasture and cows in late lactation), milk quality depends more on herbage composition than on cow breed.

Keywords: grazing behavior, simulated bites, late-lactating, Holstein, Montbéliarde, Valdostana Red Pied, α-linolenic acid (C18:3 n-3)

INTRODUCTION

Politics and consumers are increasingly concerned about the impact of livestock systems on environment and animal welfare, which triggers intensive research activities. Livestock systems need to be multi-functional. This means that they have to perform well in ecology, animal welfare and economics while producing healthy and quality food. The valorization of biodiverse pastures by ruminants is one of the key tools toward establishing this kind of livestock systems, characterized by higher resilience and fewer inputs than common systems (1). In mountain grazing dairy systems, autochthonous or robust cow breeds are commonly used to valorize these grasslands (2). Robustness is defined as an ability to adapt and carry on casual activities while facing environmental constraints (3). In the mountains, robust breeds with lower nutrient requirements, compared to specialized breeds, are expected to develop a natural resilience to their local environment and might better adapt to utilize the local grass resource, typically characterized by low nutritional value, and to valorize these resources by generating high-quality dairy products.

The ability to efficiently exploit local natural grasslands depends on grazing behavior, which can in turn influence the quality and specificity of dairy products (4). Plant secondary compounds (PSC) play a specific role in this context, such as phenols and terpenes, which are present in greater quantity in upland biodiverse grasslands (5–7). Besides species diversity, also vegetation stage and environmental conditions are involved in influencing the synthesis of terpenoids by the plants. For instance, Fraisse et al. (8) identified 170 different compounds on the same pasture, of which only 30 were common to all vegetation species and growth stages. These PSC may be transferred to the milk (9). Accordingly, milk from pastures rich in dicotyledons contains a greater quantity and wider diversity of terpenes than milk from pastures consisting mainly of grass species (10–12). Some PSC are also active in inhibiting the final reduction step of the biohydrogenation of fatty acids (FA) in the rumen (13–15). Therefore, when cows are grazing on biodiverse mountain pastures, their milk is richer in monounsaturated FA (MUFA), polyunsaturated FA (PUFA) and, especially, in conjugated linoleic acids (CLA) and n-3 FA (16–18). Terpenes and FA in milk are of interest for the sensory properties of milk and other quality attributes of the processed cheese (19).

Dairy cattle grazing on biodiverse pastures express preference or aversion for some specific plants that can vary according to their nutrient requirements and experience. Therefore, the actual diet ingested by the animals might differ from the average vegetation available on the pasture (20, 21), and this according to individual or breed-specific behavior (22, 23). Consequently, FA and terpene composition of the milk might vary among animals grazing the same biodiverse grassland. We reported a first part of the present study in Koczura et al. (24), showing that specialized dairy breeds with high nutrient requirements like Holstein cows (Ho) selected more grasses than dual-purpose breeds like Valdostana Red Pied cows (Va), an autochthonous Italian alpine breed with low requirements, when grazing on heterogenous and biodiverse pastures. The Va were generally less selective toward forbs and mature vegetation. Hence, the aim of the present study was to (i) deepen the analysis of grazing behavior of the three contrasting breeds by quantifying their diet selection on the biodiverse pasture and (ii) further investigate the consequences of this behavior on FA profile and prevalence of terpenes in the milk. We hypothesized that, as a result of our behavioral observations (24), the ingested diet of autochthonous Va cows would be more diverse than that of highly specialized breeds like Ho. Diet of Montbéliarde cows (Mo), which are less specialized for high milk production than Ho and supposedly better adapted to grazing on upland pastures, would be intermediate. We expected that the milk of the individual cows and breeds selecting less grasses and more forbs would have a higher prevalence of MUFA, PUFA, n-3 FA and terpenes.

MATERIALS AND METHODS

Animals and Experimental Pasture

The present experiment was carried out in 2017 at Marcenat, INRAE's experimental farm. Other aspects and details of the experiment were described in Koczura et al. (24), in which data obtained in the beginning of June 2017 had also been included. Twelve late-lactating dairy cows (four Ho, four Mo, and four Va) grazing on a biodiverse pasture since the beginning of June were monitored during two consecutive days in the end of June (end of first grazing cycle) and then in July (beginning of the second grazing cycle). Briefly, cows grazed (extensive continuous grazing conditions) a natural and highly biodiverse pasture (65 species; 48% of grasses, 13% of legumes, and 39% of forbs on ground cover), dominated by *Festuca* gr. *rubra* (18%), and *Agrostis capillaris* (15%). Before the start of the experiment, the botanical composition (% of ground cover) was determined using the vertical point-quadrat method (25) (**Table 1**). The characteristics of the herbage on the experimental plots is described in **Table 2**. Cows did not receive any supplementation with concentrate, and had free access to water and mineral supplements. The individual cow's potential intake capacity, calculated according to INRA (26), was used as an estimation of herbage intake.

TABLE 1 | Botanical composition of the experimental plot.

Species[a]	**Specific contribution (% of ground cover)**
Festuca gr. *rubra*	18.4
Agrostis capillaris	15.3
Trifolium repens	7.4
Achillea gr. *millefolium*	5.3
Anthoxanthum odoratum	4.2
Thymus gr. *serpyllum*	3.4
Avenula pubescens	3.3
Trifolium pratense	2.7
Dactylis glomerata	2.5
Veronica arvensis	2.5
Plantago lanceolata	2.4
Luzula gr. *campestris*	1.9
Helianthemum nummularium	1.8
Lathyrus pratensis	1.8
Cynosurus cristatus	1.7
Galium verum	1.6
Carex sempervirens	1.6
Viola tricolor	1.5
Festuca gr. *ovina*	1.4
Stellaria graminea	1.3
Cerastium arvense	1.3
Ranunculus gr. *montanus*	1.3
Lotus corniculatus	1.2
Cerastium holosteoides	1.2
Daucus carota	1.2
Rumex acetosella	1.2
Chamaespartium sagittale	1.1
Potentilla erecta	1.1
Cirsium eriophorum	1.0

[a] *Species with specific contribution <1%, in decreasing specific contribution order: Ajuga reptans, Meum athamanticum, Poa pratensis, Silene vulgaris, Hieracium gr. pilosella, Saxifraga granulate, Alchemilla gr. vulgaris, Stachys gr. officinalis, Hypericum hirsutum, Leucanthemum vulgare, Deschampsia flexuosa, Knautia arvensis, Crepis capillris, Dianthus deltoids, Ranunculus bulbosus, Tragopogon pratensis, Dianthus sylvestris, Gentiana lutea, Trisetum flavescens, Ranunculus repens, Rumex longifolius, Sanguisorba minor, Briza media, Poa chaixii, Centaurea gr. jacea, Pimpinella major, Veronica chamaedrys, Poa trivialis, Vicia cracca, Galium album, Spergula arvensis, Cytisus scoparius, Conopodium majus, Bromus erectus.*

TABLE 2 | Characterization of vegetation offered on the experimental plots.

Item	**Period**		**SEM**
	June	**July**	
Proportion of vegetation type (%)			
Grasses	60.0	47.1	3.64
Legumes	5.6	6.8	1.22
Forbs	34.5	46.1	4.28
Short vegetation	42.0	54.1	4.05
Tall vegetation	45.9	26.2	3.56
Mature vegetation	12.1	19.7	2.50
Nutritional value (g/kg DM)			
Crude protein	8.3	9.1	0.24
Neutral detergent fiber	62.1	64.6	1.11
Acid detergent fiber	33.9	34.0	0.42
Digestibility of organic matter (g/kg)	45.6	42.3	0.94

Grazing Behavior: Selectivity Index and Composition of Simulated Bites

During the two-day experimental periods, behavioral observations were performed each day by scan-sampling of the cows' bites at 5-min intervals. From these data, Jacob's index of selectivity (IS) was calculated as described in detail by Koczura et al. (24). These indices range from −1 (aversion) to +1 (preference). Additionally, on the same observation days, simulated bites were collected, according to the procedure described by Coppa et al. (27). Individual simulated bites were sampled several times for each cow during the days of observation, and immediately stored at 4°C. The different simulated bite samples were pooled to constitute one sample per period per cow, in order to be representative for the grazed herbage. From this herbage sample, two sub-samples were created: one of them was oven-dried at 60°C for 72 h and analyzed for crude protein (CP) (28), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents (29), as well as solubility in pepsin and cellulase (30) as an estimate of the organic matter digestibility (OMD). The second sub-sample was sorted into green and dry vegetation. Then, the green herbage was sorted into the three main botanical groups: grasses, legumes and forbs. All fractions were separately oven-dried (in the same conditions as these applied to the first sub-sample) and weighed. The proportion of each fraction to total dry matter (DM) was calculated and was referred to potential intake capacity to estimate the relative intake.

Milk Sampling

In each experimental period, the individual milk yield of the cows was monitored at each milking during the two consecutive observation days, and 100-mL milk samples were collected. A 30-mL sub-sample was preserved with bronopol-B2, stored at 4°C and analyzed for fat and protein contents by Fourier transformed infrared spectroscopy [MilkoScan 4000, Foss System, Hillerød, Denmark, (31)] following the International Dairy Federation (32). Another sub-sample was stored at −20°C until further analyses.

Milk Fatty Acids Analysis

A 3-mL milk sample was stored at −20°C before lyophilization (Thermovac TM-20, Froilabo, Ozoir-La-Ferrière, France) for FA analysis, performed as described by Ferlay et al. (33). Lipids were directly methylated using 2 mL of 0.5 M Sodium methoxide plus 1 mL of hexane at 50°C for 5 min, followed by cooling with the addition of 75µl of 12M HCl at room temperature for 10 min. The FA methyl esters were recovered in 3 mL hexane and washed with 3 mL water. Samples were injected by auto-sampler into a TraceGC 2000 series gas chromatograph equipped with a flame ionization detector (Thermo Finnigan, Les Ulis, France). Methyl esters from all the samples were separated on a 100 m 30.25 mm i.d. fused-silica capillary column (CP-Sil 88, Chrompack, Middelburg, The Netherlands). The injector temperature was maintained at 250°C and the detector temperature at 255°C. The initial oven temperature was held at 70°C for 1 min, increased by 5°C/min to 100°C (held for 2 min), and then increased by 10°C/min to 175°C (held for 40 min), and 5°C/min to a final temperature of 225°C (held for 15 min). The carrier gas was hydrogen. Identification of trans isomers of 18:1, non-conjugated 18:2, and CLA isomers was as described in Loor et al. (34). A reference standard butter (CRM 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain FA (C4:0 to C10:0). The *de novo* synthesized FA were defined as the sum of individual FA synthesized in the mammary gland.

Milk Terpene Analysis

A balanced sample of milk from the four 50-mL samples from morning and evening milking that had been stored in 200-mL glass bottles at −20°C was thawed at ambient temperature for 6 h. The supernatant was then collected and centrifuged at 20,000 rpm and 25°C for 2 h. The anhydrous fat of these samples was collected and stored in 2 mL glass vials until further analysis for terpene composition. Then, headspace-solid phase microextraction (HS-SPME) was carried out using a 50/30 µm divinylbenzene-carboxen-polydimethylsiloxane (DVB/Carboxen/PDMS) fiber (Supelco, Bellefonte, PA, USA). The extraction conditions were the following: equilibration temperature: 60°C for 15 min; extraction temperature: 60°C for 60 min. The fiber was exposed into a gas chromatograph-mass spectrometer (GC-MS) injector for 2 min at 230°C, to desorb the terpenes. Analyses were performed on a Thermo Scientific Trace 1,300 gas chromatograph coupled to a Thermo Scientific ISQ single quadrupole mass spectrometer (both Thermo Fisher, Waltham, Massachusetts), equipped with an electronic impact source. All samples were injected in splitless mode, maintaining the valve closed for 3 min. The carrier gas was helium, with a total flow of 1 mL/min. The separation was performed on a SUPELCOWAX 10 capillary column (Supelco, Bellefonte, PA, USA, 30 m × 0.25 mm × 0.25 µm), using the following temperature program: starting at 35°C for 8 min; increasing by 4°C/min up to 60°C; increasing by 20°C/min up to 200°C; maintaining this final temperature for 10 min. The transfer line temperature was 230°C. The signal acquisition mode was SIM (93, 121, 136 m/z for the first 20 min, from 20 to 20,49 min were 93, 105, 133, 164, 204 m/z; from 20,50 min to the end were 93, 121, 147, 204 m/z). Terpenes identification was performed by comparing their retention times with those of pure standard compounds injected under the same chromatographic conditions, and comparing ratios between selected ions intensity with those of pure compounds.

Statistical Analysis

Statistical analysis was performed with SAS software (version 8.6, SAS Institute Inc., Cary, NC). A repeated mixed model was applied, in which breed (Ho, Mo, Va), period (June, July) and their interaction were included as fixed factors. The repeated factor was the period, with the individual cow as subject. Results are presented as Least Square means and standard errors of the mean (SEM). In addition, Pearson's correlation coefficients between the variables describing grazing behavior and those of milk composition were calculated in order to be able to distinguish among individual cows.

RESULTS

Grazing Behavior: Selectivity Index and Composition of the Simulated Bites

Only a few differences were observed among breeds in terms of grazing behavior (**Table 3**). Based on the IS, both Ho and Mo avoided mature vegetation whereas Va were indifferent to it. In June, Ho tended to express a stronger aversion to legumes than the other breeds (IS Ho = −0.78, IS Mo = −0.38, and IS Va = −0.25, $p = 0.054$; data not shown in table). No changes in the botanical and nutritional composition of the simulated bites was observed, except for a tendency of Mo to select herbage with a higher ADF content in June (+23 and +22 compared to that selected by Ho and Va cows, respectively, $p = 0.093$; data not shown in table). These tendencies observed in June among breeds were not observed in July. The daily potential intake capacity of Va was 4.2 kg of DM lower than those of Ho and Mo, and, as a consequence, their daily estimated grasses intake was lower (3.6 kg of DM). No difference among breeds were observed for legumes and forbs intake. The intake of dry material was also estimated higher for Ho compared to Mo and Va (+2.0 kg of DM). Regardless of breed, grazing behavior of all cows changed between the two experimental periods. In July, the IS for forbs and mature vegetation decreased by 0.31 and 0.62, respectively, whereas it increased by 0.17 and 0.25 for legumes and short vegetative herbage. The proportion of the different vegetation types in the selected bites did not differ between June and July, except for a tendency for increased proportion of dry vegetation (+12.5%, $p = 0.059$). Overall, the selected bites had a higher crude protein content (+38 g/kg DM) and a lower ADF content (−24 g/kg DM) in July compared to June. The estimated OMD tended to be higher by 2.9 g/kg DM in July than in June ($p = 0.086$). The proportion of legumes in the selected bites was negatively correlated to ADF and NDF contents (-0.609^{**} and -0.612^{**}, respectively), and positively correlated to the crude protein content (0.673^{**}) and estimated OMD (0.591^{**}). The proportion of forbs in the simulated bites was negatively correlated to the NDF content (-0.462^{*}). The proportion of dry material was negatively correlated to the ADF content (-0.432^{*}).

Milk Yield and Gross Composition, and Their Relation With Grazing Behavior

Milk yield and protein content did not significantly differ among breeds. In Mo, the milk fat content was higher than in Ho and Va, by 5.9 and 9.2 g/kg, respectively. The daily milk fat yield was similar between Ho and Mo and lower by 126 g/day in average for Va. There was a similar tendency for the daily protein yield (−89 g/day in average for Va compared to both other breeds, $p = 0.077$). In June, the SCC of Mo's milk was lower than that

TABLE 3 | Indices of selectivity, characterization of vegetation eaten (simulated bites) by the cows, and performance distinguished by breed and period.

Item	Breed			Period			Significance		
	Ho	Mo	Va	June	July	SEM	Breed	Period	Breed × period
Jacob's index of selectivity ($-1 \le IS \le 1$)									
Grasses	0.45	0.41	0.29	0.41	0.36	0.030	ns	ns	ns
Legumes	−0.56	−0.36	−0.23	−0.47	−0.30	0.055	†	*	†
Forbs	−0.26	−0.27	0–.18	−0.08	−0.39	0.055	ns	*	ns
Short vegetation	0.39	0.47	0.28	0.26	0.51	0.046	ns	*	ns
Tall vegetation	−0.23	−0.28	−0.21	−0.27	−0.21	0.045	ns	ns	ns
Mature vegetation	-0.60^{a}	-0.69^{a}	-0.24^{b}	−0.20	−0.82	0.093	*	***	**
Proportion of vegetation type in the simulated bites (% of DM)									
Green vegetation									
Grasses	86.6	81.2	84.4	84.1	84.0	4.05	ns	ns	ns
Legumes	1.2	2.2	1.3	0.5	2.6	0.75	ns	*	ns
Forbs	12.2	16.7	14.3	15.3	13.4	3.7	ns	ns	ns
Dry material	22.5	20.0	21.5	15.1	27.6	2.62	ns	†	ns
Potential intake capacity and estimated intake of vegetation type (kg of DM)									
Total potential intake capacity	22.7^{a}	22.1^{a}	18.2^{b}	20.9	21.1	0.65	**	ns	ns
Green vegetation									
Grasses	15.7^{a}	15.8^{a}	12.1^{b}	16.2	12.9	1.01	*	**	ns
Legumes	0.3	0.2	0.1	0.1	0.3	0.07	ns	ns	ns
Forbs	1.8	1.6	1.5	2.0	1.2	0.33	ns	*	ns
Dry material	4.3^{a}	2.4^{b}	2.1^{b}	2.6	3.3	0.39	**	ns	†
Nutritional value of the diet eaten by the cows (simulated bites; g/kg DM)									
Crude protein	110	102	111	87	125	5.2	ns	**	ns
Neutral detergent fiber	658	653	642	657	645	4.5	ns	ns	ns
Acid detergent fiber	331	339	329	345	321	4.1	ns	**	†
Digestibility of organic matter (g/kg)	461	456	475	449	478	8.4	ns	†	ns
Milk yield and composition									
Milk yield (kg/day)	12.1	11.4	9.2	12.2	9.6	0.61	ns	*	ns
Protein content (g/kg)	30.6	33.0	30.6	31.6	31.2	0.74	ns	ns	ns
Fat content (g/kg)	39.5^{b}	45.4^{a}	36.2^{b}	39.6	41.2	1.13	**	ns	ns
logSCC	2.4	3.2	2.2	2.2	2.4	0.07	ns	*	*
Protein yield (g/day)	363^{a}	372^{a}	278^{b}	377	298	17.3	†	**	ns
Fat yield (g/day)	471^{a}	510^{a}	334^{b}	484	393	25.4	*	*	ns

*Significance levels: ***$p \le 0.001$, **$p \le 0.01$, *$p \le 0.05$, †$p \le 0.10$, ns $p > 0.10$.*

^{a,b}Least square means with different superscript letters significantly differ at $p < 0.05$.

Ho, Holstein; Mo, Montbéliarde; SCC, somatic cell count; Va, Valdostana Red Pied.

of Ho and Va by 0.5 and 0.3 log units, respectively. Regardless of breed, the milk yield decreased by 2.6 kg from June to July. Concomitantly, the daily fat and protein yields decreased by 79 and 91 g/day, respectively. Milk yield and composition were not correlated to the proportions of the different botanical groups in the bites of the cows, but milk yield was positively correlated to the estimated grasses intake (0.594**), as well as milk fat and protein yields (0.620** and 0.575**, respectively). However, both yields were also positively correlated to the total potential intake capacity (0.508* and 0.575**, respectively), as well as milk fat content (0.406*). Concerning herbage composition, fat and protein yield were positively correlated to the ADF content (0.466* and 0.497*, respectively). The SCC (log unit) of the milk was negatively correlated to the ADF and NDF contents of the herbage (−0.564** and −0.457*, respectively).

Milk Fatty Acid Profile and Its Relation With Grazing Behavior

The milk fat of Va tended to have a higher content of C18:2 n-6 (+0.11 g/100 g of total FA, $p = 0.070$) and had a higher content of C18:3 n-3 (+0.12 g/100 g of total FA) than the milk fat of both other breeds (**Table 4**). Milk fat of Mo tended to have the lowest content of total n-6 FA (−0.14 g/100 g of total FA in average, p = 0.097), and the n-6 to n-3 and C18:2n-6 to C18:3n-3 ratios were 10% higher in milk fat of Ho than that of the other breeds. In June, the milk fat of Ho tended to have a higher content of C16:0 ($p = 0.071$) than that of Mo (+0.30 g/100 g of total FA), and then Va (+1.5 g/100 g of total FA), but this was no longer the case in July (data not shown in table). Most of the changes in milk FA profile occurred between June and July. In June, the milk fat of all cows had a higher content of saturated FA (total SFA +2.9 g/100 g of total FA) than in July. More specifically, the content of C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, and C16:0 was higher. On the contrary, the content of mono-unsaturated FA was higher in milk fat in July (+2.4 g/100 g of total FA), in particular that of C18:1c9 (+1.97 g/100 g of total FA), as well as the sum of all C18:1 *cis* isomers (+2.1 g/100 g of total FA). The content of poly-unsaturated FA in milk fat was also higher in July (+0.41 g/100 g of total FA), in particular that of n-3 FA (+0.09 g/100 g of total FA). Accordingly, the ratio n-6 to n-3 decreased in July. There was a decrease in the proportion of even-chained FA in July (−2.94 g/kg), whilst that of the odd-chained FA increased (+0.26 g/kg). The C14:1c9 to C14:0 and C18:1c9 to C16:0 ratios increased by 0.02 and 0.17 from June to July, respectively. *De novo* synthesized FA decreased in July, by 1.79 g/kg. Overall, milk FA proportions had little correlation with the proportions of the botanical groups in the diet of the cows. The proportion of grasses in the simulated bites was correlated only to the proportion of one FA, C18:0 (0.444*). The estimated grasses intake was also negatively correlated to C18:3n-3 (−0.482*) and n-3 FA (−0.412*) concentration in milk. In the same way, legume proportion was only correlated to the proportion of C18:3 n-6 (0.408*). Forb proportion was positively correlated to proportions of C12:0 and C14:0 (0.419* and 0.423*, respectively), and negatively correlated to proportions of C14:1t9 and C18:0 (−0.444* and −0.470*, respectively). The proportion of dry material in the bites was positively correlated to proportions of C18:1c9 (0.487*), MUFA (0.552**), sum of C18:1 *cis* isomers (0.490*), and to the C14:1c9 to C14:0 and C18:1c9 to C18:0 ratios (0.445* and 0.462*, respectively). It was negatively correlated to the proportions of C5:0 (−0.407*), C6:0 (−0.458*), total SFA (−0.558**) and *de novo* FA (−0.441*). Two correlations were also found between milk FA profile and the nutritional value of the diet as calculated from the simulated bites: the crude protein content of the herbage was positively correlated to C18:3 n-6 proportion (0.531*) and the NDF content was negatively correlated to C12:1c9 proportion (−0.461*).

Prevalence of Milk Terpenes and Their Relation With Grazing Behavior

A total of 16 different terpenes were identified in the milk of the three breeds, whereof the 11 most frequently found terpenes are presented in **Table 5**. From these 16 terpenes, α- and β-pinene, camphene, γ-terpinene, α- and β-caryophyllene were found in all 24 samples. Sabinene was detected in 22 samples out of 24, limonene in 21, β-myrcene and terpinolene in 19, and α-phellandrene in 16. The other compounds occasionally detected were α-thujene, δ3-carene, ocimene and linalool. Regardless of breed or period, the three most abundant terpenes found in milk were limonene, β-caryophyllene and γ-terpinene, with in average 24.6, 23.8, and 10.2 %, respectively. Milk terpenes did not vary among breeds, but a few differences occurred between the two experimental periods. This was Specifically an increase in camphene (+2.98 %) and α-caryophyllene (+2.64 %) prevalence in July compared to June. Terpinolene and β-caryophyllene tended to increase in July, too (+1.50 and +9.18%, p = 0.096 and 0.088, respectively), while β-myrcene tended to decrease (−1.53%, $p = 0.068$). The milk terpenes were not correlated to the proportions of different botanical groups in the bites of the cows, except for α-caryophyllene that was negatively correlated with the estimated grasses intake (−0.475*). It was also negatively correlated to the ADF content of the bites (−0.454*).

DISCUSSION

Differences Among Breeds in Grazing Selection

In continuous grazing conditions on a biodiverse pasture, according to the IS, cows of the least specialized breed (Va) were less selective on pasture than those of the highly specialized breed (Ho). This was particularly the case with mature herbage. Grazing behavior of Mo seems to be more similar to that of Ho, even though they were a little less selective. However, these differences in grazing selection were minimal compared to those expressed within breeds on pastures with different botanical composition or under different grazing management (21, 27), as was also observed in the present study, when forage of different vegetation growth stage was offered in June and July. Still, the breed differences in grazing selection were lower than expected and were in contrast to the results of Farruggia et al. (35), who showed that lactating cattle with high nutrient requirements grazed more selectively than dry cows, and Pauler et al. (22, 23), who found that more productive Angus × Holstein cattle grazed more selectively than Highland cattle. However, Dumont et al. (36) also did not find relevant differences in grazing behavior among traditional and specialized breeds. The few differences among cow breeds highlighted by the IS did not lead to a significant difference in composition and estimated digestibility of the simulated bites in our experiment. This indicates that, even though cows selected or avoided some species while grazing, the corresponding changes in DM quantities found in the simulated bites remained too low to make a clear difference in their proportion of the total daily diet. Concerning season and evolution of the herbage, cows avoided forbs and mature vegetation less in June than in July, probably because they already overgrazed the preferred patches with grasses during the beginning of the grazing cycle (24, 37, 38). In July, they selected the vegetative regrowth and avoided legumes less. This can be explained by the observation that the latter botanical group regrows rapidly (21) and therefore was more accessible at that time than at the end of the first grazing cycle. These changes in grazing selection and the increased small proportion of legumes are coherent with the higher crude protein

TABLE 4 | Proportions of fatty acids (g/100 g total fatty acids) in milk fat distinguished by breed and period.

Fatty acid	Breed			Period		SEM	Significance		
	Ho	Mo	Va	June	July		Breed	Period	Breed × period
C4:0	3.44	3.53	3.24	3.50	3.31	0.100	ns	ns	ns
C6:0	1.94	1.93	1.81	2.00^{a}	1.79^{b}	0.050	ns	*	ns
C8:0	0.90	0.91	0.87	0.95^{a}	0.83^{b}	0.027	ns	*	ns
C10:0	1.69	1.64	1.59	1.77^{a}	1.51^{b}	0.059	ns	**	ns
C12:0	1.88	1.79	1.86	1.98^{a}	1.76^{b}	0.061	ns	**	ns
C12:1c9	0.048	0.051	0.052	0.050	0.051	0.002	ns	ns	ns
C14:0	8.21	8.37	8.05	8.62^{a}	7.81^{b}	0.221	ns	**	ns
C14:1c9	0.77	0.73	0.82	0.75	0.79	0.037	ns	†	ns
C15:0	1.28	1.28	1.35	1.22^{b}	1.39^{a}	0.040	ns	**	ns
C16:0	23.9	23.9	23.1	24.5^{a}	22.7^{b}	0.41	ns	***	†
C16:1c9	1.35	1.38	1.26	1.29	1.37	0.052	ns	ns	ns
C17:0	0.81	0.78	0.82	0.80	0.80	0.015	ns	ns	ns
C18:0	13.1	11.7	12.2	12.2	12.6	0.30	ns	ns	ns
C18:1t10	0.21	0.21	0.23	0.23	0.21	0.009	ns	ns	ns
C18:1t11	2.15	2.25	2.50	2.28	2.31	0.132	ns	ns	ns
C18:1c9	25.1	26.2	26.3	24.9^{b}	26.8^{a}	0.444	ns	**	ns
C18:2n-6	1.19	1.14	1.28	1.18	1.22	0.023	†	ns	ns
C18:3n-3	0.82^{b}	0.87^{ab}	0.97^{a}	0.85	0.92	0.022	*	†	ns
C18:2c9t11	0.89	1.08	1.26	1.03	1.129	0.056	ns	ns	ns
ECFA	55.6	54.2	53.1	55.9^{a}	53.0^{b}	0.61	ns	**	ns
OCFA	2.89	2.87	3.01	2.79^{b}	3.05^{a}	0.060	ns	***	ns
BCFA	3.01	3.00	3.08	2.99	3.08	0.076	ns	ns	ns
SFA	60.9	59.6	58.6	61.1^{b}	58.2^{a}	0.54	ns	**	ns
MUFA	33.7	35.0	35.3	33.5^{b}	35.9^{a}	0.47	ns	**	ns
PUFA	4.53	4.62	4.16	4.56^{b}	4.97^{a}	0.124	ns	*	ns
Σcis18:1	26.4	27.5	27.6	26.1^{b}	28.2^{a}	0.46	ns	**	ns
Σtrans18:1	3.46	3.60	3.96	3.60	3.75	0.168	ns	ns	ns
Σn-6	1.52	1.39	1.55	1.46	1.51	0.029	†	ns	ns
Σn-3	1.12	1.12	1.25	1.12^{b}	1.21^{a}	0.027	ns	*	ns
Ratios									
n-6 to n-3	1.37^{a}	1.24^{b}	1.25^{b}	1.31^{a}	1.26^{b}	0.019	*	*	ns
C14:1c9 to C14:0	0.094	0.086	0.102	0.087^{b}	0.102^{a}	0.004	ns	**	ns
C18:1c9 to C16:0	1.05	1.12	1.15	1.02^{b}	1.19^{a}	0.034	ns	***	ns
Σ *de novo* synthesized fatty acids	18.0	18.3	17.4	18.8^{a}	17.0^{b}	0.42	ns	*	ns

*Significance levels: ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, $^{\dagger}p \leq 0.10$, ns $p > 0.10$.*

^{a,b}*Least square means with different superscript letters significantly differ at $p < 0.05$.*

BCFA, branched-chained fatty acids; ECFA, even-chained fatty acids; Ho, Holstein; Mo, Montbéliarde; MUFA, mono-unsaturated fatty acids; OCFA, odd-chained fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Va, Valdostana Red Pied.

content in the simulated bites, together with their lower ADF content, in July compared to June. Finally, cows seem to have adapted their grazing behavior to what was offered during the respective season, regardless of breed. Further studies would be required to strengthen our findings over a larger number of animals.

Differences Among Breeds in Milk FA Profile

The differences among cow breeds in milk yield and gross composition were previously discussed in Koczura et al. (24). Briefly, the lower milk fat content in Va milk compared to Mo is typical of Va breed (39, 40), as this dual-purpose breed is not specifically selected for a high milk fat content. The lower fat content of Ho compared to Mo is in accordance with previous results comparing Ho and Mo reared in the same conditions (41). The lower fat and protein yields of Ho compared to Mo could be due to the lower adaptability of Ho breed to cover their high requirements without concentrate supplements, resulting in a negative energy balance, and lower fat and protein yield (24). Regarding FA composition of milk fat, several studies already investigated the link between the composition of diverse pastures

TABLE 5 | Prevalence of the most abundant terpenes in milk (proportion of individual peak area over total peak area, %) distinguished by breed and period.

Terpene	Breed			Period		SEM	Significance		
	Ho	Mo	Va	June	July		Breed	Period	Breed × period
Limonene	26.7	30.1	17.0	29.4	19.8	3.96	ns	ns	ns
β-Caryophyllene	21.2	23.5	26.8	19.2	28.4	2.73	ns	†	ns
γ-Terpinene	10.56	12.15	7.96	8.98	11.47	1.323	ns	ns	ns
α-Pinene	8.75	9.61	10.72	10.03	9.36	0.599	ns	ns	ns
β-Pinene	8.71	8.80	10.85	7.90	11.01	0.891	ns	ns	ns
α-Caryophyllene	3.49	3.15	5.03	2.56^b	5.21^a	0.602	ns	*	ns
Camphene	3.37	3.43	4.55	2.29^b	5.27^a	0.639	ns	*	ns
β-Myrcene	2.30	2.82	2.24	3.22	1.69	0.363	ns	†	ns
Terpinolene	2.00	1.74	2.83	1.44	2.94	0.394	ns	†	ns
Sabinene	1.29	1.43	1.96	1.76	1.36	0.170	ns	ns	ns
α-Phellandrene	0.32	0.61	0.63	0.30	0.74	0.129	ns	ns	ns

*Significance levels: *$p \leq 0.05$, †$p \leq 0.10$, ns $p > 0.10$.*

[a,b]Least square means with different superscript letters significantly differ at $p < 0.05$.

Ho, Holstein; Mo, Montbéliarde; Va, Valdostana Red Pied.

and milk quality [e.g., (16, 18, 42)], but only a few allowed to link the selective behavior that ruminants exhibit on pasture with their milk composition (27, 38). These authors illustrated how the ability of an animal to feed on forbs instead of grasses has an effect on the ruminal microbial population, with PSC partially inhibiting the biohydrogenation of dietary PUFA, entirely or at certain steps. This results in milk richer in these FA, and particularly in n-3 FA (43). However, this effect is especially visible when comparing selection on highly biodiverse pastures with grasslands with very different proportions of grasses and forbs (18). In the present experiment, all cows grazed on the same biodiverse pasture. The results, therefore, suggest that, even though some preferences were expressed, the differences in the material eaten among breeds remained too low to affect the milk FA profile. This is consistent with the similar FA profile of the milk found for the three breeds, especially in terms of MUFA, PUFA, and CLA proportions of the milk fat. These groups of FA were actually correlated to the herbage evolution over the season and, more specifically, to the proportion of dry material in the simulated bites, regardless of breed. The cause for that is the advanced herbage phenological stage in June compared to July measurement period, which is known to increase MUFA and decrease PUFA and CLA proportions of the milk fat (18, 42). Only one particular FA, the major n-3 FA C18:3 n-3, was found to be always higher in Va milk fat compared to Ho milk fat, without any correlation to diet selection or period. This FA is a substrate for ruminal biohydrogenation, leading to the production of C18:1t11, ultimately being saturated to C18:0 (43). Although not significant, C18:1t11 was also numerically higher in Va than Ho milk fat. This suggests that C18:3 n-3 is less biohydrogenated in the rumen of Va than Ho cows, and this could probably be explained by breed differences in the rumen microbial population and metabolism rather than grazing behavior. This aspect should be further investigated, especially on a larger number of animals.

Differences Among Breeds in the Prevalence of Milk Terpenes

To our knowledge, this is the first study directly linking diet selection of dairy cows and the occurrence of terpenes in their milk. Terpenes are absorbed directly from the diet (9), and most of them are transferred directly into milk and are subject to no or minor changes (44, 45). Some authors demonstrated that terpenes can also be partially biohydrogenated and isomerized by the rumen microbial population, leading to additional terpenes in milk (46). In the present experiment, the investigated pasture was rich in forbs, such as plants from the *Apiaceae* family and *Thymus serpyllum*, both containing pinenes and caryophyllenes (47, 48). The presence of this kind of compounds in the milk of the experimental cows suggests that pinenes and caryophyllenes indeed originated from the diet. Several previous studies already identified β-caryophyllene in the milk of grazing cows (10, 11). However, unlike our hypothesis, it seems difficult to directly relate terpene prevalence in milk and grazing behavior of the cows. Accordingly, in the present study no significant correlations were found between terpene prevalence and the proportion of the different botanical groups in the simulated bites. This could be partially due to the limited number of animals in our experiment. However, if a biological link was underlying between grazing selection and milk composition, significant correlations would have been expected even with few animals, probably with a poor correlation coefficient that would have been improved by increasing the number of animals, but it was not the case. This suggests that the selection of a single species or family could be responsible for the transfer of such molecules (5), rather than the overall proportion of forbs or mature vegetation in the sward. Besides, Lejonklev et al. (49) showed that terpenes from essential oils can be transferred to milk by both ingestion and inhalation. Cows may therefore also have taken in terpenes while breathing, which would explain why the latter are not correlated to the simulated bites. The few differences that occurred in milk terpene prevalence were related to the grazing period: the vegetative stage of the pasture evolved after the first grazing cycle and most probably led to a different terpene composition of the herbage (5, 50). Indeed, a parallel increase was observed by Tornambé et al. (10) between milk terpenes concentration and the variation in the phenological stage of the herbage. Even though concentrations of terpenes found in milk are low, it would be interesting to manufacture cheeses with the milk of the three breeds and investigate the link between terpene profile, microbial development and potential further influences on cheese sensory properties. Such an effect could be expected because some terpenes have been found to have antimicrobial effects. Rivas da Silva et al. (47) for instance demonstrated that positive enantiomers of α- and β-pinene used in synergy (250 μg/mL) can have a bactericidal effect against methicillin-resistant *Staphylococcus aureus*.

Implications

The overall small differences among the autochthonous, more robust Va, the intermediate Mo and the Ho highly specialized for milk production may suggest that in low-input mountain grazing systems, the individual animal's adaptability in the short term could actually be more important than its breed. It has to be mentioned, though, that all cows were in their late lactation and therefore even the Ho were not in a situation of high nutrient requirements. Breed differences could be clearer in other stages of lactation. Breed also seems to have less influence on milk quality than herbage composition. Other criteria than grazing behavior should be investigated in order to assess the role of autochthonous breeds in the multi-performance of future low-input grazing systems. Indeed, individual adaptation in the short term may result in different long-term breed responses, i.e., in reproduction or productive lifespan. Further investigation on a larger number of animals would reinforce our findings.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

No ethical approval was required because no invasive samples have been taken on the animals and all procedures carried out followed the Certificate of Authorization to Experiment on Living Animals No. D 15-114-01 delivered by the French government to Marcenat INRAE experimental facility.

AUTHOR CONTRIBUTIONS

MKo, MC, BM, MKr, and JB contributed to the conception and design of the study. MKo, MB, and GT organized the experiment. MKo supervised the experiment. MKo, MB, and BM participated in behavioral observation and milk sampling. MC performed the fatty acids analysis. MMu and MMa performed the terpene analysis. MKo and MC processed the data and equally contributed to the writing of the manuscript. MC performed the statistical analysis. MKr and JB supervised MKo in the frame of her doctoral thesis. All the authors contributed to manuscript revision, and all read and approved the submitted version.

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frontiers in Veterinary Science

BRIEF RESEARCH REPORT
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A Note on the Tracing of Herbage Contribution to Grazing Sheep Diet Using Milk and Feces Biomarkers

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Milk from grazing ruminants is usually rich in beneficial components for human health, but distinguishing milks sourced from grazing is difficult, and this hinders the valuing of the grazing benefit. This study aimed at evaluating the ability of milk biomarkers (1) to trace milks sourced from sheep submitted to different access times (ATs) to pasture and (2) to estimate sheep herbage dry matter intake (HDMI, g DM ewe^{-1} d^{-1}) and herbage percentage (HP, % DM) in sheep diet. Animal data derive from a published experiment in which six replicated groups of mid-lactation Sarda sheep had ATs of 2, 4, or 6 h d^{-1} to a ryegrass pasture. Sheep HDMI and HP of each group were measured on four dates in April 2013. Group milk was sampled, and milk fatty acids (FAs) and n-alkanes were determined by gas chromatography. The latter markers were also measured in feces samples bulked by group. The data ($N = 24$ records) were submitted to Linear Discriminant Analysis (LDA) aimed at distinguishing the AT to pasture based on biomarkers previously selected by Genetic Algorithms (GA). Partial Least Square Regression (PLSR) models were used to estimate HDMI and HP using biomarkers selected by GA. Based on one milk alkane and six milk FAs as biomarkers, estimates of the AT using GA-LDA were 95.8% accurate. The estimation of HDMI by GA-PLSR based on five milk FAs was moderately precise [explained variance = 75.2%; percentage of the residual mean square error of cross-validation over the mean value (RMSECV%) = 15.0%]. The estimation of HP by GA-PLSR based on 1 milk alkane and 10 FAs was precise (explained variance = 80.8%; RMSECV% = 7.4%). To conclude, these preliminary results suggest that milks sourced from sheep flocks with AT to pasture differentiated by 2 h in the range 2–6 h d^{-1} can be precisely discriminated using milk biomarkers. The contribution of herbage to sheep diet can also be precisely estimated.

Keywords: dairy sheep, nutrition, traceability, alkanes, fatty acids, pasture, chemometrics, authentication

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INTRODUCTION

Grazing delivers high-quality ruminant products at low cost as compared with stall feeding, as highlighted by recent reviews (1, 2). Positive implications were often found on these products, with reference to their nutritional and health value as well as technological and sensory attributes, such as texture, oxidative stability, and flavor (1) with few exceptions [e.g., (3)]. Moreover, consumers perceive pasture-based livestock systems as more friendly for both environment and animal welfare than housed systems (4).

Unfortunately, pasture availability is seasonal and often scarce in many grazing areas, such as the Mediterranean regions; hence, supplementation is necessary, at least for part of the pasture growth cycle. For this reason, part-time grazing (PTG), i.e., a time-restricted allocation to pasture, is often implemented in Mediterranean dairy sheep systems. This technique has revealed several beneficial implications compared with stall feeding and 24-h grazing, among them, the saving of herbage when herbage growth is low and a more balanced diet (5). Moreover, a moderate restriction of access time (AT) to pasture (6 h/d) can suffice to reach levels of beneficial FA in milk as high as those achieved with longer allocations (9 h/d), as shown in dairy cattle (6).

In order to increase the value of grazing in the sheep supply chain, tracing the milk back to the feeding system is fundamental. Tracing can be based on biomarkers, such as milk FA and fecal and milk concentration of n-alkanes, which are long-chain hydrocarbons contained in plant epicuticular or waxes (7). N-alkanes, particularly those with a short chain, can be uptaken by the gut mucosa to some extent and pass without changes in ruminant milk (8). In fact, milk alkane profile was successfully used to discriminate cheeses derived from cows grazing pastures with different botanical compositions (9).

Despite the growing body of knowledge on biomarkers of dairy products, the impact of these findings is still limited, because milk FAs and alkanes composition can possibly fail to discriminate milks coming from semi-intensive systems, where PTG is practiced (10).

This paper is an outcome of a wider research program undertaken at Agris Sardegna in 2013–2016 for evaluating the impact of PTG of dairy ewes on their ingestive behavior and milk production (5, 11). This specific study is aimed at evaluating the ability of milk FAs and n-alkanes measured in both milk and feces 1) to trace bulk milks sourced from sheep submitted to PTG with different ATs to pasture and 2) to estimate sheep herbage dry matter intake (HDMI, g DM ewe^{-1} d^{-1}) and herbage percentage (HP, % DM) in sheep diet. Since Genetic Algorithms (GA) had already been successfully used to select the informative variables for the estimation of the sheep milk fatty acids (FAs) by mid-infrared spectroscopy (12) and in the selection of the FAs able to trace the geographical origin of sheep milk (13), we investigated their use to the aims of the present work.

MATERIALS AND METHODS

The animal protocol and implemented procedures were in accordance with the ethical guidelines in force at Agris, in compliance with the EU directive 86/609/EC and the recommendation of the EU Commission 2007/526/EC.

The study was conducted at the Bonassai research station, north-western Sardinia (40°N, 8°E, 32 m a.s.l.). The whole experiment lasted from February to April 2013, but, for the purpose of this work, the experimental period ranges from 10 to 23 April. This short period was chosen to represent the spring period in which neither availability nor quality of grazed grass limits animal performance. The climate is Mediterranean with a long-term (1995–2013) average annual rainfall of 568 mm. A randomized block design was adopted, with two replicates per treatment. Pasture consisted of 1.5 ha of Italian ryegrass (*Lolium multiflorum* Lam. cv. Teanna). The area was split into two blocks of 7,500 m^2 each, which were in turn divided into three experimental plots ($n = 6$ plots in total) randomly allocated to the treatments. Each plot was then divided by electric fences into four sub-plots of 625 m^2 each, which were rotationally grazed, with 7 days of occupation per sub-plot and a recovery period of 21 days.

Six groups of six ewes each, all belonging to Sarda breed, lambed in autumn (November–December) and at the mid-lactation stage (mean ± standard deviation 76 ± 14 days in milk) at the beginning of the experiment, were balanced for sheep age (3.7 ± 1.2 years), pre-experimental milk yield (1,449 ± 206 g/d), and body weight (42.5 ± 4.0 kg) and randomly assigned to the experimental plots. The ewes were machine milked twice daily at 07:00 and 15:00 h. After morning milking, the groups were carried on a trailer to the plots where they spent the scheduled time. Treatments were three different levels of ATs to pasture: 2 (08:00–10:00), 4 (08:00–12:00), and 6 h/d (08:00–14:00). During the remaining daytime, the ewe groups were kept indoors in separate pens. Supplementation consisted for all ewes of pelleted concentrate (400 g/head day split in two meals at milkings), lupin seed (300 g/head day) at pasture turnout, and ryegrass hay (700 g/head day) overnight. The flat supplementation rate was set in order to meet 100% of energy requirement of the 4 h/d treatment and 100% metabolic protein requirements of 2 h/d treatment. For details on pasture establishment and animal management, refer to Molle et al. (11).

Measurements and Samplings

Supplements intake was measured at group level, weighing the offer and the refusals either after each meal (concentrates) or after 24 h (hay). On four occasions during the experimental period (test days), short-term herbage intake rate was measured on three ewes per group using the double-weighing technique as detailed in depth by Molle et al. (5). The day after each test day, individual milk yield was measured and milk sampled for determining milk fat, protein, and lactose contents (MilkoScan FT+; Foss Electric, Hillerød, Denmark). Bulk milk samples of each group of ewes for each treatment were also collected for milk FA and n-alkane determinations. Samples of supplements and hand-plucked samples of ryegrass potentially eaten by the sheep were taken on the intake measurement days. Moreover, feces were also individually grab-sampled from each ewe tested for intake measurement after each milking on the days of milk recording. All these samples were immediately frozen and then freeze-dried prior to analysis. The sample of feces was bulked per group before chemical determinations.

Chemical Analysis

The FA composition of the milk samples was determined as reported in Caredda et al. (12). The FA content of herbage and supplements was determined according to Addis et al. (14). Supplement FA composition was measured on a composite sample per feedstuff. The n-alkane analysis of herbage and feces followed the protocol by Dove and Mayes (15). A similar protocol was implemented for milk alkane determination. Milk, feeds, and feces alkane analytical method and gas chromatographic conditions are reported as **Supplementary Material**. Individual n-alkanes from C23 to C36 were identified by the comparison of the retention time of a standard mixture of pure components. Furthermore, indices were calculated with reference to n-alkane, such as the ratio between the concentrations of adjacent alkanes with carbon chain length ranging between C27 and C33 (C27/C25, C29/C27, C31/C29, and C33/C31). Feedstuff samples were also submitted to analyses for the evaluation of their nutritional value according to the methods detailed by Molle et al. (5). Data on feedstuff nutritional, FA, and n-alkane compositions are reported in **Supplementary Table 1**.

Statistical Analysis

The database was constituted of $N = 24$ group records (3 treatments × 2 replicates × 4 dates), inclusive of treatment (AT, h/day), replicate (1, 2), date, and all the measured biomarkers (alkane in feces and milk and FA in milk and their classes and indices). Since the two replicates actually consisted of two different groups of ewes, the obtained milk samples were not considered replicates from a chemometric point of view but different samples belonging to the same treatment category. Means with standard deviation of the distribution and ranges of the data across the experimental period are shown in **Table 1**. Correlation analysis was used to explore the relationships among variables.

Linear Discriminant Analysis (LDA) was used for classifying the samples coming from sheep submitted to different feeding regimens, and the resulting confusion matrix was evaluated both in terms of accuracy (calculated as the average of the percentages of correct predictions of each category) and in terms of Cohen's kappa (κ) that takes into account the possibility of correct classifications occurring by chance (16). The Mahalanobis distance between each sample and the centroids of the three treatment groups were also evaluated. Partial Least Square Regression (PLSR) was used to predict sheep HDMI and HP in sheep diet. The models were built both using all the identified biomarkers in milk and feces as predictors and using the informative biomarkers selected, separately for each dependent variable, by means of GA (17, 18). The validation of the models was achieved through the cross-validation approach. LDA and PLSR were run on the CAT (Chemometric Agile Tool) software, developed by the Group of Chemometrics of the Division of Analytical Chemistry of the Italian Chemical Society, freely downloadable from the site gruppochemiometria.it.

RESULTS

Although comparing the effects of treatment on performance goes beyond the scope of this study, it is worth noting in **Supplementary Table 1** that the herbage contents of crude protein (CP, 137–142 g/kg DM) and neutral detergent fiber (NDF, 443–460 g/kg DM) showed a narrow range between groups, differently from herbage intake [mean ± SD, 718 ± 105 g DM (2 h/d), 1,248 ± 227 g DM (4 h/d), and 1,372 ± 216 g DM (6 h/d)], total intake [1,891 ± 128 g DM (2 h/d), 2,380 ± 217 (4 h/d), and

TABLE 1 | Mean ± standard deviation of the distribution (SD) and range of the variables under study.

		Mean ± SD	**Range**	
			Max	**Min**
Herbage intake	g DM/d	1,113 ± 342	1,768	580
Total intake	g DM/d	2,268 ± 337	2,947	1,686
Herbage in diet	% DM	47.9 ± 8.2	60.00	34.42
n-Alkane in feces				
C24	mg/kg DM	1.0 ± 1.7	6.74	0.00
C25	mg/kg DM	16.6 ± 2.7	24.69	12.98
C26	mg/kg DM	2.4 ± 1.0	6.07	1.58
C27	mg/kg DM	54.3 ± 4.6	61.93	45.25
C28	mg/kg DM	24.3 ± 9.1	44.35	10.01
C29	mg/kg DM	274.9 ± 34.3	350.73	213.83
C30	mg/kg DM	16.3 ± 2.3	21.52	12.75
C31	mg/kg DM	446.9 ± 60.4	565.75	332.93
C32	mg/kg DM	8.6 ± 2.7	17.19	5.02
C33	mg/kg DM	84.7 ± 16.3	115.00	56.40
C35	mg/kg DM	3.4 ± 2.2	6.18	0.00
R2725F		3.32 ± 0.45	4.40	2.41
Ratio 29/27		5.0 ± 0.4	5.66	4.37
Ratio 31/29		1.62 ± 0.06	1.77	1.54
Ratio 33/31		0.19 ± 0.03	0.27	0.15
n-Alkane in milk				
C24	mg/L	1.1 ± 0.4	1.89	0.00
C25	mg/L	3.0 ± 1.1	5.31	1.01
C26	mg/L	1.2 ± 0.4	1.90	0.00
C27	mg/L	4.0 ± 0.5	4.81	3.12
C29	mg/L	7.6 ± 0.8	8.74	6.36
C30	mg/L	0.9 ± 1.2	5.80	0.00
C31	mg/L	4.7 ± 1.5	6.24	0.00
Ratio 27/25		1.5 ± 0.7	3.55	0.87
Ratio 29/27		1.9 ± 0.2	2.20	1.62
Ratio 31/29		0.6 ± 0.2	0.78	0.00
Fatty acids in milk				
C4:0	% FAME	3.96 ± 0.15	4.30	3.71
C6:0	% FAME	2.80.1	2.99	2.49
C7:0	% FAME	0.03 ± 0.01	0.05	0.02
C8:0	% FAME	2.33 ± 0.15	2.60	1.99
C10:0	% FAME	6.7 ± 0.5	7.88	5.76
C11:0	% FAME	0.34 ± 0.03	0.44	0.27
C12:0	% FAME	3.7 ± 0.3	4.44	3.22
C13:0 *iso*	% FAME	0.035 ± 0.005	0.05	0.03
C13:0 *anteiso*	% FAME	0.046 ± 0.004	0.06	0.04
C14:0 *iso*	% FAME	0.13 ± 0.01	0.16	0.11
C14:0	% FAME	11.2 ± 0.6	12.77	10.40
C15:0 *iso*	% FAME	0.40 ± 0.04	0.46	0.32
C15:0 *anteiso*	% FAME	0.65 ± 0.04	0.76	0.58
C14:1 9c	% FAME	0.20 ± 0.02	0.25	0.15
C15:0	% FAME	1.18 ± 0.05	1.31	1.10
C16:0 *iso*	% FAME	0.33 ± 0.03	0.41	0.28

(Continued)

TABLE 1 | Continued

		Mean ± SD	Range	
			Max	Min
C16:0	% FAME	25.0 ± 1.1	27.21	22.59
C17:0 *iso*	% FAME	0.58 ± 0.05	0.67	0.49
C16:1 7c	% FAME	0.28 ± 0.02	0.33	0.25
C17:0 *anteiso*	% FAME	0.62 ± 0.04	0.71	0.54
C16:1 9c	% FAME	0.75 ± 0.07	0.90	0.62
C17:0	% FAME	0.69 ± 0.05	0.80	0.58
C17:1 10c	% FAME	0.18 ± 0.02	0.20	0.16
C18:0	% FAME	10.2 ± 0.7	11.75	8.71
C18:1 4t	% FAME	0.013 ± 0.003	0.02	0.01
C18:1 5t	% FAME	0.013 ± 0.004	0.02	0.00
C18:1 6t ÷ 8t	% FAME	0.18 ± 0.02	0.23	0.15
C18:1 9t	% FAME	0.20 ± 0.02	0.24	0.17
C18:1 10t	% FAME	0.30 ± 0.05	0.43	0.23
C18:1 11t	% FAME	1.2 ± 0.2	1.73	0.92
C18:1 12t	% FAME	0.35 ± 0.05	0.42	0.26
C18:1 13t ÷ 14t	% FAME	0.9 ± 0.1	1.22	0.67
C18:1 9c	% FAME	17.5 ± 0.9	19.32	15.81
C18:1 15t + 10C	% FAME	0.6 ± 0.2	1.04	0.40
C18:1 11c	% FAME	0.28 ± 0.02	0.31	0.24
C18:1 12c	% FAME	0.15 ± 0.03	0.20	0.11
C18:1 13c	% FAME	0.06 ± 0.01	0.07	0.05
C18:1 14c + 16t	% FAME	0.48 ± 0.05	0.59	0.38
C18:2 9t, 12t	% FAME	0.03 ± 0.01	0.05	0.02
C18:2 9c, 13t	% FAME	0.39 ± 0.05	0.49	0.30
C18:2 9c, 12t	% FAME	0.16 ± 0.02	0.18	0.13
C18:1 16c	% FAME	0.13 ± 0.02	0.17	0.11
C18:2 9t, 12c	% FAME	0.024 ± 0.004	0.04	0.02
C18:2 11t, 15c	% FAME	0.21 ± 0.05	0.31	0.14
C18:2 9c, 12c	% FAME	1.8 ± 0.2	2.08	1.55
C18:2 9c, 15c	% FAME	0.03 ± 0.01	0.04	0.02
C20:0	% FAME	0.28 ± 0.02	0.35	0.25
C18:3 6c, 9c, 12c	% FAME	0.05 ± 0.01	0.07	0.03
C20:1 9c	% FAME	0.02 ± 0.01	0.04	0.01
C20:1 11c	% FAME	0.05 ± 0.01	0.09	0.04
C18:3 9c, 12c, 15c	% FAME	0.60 ± 0.08	0.82	0.47
CLA 9c, 11t	% FAME	0.60 ± 0.06	0.72	0.48
CLA 9t, 11c	% FAME	0.08 ± 0.01	0.10	0.07
CLA 11t, 13c	% FAME	0.014 ± 0.003	0.02	0.01
CLA 12t,14t	% FAME	0.03 ± 0.01	0.04	0.01
CLA 11t, 13t	% FAME	0.03 ± 0.01	0.05	0.02
CLA 9t, 11t	% FAME	0.022 ± 0.003	0.03	0.02
C20:2 11c, 14c	% FAME	0.03 ± 0.01	0.05	0.01
C20:3 5c, 8c, 11c	% FAME	0.21 ± 0.02	0.27	0.17
C22:0	% FAME	0.023 ± 0.002	0.03	0.02
C20:3 8c, 11c, 14c	% FAME	0.03 ± 0.02	0.11	0.01
C20:3 11c, 14c, 17c	% FAME	0.01 ± 0.01	0.04	0.00
C20:4 5c, 8c, 11c, 14c	% FAME	0.17 ± 0.01	0.19	0.15
C23:0	% FAME	0.06 ± 0.01	0.08	0.04
C24:0	% FAME	0.05 ± 0.01	0.06	0.03
C20:5 5c, 8c, 11c, 14c, 17c	% FAME	0.05 ± 0.01	0.06	0.03
C26:0	% FAME	0.033 ± 0.004	0.04	0.02
C22:5 7c, 10c, 13c, 16c, 19c	% FAME	0.10 ± 0.01	0.13	0.09
C22:6 4c, 7c, 10c, 13c, 16c, 19c	% FAME	0.04 ± 0.01	0.05	0.03
Saturated FA	% FAME	71.4 ± 1.2	74.51	69.83
Unsaturated FA	% FAME	28.6 ± 1.2	30.17	25.49
Monounsaturated FA	% FAME	24.0 ± 1	25.36	21.41
Polyunsaturated FA	% FAME	4.7 ± 0.3	5.25	4.08
n−6 FA	% FAME	2.3 ± 0.2	2.59	1.97
n−3 FA	% FAME	0.96 ± 0.09	1.21	0.80
Ratio n3/n6		0.42 ± 0.04	0.49	0.35

(Continued)

TABLE 2 | Confusion matrix obtained by GA-LDA in tracing the diet of lactating ewe groups submitted to different part-time grazing regimens differing for daily access time (2, 4, and 6 h/d).

	2 h/d	4 h/d	6 h/d
2 h/d	8	0	0
4 h/d	1	7	0
6 h/d	0	0	8

2,532 ± 230 (6 h/d)], percentage of herbage in the diet [38 ± 3% DM (2 h/d), 52 ± 5% DM (4 h/d), and 54 ± 4% DM (6 h/d)], and milk yield [921 ± 130 g (2 h/d), 904 ± 109 g (4 h/d), and 1,068 ± 123 g (6 h/d)]. In the whole experiment, herbage and total intake as well as milk yield were higher in 6 h/d than in the other treatment groups, being the 4 h/d group means intermediate (11).

Using all the variables as predictors, the LDA performance was not accurate in estimating the AT to pasture, with only 25% of correct predictions ($\kappa = -0.125$). In contrast, the GA-LDA showed high accuracy (95.8% of correct classification and a $\kappa = 0.9375$ that indicates an almost perfect agreement, being close to 1) using one milk n-alkane and six milk FAs as biomarkers (**Table 2**). The selected biomarkers were the n-alkane C24 and the following milk FAs C13:0 *iso*, C14:0, C16:1 7c, C18:1 11t, CLA 11t 13c, and C22:5 7c 10c 13c 16c 19c. Only one milk sample belonging to the 4 h/d group was misclassified as 2 h/d.

Figure 1 shows the Mahalanobis distances of each sample from the centroids of the three groups of samples that differ for AT to pasture. In particular, **Figure 1a** plots the 2 h/d samples, **Figure 1b** plots the 4 h/d samples, and **Figure 1c** plots the 6 h/d samples. It can be seen that every sample is well classified since its distance to the centroid of the group it belongs to is way shorter than the distances to the centroids of the other groups. The exception is the only misclassified sample (#13) in **Figure 1b** (4 h/d group), which

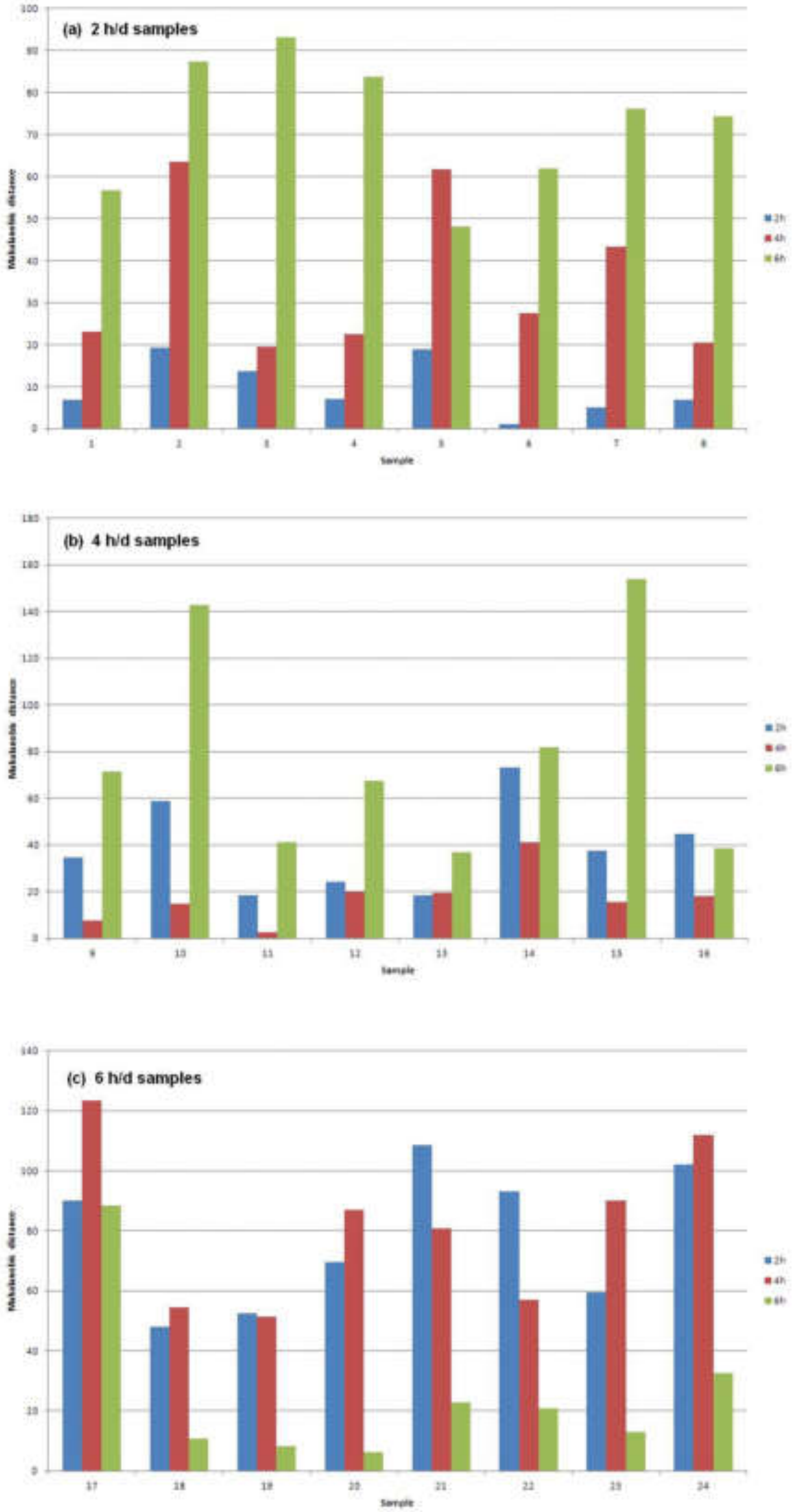

FIGURE 1 | Mahalanobis distances of the **(a)** 2, **(b)** 4, and **(c)** 6 h/d samples, from the centroids of the three treatment groups of samples.

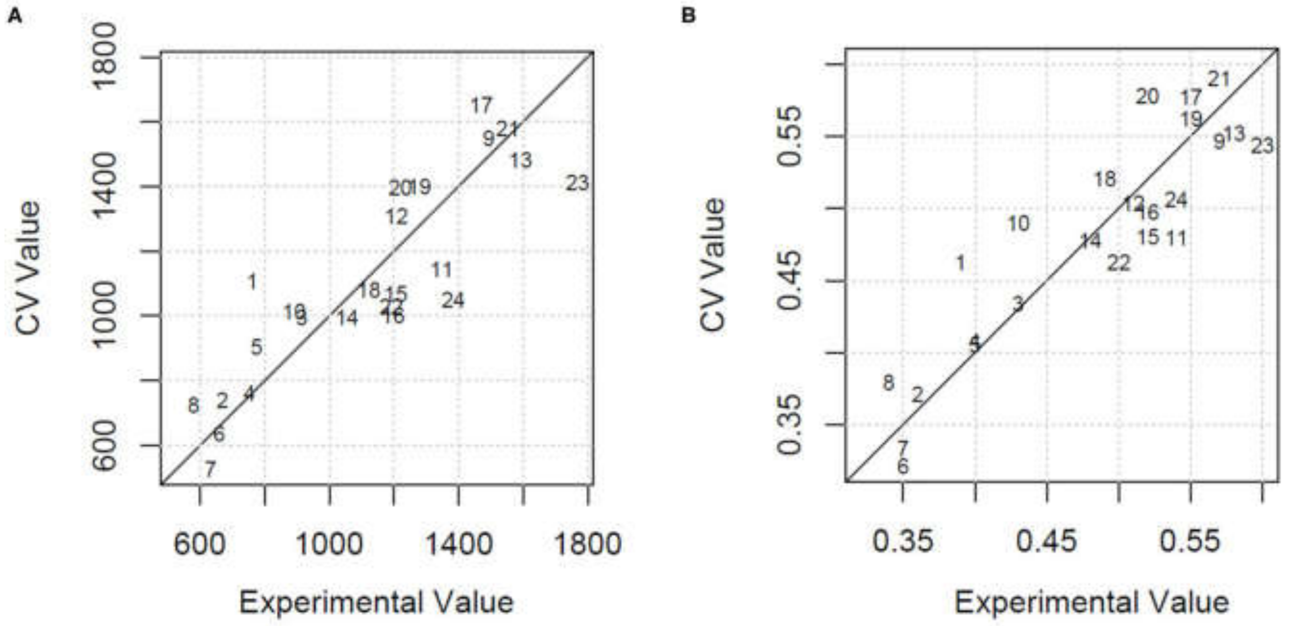

FIGURE 2 | (A) Cross-validated (CV) vs. experimental value plot obtained by the GA-PLSR model to estimate the herbage intake of lactating ewe groups submitted to different part-time grazing regimens differing for daily access time (2, 4, and 6 h/d). **(B)** Cross-validated (CV) vs. experimental value plot obtained by the GA-PLSR model to estimate the herbage percentage in the diet of lactating ewe groups submitted to different part-time grazing regimens differing for daily access time (2, 4, and 6 h/d).

is only slightly further from the centroid of its true group than from that of the group it has been assigned to (2 h/d), meaning that the only error in the assignment has not been great.

Using all the variables as predictors, the estimation of HDMI was moderately precise, with a percentage of the residual mean square error of cross-validation over the mean value (RMSECV%) equal to 22.1% and an explained variance of 46.3%. GA applied to PLSR selected only four milk FAs (C13:0 *iso*, C20:0, CLA 11t 13t, C20:5 5c 8c 11c 14c 17c) and the ratio n3/n6. Using these few variables, the HDMI estimation improved markedly, with a lowering of the RMSECV% to 15.0% and an increase of the explained variance to 75.2%. **Figure 2A** shows the cross-validated vs. the experimental value plot obtained by the model built with the selected variables.

The model built by PLSR using all the variables for the estimation of HP explained 56.5% of variance and predicted the HP values with an RMSECV% of 11.1%. This result, already acceptable, was further improved with the selection of the informative variables by means of GA. In particular, the retained explanatory variables were 1 milk alkane (C24) and 10 milk FA concentrations, classes, or ratios: C13:0 *iso*, C16:1 7c, C18:1 12c, C18:2 9c 12c, C20:0, CLA11t 13t, C20:3 5c 8c 11c, C20:5 5c 8c 11c 14c 17c, and n6, n3/n6. The model had an RMSECV% of 7.4% and an explained variance of 80.8%. **Figure 2B** shows the cross-validated vs. the experimental value plot. The former data were obtained in the prediction of HP using the selected variables.

DISCUSSION

The discrimination of milk sourced from mixed diets of ewes with ATs to pasture of 2, 4, or 6 h/d performed better than the discrimination of cow milk from animals fed fresh forages (>50% DM from fresh forage) or mixed diets [diets in which none of the forages under scrutiny–fresh forage, grass silage, and corn silage–reached at least half of the dietary DM, (19)]. In that case, the milk samples were correctly classified in 84% of cases with fresh forages and in 57.6% of cases with mixed diets. In contrast, results of this note are similar to those based on the analysis of visible and near-infrared spectra of milk samples by Coppa et al. (20): in that case, the error of classification of pasture-based compared with hay-based diets was only 8.5%.

The FAs most relevant for the discrimination (C13:0 *iso*, C14:0, C16:1 7c, C18:1 11t, CLA 11t 13c, C22:5 7c 10c 13c 16c 19c) partially align with those found by Coppa et al. (18) as biomarkers of fresh forages in milk of dairy cows. In our case, an important role was played by some FAs sourced from *de novo* synthesis (C13:0 and C14:0) or from mixed origin (C16:1 7c). According to Vlaminck et al. (21), odd-chain FAs, such as C13:0 and C17:0, are potential markers of microbial activity, assuming their ruminal origin. In contrast, long-chain FAs, such as C18:1 11t and CLA 11t 13c, are in line with previous results in sheep (22) and goats (23) that showed higher levels in milk sourced from fresh herbage-based diets. The higher polyunsaturated n−3 FA concentration in the diet richest in grazed herbage (6 h/d group, **Table 1**) explains the presence also of C22:5 7c 10c 13c 16c 19c (DPA), a long-chain n−3 polyunsaturated fatty acid (PUFA) that comes from the elongation of α-linolenic acid.

The only alkane selected by GA for milk authentication was C24 in milk, which was also the shortest chain alkane detected in milk. Its amount and its proportion on total milk alkanes are both low (1.08 mg/L and 4.08%), with numerically higher levels in milks of sheep with the lowest allocation to pasture (1.33, 0.86, and 1.06 mg/L in 2, 4, and 6 h/d treatment groups). Although grasses contain mainly long-chain alkanes (C29–C33, **Supplementary Table 1**), their digestibility is low, and this explains their low milk concentration in our milk dataset. The level of C24 was almost undetectable in feces, confirming its probable digestion and uptake. To the best of our knowledge, data on n-alkane concentration in sheep milk are not available; hence, this finding warrants further investigation.

The estimation of herbage intake was moderately precise after the selection of the relevant variables. The main explanatory variables are overall related to the level of PUFA in sheep diet and hence in milk, particularly n−3 FA, such as C18:3 9c 12c 15c, in the herbage (CLA 11t 13t, C20:5 5c 8c 11c 14c 17c, and n3/n6) and to the ruminal metabolism of amino acids (C13:0 iso) (21). Another FA, C20:0, was found to be moderately negatively correlated to dietary herbage contribution ($r = -0.49$, $P < 0.05$), and it could be possibly associated with the intake of lupin seed (see below).

The estimation of the proportion of herbage in the diet after GA-LDA was similar to that obtained by Coppa et al. (19) in bulk cow milk samples gathered across Europe and submitted to conventional FA analysis ($R^2 = 0.81$ in calibration and 0.79 in validation). In our study, as expected, the most relevant biomarkers for the prediction of HP were partially the same selected for the prediction of HDMI. They were the alkane in milk C24 and the FAs C13:0 iso, C16:1 7c, C20:0, CLA11t 13t, C20:5 5c 8c 11c 14c 17c, and n3/n6. The other FA estimators, such as C18:2 9c 12c and C20:3 5c 8c 11c, are possibly metabolites sourced from lupin seed intake. Linoleic acid, together with oleic acid (**Supplementary Table 1**), is the most relevant FA in the lupin seed used in our study, and C20:3 5c 8c 11c could derive from the metabolism of linoleic acid by the elongase/desaturase activities that occur at the mammary level.

CONCLUSION

This note shows that using milk FA and n-alkane to trace milks from dairy sheep submitted to PTG provides encouraging results. Firstly, the authentication performance based on one milk n-alkane and six milk FAs as biomarkers was very good, with almost 96% of samples correctly classified. Fecal alkanes were not selected as biomarkers of feeding regimen. This means that the combined use of FAs and alkane biomarkers in milk allows to successfully distinguish milks that come from pretty similar mixed feeding regimens, differing for only 2 h/d AT to pasture. Secondly, this study shows that the proportion of herbage in sheep diet can be precisely estimated using the above biomarkers. Finally, herbage intake can also be predicted, although estimates are only moderately precise. These results need to be confirmed on a longer grazing period using a wider database, possibly including other forage species and different supplementation levels.

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 Agris ethical committee.

AUTHOR CONTRIBUTIONS

GM planned the experiment with AC and MAd and contributed to the on-field data collection and to the writing of the manuscript. MD and MAc contributed to the on-field experimental setting. GS and MF carried out the n-alkanes and FA analyses, respectively. MC carried out the statistical analysis and contributed to the writing of the paper. AC and MAd edited a preliminary release of the paper. All authors contributed to the last edition.

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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.2021.623784/full#supplementary-material

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Can FT-Mid-Infrared Spectroscopy of Milk Samples Discriminate Different Dietary Regimens of Sheep Grazing With Restricted Access Time?

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Milk obtained from sheep grazing natural pastures and some forage crops may be worth a plus value as compared to milk obtained from stall-fed sheep, due to their apparently higher content of beneficial fatty acids (FAs). Fourier transformed mid-infrared (FT-MIR) analysis of FA can help distinguish milk from different areas and diverse feeding systems. The objective was to discriminate milk from sheep and milk from dairy sheep rotationally grazing Italian ryegrass or berseem clover for 2, 4, or 6 h/day. To test this hypothesis, a data-mining study was undertaken using a database of 1,230 individual milk spectra. Data were elaborated by principal component analysis (PCA) and analyzed by linear discriminant analysis (LDA) with or without the use of genetic algorithm (GA) as a variable selection tool with the primary aim to discriminate grazed forages (grass vs. legume), access time (2, 4, or 6 h/day), grazing day (first vs. last grazing day during the 7-day grazing period), and the milking time (morning vs. afternoon milking). The best-fitting discriminant models of FT-MIR spectra were able to correctly predict 100% of the samples differing for the pasture forage, 91.9% of the samples differing for grazing day, and 97.1% of the samples regarding their milking time. The access time (AT) to pasture was correctly predicted by the model in 60.3% of the samples, and the classification ability was improved to 77.0% when considering only the 2 and 6 h/day classes.

Keywords: authentication, fatty acids, pasture, chemometrics, FT-MIR, linear discriminant analysis

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INTRODUCTION

Grazing delivers high-quality ruminant products usually at a lower cost as compared to stall feeding (1). Grazing diets of dairy sheep result in positive effects on nutritional and health value, texture, oxidative stability, and flavor of dairy products (2). Moreover, products from pasture are perceived by consumers as more friendly for the environment and animal welfare than those coming from housed systems.

In Mediterranean dairy sheep production systems, diets only with pastures are rather rare because pasture availability is low, at least for part of the pasture growth cycle. Therefore, part-time grazing (PTG) i.e., a time-restricted allocation of ruminants to pasture is a widespread grazing technique in many areas of dairy sheep production. This technique has several benefits compared to 24-h grazing such as a better balancing of ruminant diet and a higher efficiency and evenness of herbage utilization, due to lower sward damages by animal trampling (3).

The allocation to pasture between 4 and 7 h/day can optimize the intake and performance of the dairy sheep (3–5). Besides, PTG can improve the fatty acid (FA) composition of sheep milk (6), particularly if grazing is postponed to afternoon when the grazed herbage is higher in the beneficial polyunsaturated fatty acid (PFA) (n-3) (7).

A key to authenticate and value the grazing feeding regimens in the supply chain of meat and dairy ruminant is to trace milk back to the feeding system (8). The authentication of feeding regimens can be based on biomarkers such as milk FA (9–11), secondary plant metabolites [terpenoids, n-alkanes, and derivatives of chlorophyll (e.g., phytanic and pristanic acids)], and isotopes (12).

The multivariate analysis of spectra captured by Fourier transformed mid-infrared (FT-MIR) spectroscopy has the potential to trace the feeding regimens of cows since spectra contain information that goes beyond that resulting from the analyses of biomarkers (13). Moreover, these methods, if properly calibrated and validated, open up new avenues for the implementation of authentication technology in the dairy industry.

Despite the growing body of knowledge on biomarkers and the development of rapid, low-cost analytical techniques and associated chemometrics that are able to discriminate the feeding regimens of ruminants, tracing of dairy sheep supply chains is still in its infancy.

This paper is an outcome of a wider research program undertaken at Agris Sardegna between 2013 and 2016 for evaluating the impact of PTG on their ingestive behavior and milk production of dairy ewes (3, 4, 14, 15).

This study aims at evaluating the ability of FT-MIR spectra to authenticate individual milk sourced from dairy sheep submitted to PTG at different access time (AT) to different forage crops: a grass (Italian ryegrass, *Loliumitalicum,* Lam) and a legume (berseem clover, *Trifolium alexandrinum* L.).

Genetic algorithms (GAs) were successfully used by our laboratories to select the informative variables for the estimation of the sheep milk fatty acids in FT-MIR spectroscopy (16) and in the selection of the FA and the FT-MIR spectral regions that are able to trace the geographical origin of sheep milk (17).

The specific objective of this study was to assess the ability of linear discriminant analysis (LDA) with or without the use of GA to discriminate (a) grass vs. legume pastures; (b) AT (2, 4, or 6 h/day), (c) grazing day (first vs. last grazing day during a 7-day grazing period); (d) milking time (morning vs. afternoon). To this aim, in order to set a benchmark for model interpretation, the effects of the factors under study were evaluated using both univariate analysis and multivariate principal component analysis (PCA).

MATERIALS AND METHODS

Pasture and Sheep Feeding

The milk samples were collected in 2013 (Experiment 1, E1) and 2014 (Experiment 2, E2) from Sarda ewes under PTG of grass (G, E1), Italian ryegrass (*Lolium multiflorum* Lam, cultivar Teanna), and a legume (L, E2) berseem clover (*Trifolium alexandrinum* L, cultivar Laura). The experiments were conducted at the Bonassai research station, north-western Sardinia [40° N, 8° E, 32 meters above sea level (m.a.s.l.)] from the end of February to early May in both years (growth period of the pasture). In both studies, 36 mid-lactation Sarda ewes, divided into replicated groups (two groups per treatment) part-time grazed their pasture for an AT of 2 h/day (8:00–10:00), 4 h/day (8:00–12:00), or 6 h/day (8:00–14:00). The pasture plots, divided by electric fences into four subplots, were rotationally grazed, with 7 days of occupation per subplot and a recovery period of 21 days.

The ewes were machine-milked twice daily at 07:00 h and 15:00 h. After morning milking, the groups were carried on a trailer to the plots where they spent the scheduled time. During the remaining daytime, the ewes were kept indoor in separate pens. The ewes were supplemented daily with pellet concentrate (400 g/head, divided into two meals at milking), lupin seed (300 g/head, E1), or whole maize grain (300 g/head, E2) after grazing, and 700 g/head of ryegrass-based hay overnight. The flat supplementation rate was set in order to meet 100% of the energy requirement of the 4 h/day treatment and 100% metabolic protein requirements of the 2 h/day treatment. For details on pasture establishment, animal management, methods adopted, and performance results, the reader can refer to Molle et al. (3) (E1) and Molle et al. (4) (E2). A summary of the average group diet composition and energy intake on the first and last days of the grazing period is given in **Table 1**.

Samplings

Milk yield and milk composition were measured on all ewes on day 1 and 7 of each grazing period. Milk samples were assayed for milk fat, protein, and lactose contents (Milkoscan FT+, Foss Electric, Hillerød, Denmark). Excluding the pre-experimental, adaptation period, 632 samples were gathered from March 20, 2013 to April 28, 2013 (E1), and 598 samples were gathered from 11 March 2014 to 22 April 2014 (E2), resulting in a total of 1,230 samples.

Fourier Transformed Mid-Infrared Spectra

Fourier transformed mid-infrared spectra of the sheep milk samples were recorded on a Spectrometer Milkoscan FT6000 (Foss Electric, Hillerød, Denmark) in the spectral region between 925.9 and 5,011.5 cm^{-1}. The instrumental resolution was 3.858 cm^{-1}, and each spectrum consisted of 1,060 data points. The acquisition of each sample was carried out as duplicate and then averaged. **Figure 1** shows the overlapping of the 1,230 milk spectra.

TABLE 1 | Diet composition and energy intake of dairy ewes part-time grazing (PTG) with different access time (AT, h/day) to pastures of Italian ryegrass or berseem clover as measured on the first (day 1) and last day (day 7) of the grazing period of 7 days.

Forages/trial	AT	Grazing day	Ash		EE		CP		NDF		ADF		NFC		IVDMD		Intake NE$_L$	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ryegrass/E1	2	1	105	7	34	2	156	9	461	10	247	7	245	17	754	23	2.7	0.3
		7	104	7	32	2	148	7	474	21	254	15	243	20	734	45	2.5	0.4
	4	1	107	10	35	2	154	12	456	12	241	9	249	27	773	32	3.4	0.6
		7	107	11	32	2	141	8	479	27	256	16	241	28	735	59	2.9	0.5
	6	1	107	11	35	2	158	13	452	15	236	10	248	24	775	33	3.8	0.6
		7	111	10	31	2	140	8	485	22	258	15	233	18	726	49	3.0	0.5
Clover/E2	2	1	102	6	41	4	173	12	382	42	229	21	301	33	764	38	3.5	0.5
		7	104	7	39	4	165	6	381	28	232	14	311	27	760	20	3.5	0.5
	4	1	108	3	42	3	181	11	371	23	225	10	298	19	779	24	4.5	0.4
		7	116	14	39	6	169	12	377	25	232	18	299	15	772	24	4.6	0.7
	6	1	106	4	44	4	187	12	360	36	214	15	303	27	792	25	4.5	0.2
		7	112	8	40	7	178	14	363	25	221	15	307	18	781	27	4.4	0.7

Each mean refer to n = 8 group data; Means and SD.

Diet composition expressed as g/kg DM and net energy intake (NE$_L$) as Mcal/head day.

EE, ether extract; CP, crude protein; NDF, neutral detergent fiber (ash excluded); ADF, acid detergent fiber; NFC, non-fiber-carbohydrates; IVDMD, pepsine–cellulase in vitro dry matter digestibility.

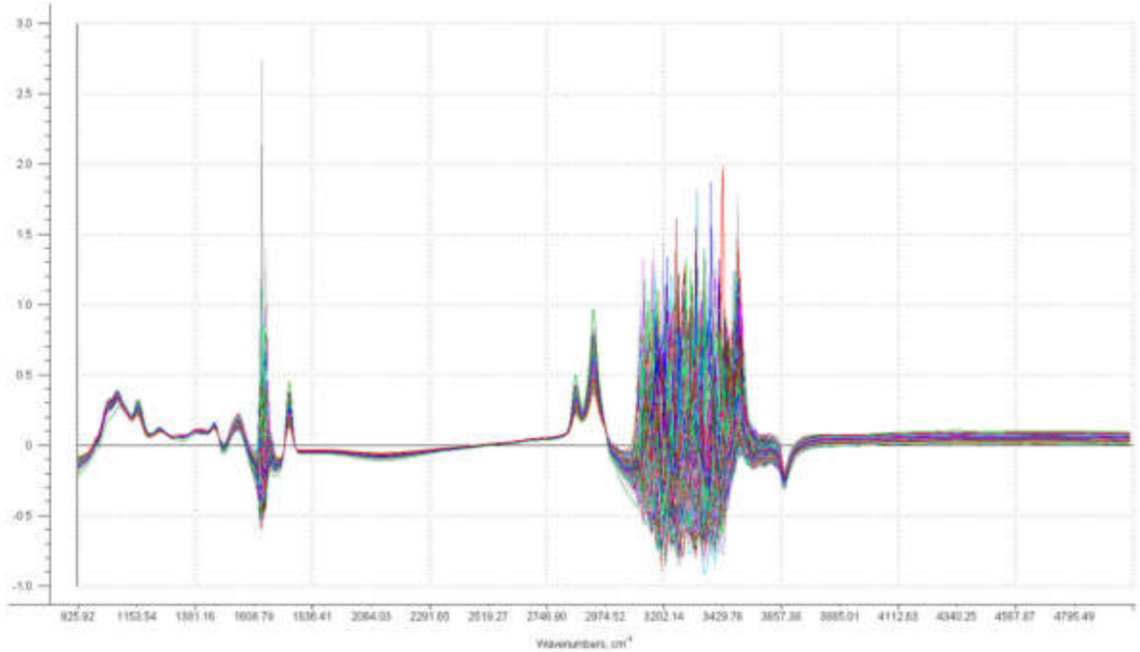

FIGURE 1 | Plot of the overlapped 1,230 milk sample spectra.

Since the regions from 1608.8 to 1697.5 cm^{-1} and from 3044.0 to 3850.3 cm^{-1} were characterized by a strong instrumental noise and the region from 3850.3 to 5011.5 cm^{-1} was characterized by pure baseline, these regions were not used in the data analysis. Therefore, we considered the regions between 925.9 and 1604.9 cm^{-1} and between 1701.4 and 3040.1 cm^{-1} as "whole spectrum," totaling 525 spectral variables.

Principal Component Analysis of the Spectral Data Set

Principal component analysis was performed on the database consisting of 1,230 samples and 525 spectral variables. Data were centered and scaled. The obtained score plot (**Figure 2A**) and diagnostic plot T^2 vs. Q (**Figure 2B**) were used to identify possible outliers. Five samples were considered as outliers and

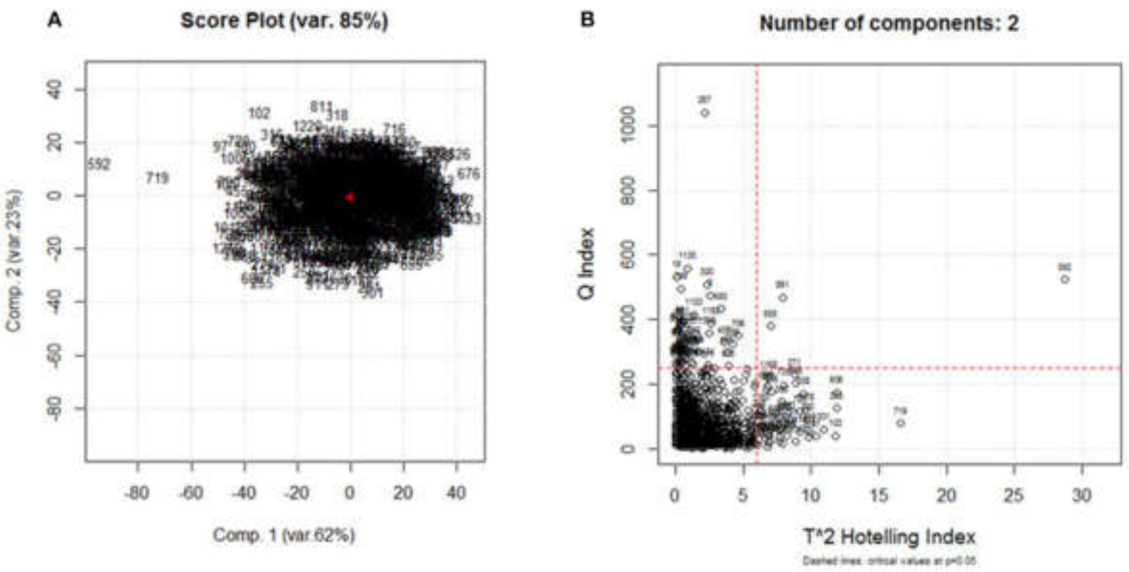

FIGURE 2 | (A) Score plot obtained on the principal component analysis (PCA) of the spectral data set (1,230 samples × 525 variables) and **(B)** T^2 vs. Q diagnostic plot.

removed from the data set; the data now contain 1,225 samples. PCA was run on the chemometric agile tool (CAT) software, developed by the Group of Chemometrics of the Division of Analytical Chemistry of the Italian Chemical Society, freely downloadable from the site, gruppochemiometria.it.

Prediction of Fatty Acids in Milk by FT-MIR

The fatty acid (FA) composition of the 1,225 milk samples was predicted by FT-MIR spectroscopy, using previously published prediction models (16). The predicted (FAs), expressed as g/100 g fatty acid methyl esters (FAME), include C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1 9c, C18:1 11t; C18:2 9c 12c, C18:3 9c 12c 15c, C18:2 9c 11t, and the classes of saturated fatty acids (SFA), unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega 6 (n-6), and omega 3 (n-3).

Univariate Analysis and PCA of the FA Profile

The predicted FA database was subjected to a mixed model analysis for repeated measurements considering fixed factors, pasture forage species/trial (grass and legume), AT (2 h/day, 4 h/day, and 6 h/day), grazing day during grazing period (first, day 1 and last, day 7), milking time [morning (M) and afternoon (A)] and all their first- order interactions and the ewe within the treatment group as random factor. Means were compared by Tukey–Kramer *t*-test, when effects were significant at $p < 0.05$. Trends are presented and discussed if $p < 0.10$.

Principal Component Analysis was performed on the milk FA profile to visualize any trend in data. Data were centered and scaled.

Building of the Discriminant Models

First, the samples were labeled considering four different types of possible categorization, which include (1) the pasture forage, confounded with the supplementation type (named as forage/trial effect); (2) the AT to pasture; (3) the grazing day; (4) the milking time.

As for the AT to pasture, four different sample partitions were performed comparing the following factors: (a) the three categories (i.e., the treatment groups: 2, 4, and 6 h/day); (b) the 2 h/day and 4 h/day samples grouped together in one category against the 6 h/day samples; (c) the 4 h/day and 6 h/day samples grouped together in one category against the 2 h/day samples; (d) the 2 h/day samples against the 6 h/day samples.

Therefore, totally, we performed seven trials; for each one, the samples were randomly divided into a training set and a test set containing about 60 and 40% of the samples, respectively. **Table 2** summarizes the different trials and the sample partitioning into two sample sets (training and test).

Linear discriminant analysis (LDA) was used for discriminating the sheep milk samples based on their respective categories. For each trial, we proceeded as follows. In the first step, we built discriminant models using all FA or only the informative FA selected by GAs as predictors. In the second step, we built discriminant models using FT-MIR spectra as predictors. Different spectral pretreatments, such as the first and second derivatives, standard normal variate (SNV) and multiplicative scatter correction (MSC) were evaluated, finding no improvement of accuracy as compared to non-pretreated spectra. Therefore, we presented only the trials performed using the non-pretreated spectra.

For each discriminant model, calibration was performed by cross-validation (CV), using samples from the training set and

TABLE 2 | Trials and sample subdivision into training and test sets.

Trial		Categories	Number of samples	
			Training set	Test set
Pasture forage		Grass	393	239
		Legume	341	252
Access time (AT) to pasture	(a)	2 h/day	244	162
		4 h/day	245	165
		6 h/day	245	164
	(b)	(2 and 4) h/day	489	327
		6 h/day	245	164
	(c)	2 h/day	244	162
		(4 and 6) h/day	490	329
	(d)	2 h/day	244	162
		6 h/day	245	164
Grazing day		Day 1	397	262
		Day 7	337	229
Milking time		Afternoon	374	236
		Morning	360	255

validation was run using samples from the test set (prediction of an external set of samples).

When we applied LDA to FT-MIR spectra, different predictors were considered, as LDA cannot use the whole spectra because the number of correlated variables would be too high:

(1) the scores obtained in the PCA of the spectral data set.

To do this, the following procedure was applied:

- principal component analysis of spectra training set;
- projecting the spectra of the test set on the PCA model obtained on the training set;
- use the obtained scores, i.e., those corresponding to the most informative components of the PCA obtained from the training set;

(2) the average of three contiguous wavelengths of each milk spectrum, obtaining a reduction from 525 to 175 spectral variables;

(3) the informative spectral variables selected by applying the GA to the spectral data set. A different selection of wavelengths was done for each trial. Since the efficiency of GA decreases when the number of variables is >200 (18), we applied GA to the averaged spectra of 175 variables. GA procedure was replicated five times in order to achieve a more consistent model. The spectral regions selected in the five runs were then compared and only those selected by the majority of the runs were retained in the final model. The selected variables were then reported on the original spectra composed of 525 variables.

Linear discriminant analysis was run on the CAT software, developed by the Group of Chemometrics of the Division of Analytical Chemistry of the Italian Chemical Society, freely downloadable from the site, gruppochemiometria.it.

RESULTS

Univariate and PCA of Predicted FA Profile

The univariate analysis of the predicted FA profile of the 1,225 sheep milk samples showed significant effects of pastures on forage species/trial and milking time on all FAs and their classes (**Table 3**). In contrast, the AT to pasture had a significant effect only on some short- to medium-chain FA (C6:0, C8:0, C10:0, and C12:0) and n-3 ($p < 0.050$), whereas the grazing day affected all variables with the exception of C12:0 ($p < 0.073$) and UFA. The content of short-chain fatty acids (with the exception of C6:0) and SFA was higher in milk samples from sheep grazing the legumes than the grass pastures. Also, the beneficial FAs (C18:1 11t; C18:2 9c 11t; C18:3 9c 12c 15c), PUFA, and n-3 had higher values in the samples from the legume-based diets. The same beneficial FAs were higher in the samples obtained from the first than the last grazing day and from the morning than the afternoon milking, with the exception of n-3 FA.

On the contrary, C18:2 9c 12c and MUFA had higher values in milk from the grass-fed sheep, afternoon milking, and the last grazing day.

Several interactions between factors affected the FA milk composition (**Table 3**) such as the one between AT and pasture forage. In fact, extending AT to pasture, the levels of C18:2 9c 11t and C18:3 9c 12c 15 c increased more in the milk of ewes grazing the grass in E1 than the legume in E2 ($p < 0.05$). Another significant interaction was between pasture forage and grazing day: C18:1 11t and C18:2 9c 11t decreased, whereas SFA increased when passing from the first to the last grazing day but only in the milk of the legume-grazing sheep ($p < 0.05$). On the contrary, C18:3 9c 12c 15c decreased during the grazing period only in the milk of the grass-grazing sheep.

Also, the milking time significantly interacted with pasture forage, AT, and grazing days. The morning milking samples had higher values of C18:1 11t than the afternoon samples, mainly in the samples of legume-grazing sheep alone ($p < 0.01$). Moreover, C18:2 9c 11t, C18:3 9c 12c 15c, and PUFA decreased in the morning samples when passing from the first to the last grazing day ($p < 0.08$ for C18:2 9c 11t, $p < 0.05$ for C18:3 9c 12c 15c, and PUFA). It is worth noting that n-3 levels were similar between afternoon and morning milking, only when AT was 6 h/day. With shorter AT, the levels were generally higher in the afternoon than in the morning milking samples.

Figure 3 shows the score plot of the PCA of the FA data set of 1,225 sheep milk samples with reference to the following four types of categorization: (a) pasture forage/trial (grass and legume); (b) AT to pasture (2, 4, and 6 h/day); (c) grazing days (day 1 and 7); (d) milking times (A and M). The PCA of FA allowed to visually distinguish only the samples differing for the pasture forage/trial (**Figure 3A**), being the first principal component the axis in which the samples are separated. In the corresponding loading plot (**Figure 4**), the first component differentiates the samples based on their content of short- and medium-chain FAs (C6:0, C8:0, C10:0, C12:0, and C14:0), SFA, and some UFAs, such as C18:1 11t and C18:2 9c 11t. All these FAs had higher values in the milk samples of the legume-fed ewes (**Table 3**). Instead, the

TABLE 3 | Fatty acid (FA) profile (means expressed as g/100 g fatty acid methyl ester (FAME), mean and standard error of the mean (SEM) of the sheep milk samples as estimated using Fourier transformed mid-infrared (FT-MIR) calibrations.

	C4	C6	C8	C10	C12	C14	C16	C18	C18:1 c9	C18:1t11	C18:2	C18:3	CLA	SFA	UFA	MUFA	PUFA	n-6	n-3
Fo/trial																			
Grass	3.93	3.67	2.18	6.25	3.54	10.09	24.00	10.42	18.60	1.32	2.31	0.67	0.85	68.33	31.19	26.45	5.89	3.00	1.31
Legume	4.16	3.05	2.62	7.92	4.33	11.13	24.54	9.39	12.90	3.08	1.83	1.04	1.48	70.46	29.75	23.49	7.02	3.09	2.13
At																			
2 h/day	4.03	2.77 a	2.30 a	6.74 a	3.78 a	10.42	24.23	10.01	16.30 b	2.14	2.11	0.84	1.13	68.66	30.99	25.44	6.38	3.07	1.68
4 h/day	4.02	2.86 ab	2.40 ab	7.13 ab	3.96 ab	10.69	24.47	9.95	15.70 ab	2.18	2.04	0.84	1.16	69.64	30.15	24.85	6.40	3.03	1.67
6 h/day	4.09	2.95 b	2.49 b	7.38 b	4.08 b	10.72	24.11	9.75	15.24 a	2.28	2.07	0.88	1.19	69.89	30.27	24.61	6.59	3.04	1.81
Gd																			
1	4.12	2.93	2.46	7.19	3.96	10.55	23.96	9.84	15.38	2.26	2.04	0.87	1.19	69.07	30.38	24.79	6.57	2.99	1.78
7	3.96	2.79	2.34	6.97	3.91	10.67	24.58	9.98	16.11	2.15	2.11	0.84	1.14	69.72	30.56	25.14	6.34	3.10	1.66
Mt																			
Afternoon	3.98	2.75	2.27	6.67	3.77	10.52	24.08	9.99	16.71	2.16	2.12	0.84	1.14	68.55	31.25	25.61	6.37	3.08	1.76
Morning	4.11	2.98	2.53	7.50	4.10	10.70	24.46	9.82	14.79	2.24	2.03	0.87	1.18	70.24	29.70	24.32	6.54	3.01	1.68
Mean	4.06	2.88	2.41	7.10	3.94	10.60	24.25	9.89	15.71	2.16	2.07	0.85	1.15	69.41	30.35	24.88	6.43	3.04	1.71
SEM	0.01	0.01	0.01	0.04	0.02	0.04	0.07	0.04	0.11	0.03	0.01	0.01	0.01	0.15	0.10	0.09	0.03	0.01	0.02
Effects, *p*<																			
Fo/trial	0.000	0.000	0.000	0.000	0.000	0.000	0.092	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.002	0.000
AT	0.662	0.018	0.018	0.007	0.013	0.165	0.665	0.566	0.027	0.332	0.114	0.221	0.405	0.099	0.311	0.207	0.167	0.403	0.051
Graze day	0.000	0.000	0.000	0.000	0.073	0.030	0.000	0.014	0.000	0.002	0.000	0.001	0.002	0.016	0.258	0.002	0.000	0.000	0.000
Mt	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.002	0.000	0.027	0.000	0.004	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Fo × AT	0.378	0.547	0.190	0.023	0.017	0.030	0.411	0.004	0.141	0.041	0.051	0.024	0.092	0.570	0.203	0.359	0.500	0.682	0.385
At × Gd	0.011	0.331	0.993	0.761	0.402	0.798	0.542	0.118	0.400	0.993	0.865	0.154	0.602	0.895	0.651	0.700	0.639	0.650	0.420
Fo × Gd	<0.0001	0.002	0.015	0.498	0.240	0.603	0.299	<0.0001	0.031	<0.0001	0.601	0.000	0.000	0.001	0.693	0.231	0.197	0.001	0.429
AT × Mt	0.440	0.468	0.585	0.729	0.774	0.958	0.843	0.000	0.007	0.108	0.720	0.181	0.157	0.817	0.093	0.073	0.122	0.074	0.036
Gd × Mt	0.164	0.308	0.878	0.705	0.215	0.001	0.001	0.272	0.321	0.419	0.669	0.030	0.080	0.010	0.585	0.622	0.024	0.418	0.214
Fo × Mt	0.272	0.732	0.240	0.010	0.007	0.116	0.176	0.002	0.000	0.003	0.000	0.969	0.162	0.175	0.002	0.000	0.762	0.000	0.687

Fo/trial, pasture forage/trial; AT, access time; Gd, grazing day; Mt, milking time.

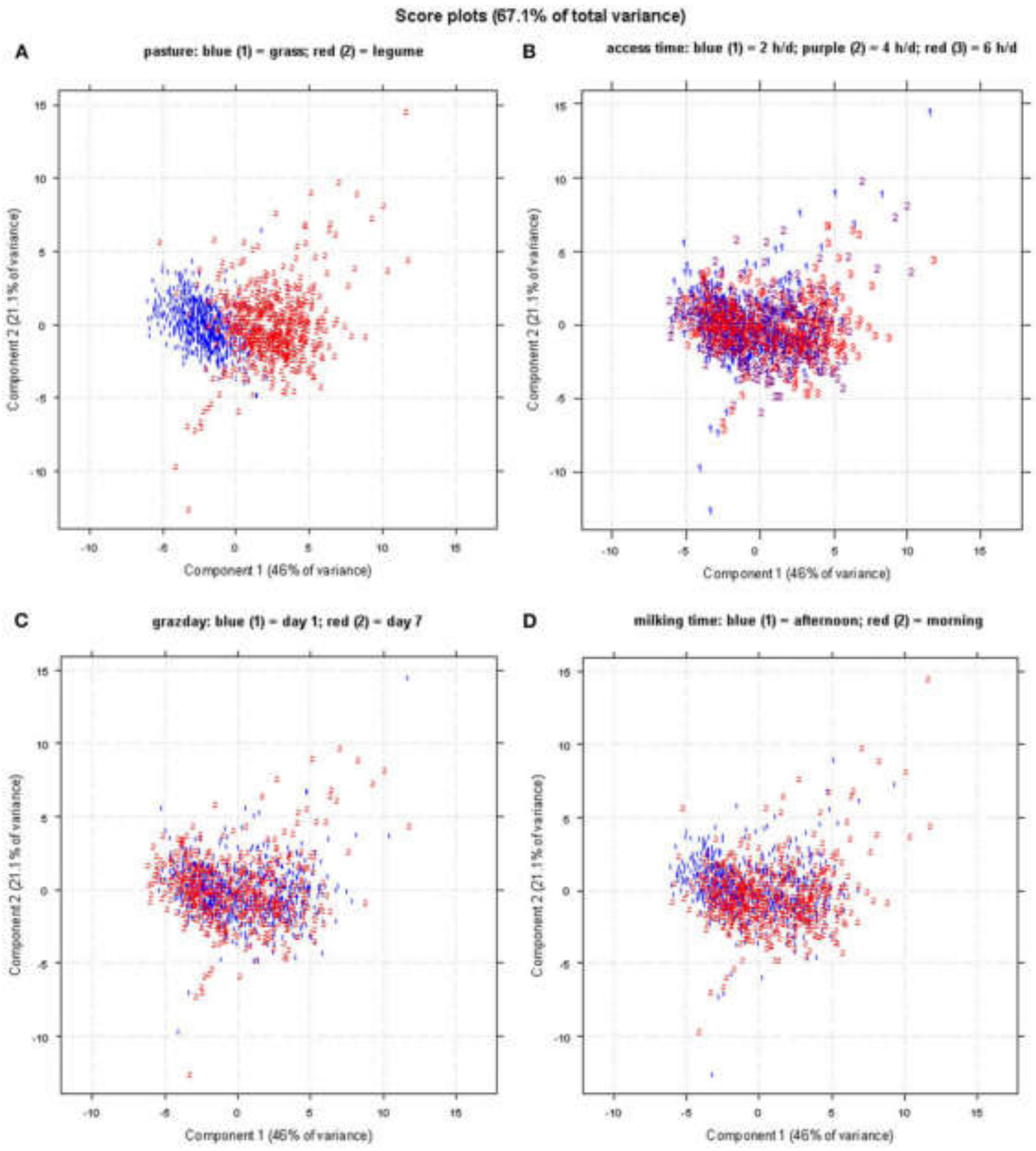

FIGURE 3 | Score plot of the principal component analysis (PCA) obtained using the fatty acid (FA) profile data set; samples are labeled and colored as for the classifications of **(A)** pasture forage, **(B)** access time, **(C)** grazing day, and **(D)** milking time.

second principal component describes the variability inside each group of samples, which is mainly due to MUFA, UFA, and n-3. For the other types of categorizations, the visual separation of samples was not possible, even when plotting other principal components.

Discriminant Analysis Using the FA Profile as Predictors

Table 4 shows the results of LDA using both the entire FA profile and the selected FAs to predict the different origins of the milk samples.

Discrimination of the Pasture Forage

When discriminating the pasture forages, the FAs were able to classify 100% of the samples of the training set and to correctly predict 100% of the test set samples. Applying GAs to the FA data set led to a reduction of the number of variables to be used in the prediction model. GA selected only C18:2 9c 12c, C18:3 9c 12c 15c, C18:2 9c 11t, PUFA, n-6, and n-3, maintaining the same accuracy of the model built using all the variables as predictors.

Discrimination of the AT to Pasture

Using the whole FA profile as the predictor, only 47.8% of the samples of the training set and 43.8% of the test set were correctly classified in the three categories, 2, 4, and 6 h/day. As can be seen in **Table 4**, an improvement was obtained grouping the 4 h/day with either 2 h/day samples or 6 h/day samples and comparing these new categories with the remaining ones (6 or 2 h/day, respectively). A further slight improvement was obtained when the LDA of FA was used to discriminate the 2 h/day from the 6 h/day samples, without considering the 4 h/day samples. In this case, 67.3% samples from the training set and 60.8% samples from the test set were correctly predicted.

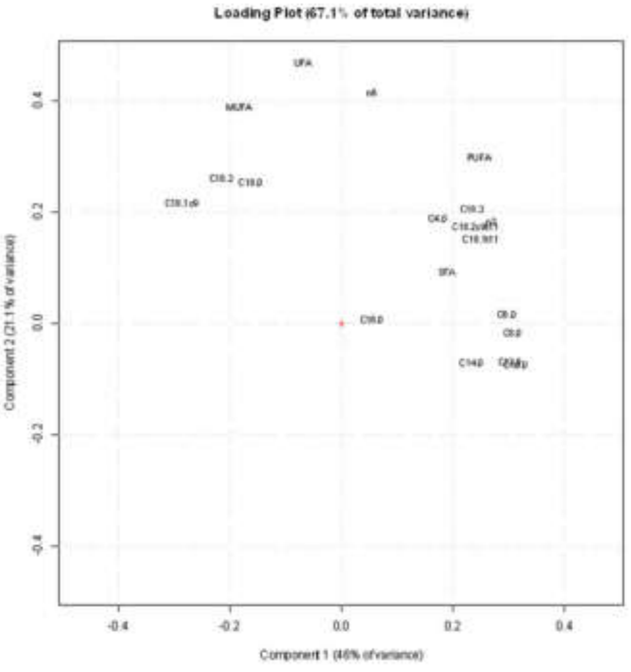

FIGURE 4 | Loading plot of the principal component analysis (PCA) obtained using the fatty acid (FA) profile data set.

Applying the GA allowed for a reduction of the number of variables in all the models. In the LDA of the three categories (2, 4, and 6 h/day), GA selected all individual SFAs (with the exception of C14:0), together with C18:1 11t, C18:2 9c 12c, and the classes, UFA n-6, and n-3. The resulting discriminant model correctly predicted 50.0 and 41.9% of the training and test set samples, respectively. As in the case of LDA, the LDA-GA of FA profile achieved slightly better discrimination accuracies when grouping the samples in order to compare only two categories (**Table 4**). The best result was obtained from the discrimination of the 2 h/day from the 6 h/day samples. The GA selected all the individual FAs (with the exception of C18:2 9c 11t), and the classes UFA, n-6, and n-3. The model built with the selected variables as predictors correctly classified 68.3% of the training set samples and 61.4% of the test set samples.

Discrimination of the Grazing Day

The model built using the whole FA profile as a predictor for the discrimination of the grazing day correctly predicted 72.3 and 74.9% of the training samples and of the test set samples, respectively. The results were similar when building the model using the GA-selected variables, with 74.8% of the training set samples and 73.0% of the test set samples correctly classified. The

TABLE 4 | Percentage of correct classifications obtained by linear discriminant analysis (LDA) using the fatty acid (FA) profile or the FA selected by genetic algorithm (GA) as predictors.

Predictors	**Pasture forage**		**AT**		**AT**		**AT**		**AT**		**Grazing day**		**Milking time**	
	Grass vs. Legume		**2 vs. 4 vs. 6**		**(2+4) vs. 6**		**2 vs. (4+6)**		**2 vs. 6**		**Day 1 vs. Day 7**		**Morning vs. Afternoon**	
	CV	**Pred.**	**CV**	**Pred.**	**CV**	**Pred.**	**CV**	**Pred.**	**CV**	**Pred.**	**CV**	**Pred.**	**CV**	**Pred.**
FA profile	100	100	47.8	43.8	63.1	57.9	64.0	60.8	67.3	60.8	72.3	74.9	88.6	88.8
FA selected by GA	100	100	50.0	41.9	65.7	59.9	65.1	59.4	68.3	61.4	74.8	73.0	89.0	88.8
Selected FA	C18:1 9c C18:2 9c 12c C18:3 9c 12c 15c C18:2 9c 11t PUFA n-6 n-3		C4:0 C6:0 C8:0 C10:0 C12:0 C16:0 C18:0 C18:1 11t C18:2 9c 12c UFA n-6 n-3		C4:0 C6:0 C8:0 C10:0 C12:0 C14:0 C16:0 C18:0 C18:1 9c C18:1 11t C18:2 9c 12c C18:3 9c 12c 15c C18:2 9c 11t UFA n-6 n-3		C4:0 C6:0 C8:0 C10:0 C12:0 C14:0 C16:0 C18:0 C18:1 9c C18:1 11t C18:3 9c 12c 15c C18:2 9c 11t SFA UFA n-6		C4:0 C6:0 C8:0 C10:0 C12:0 C14:0 C16:0 C18:0 C18:1 9c C18:1 11t C18:2 9c 12c C18:3 9c 12c 15c UFA n-6 n-3		C4:0 C6:0 C8:0 C12:0 C14:0 C18:1 9c C18:1 11t C18:2 9c 12c SFA PUFA n-6 n-3		C6:0 C8:0 C10:0 C14:0 C16:0 C18:1 9c C18:2 9c 12c C18:2 9c 11t SFA UFA PUFA n-6 n-3	
N. selected variables	7		12		16		15		15		12		13	

AT, access time; CV, cross validation; Pred., prediction; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; SFA, saturated fatty acid.

selected variables include, C4:0, C6:0, C8:0, C12:0, C14:0, C18:1 9c, C18:1 11t, C18:2 9c 12c, SFA, PUFA, n-6, and n-3.

Discrimination of the Milking Time

The model built with all the FA variables correctly predicted the milking time origin in 88.6% and 88.8% of the training and test set samples, respectively. GA selected C6:0, C8:0, C10:0, C14:0, C16:0, C18:1 9c, C18:2 9c 12c, C18:2 9c 11t, SFA, UFA, PUFA, n-6, and n-3, and the model built using these variables correctly predicted 89.0% of the training set samples and 88.8% of the test set samples.

Discriminant Analysis Using the FT-MIR Spectra as Predictors

The results expressed as percentages of correct classifications both in the training set (CV) and in the test set (prediction) are shown in **Table 5**, which also shows the spectral regions selected by GA for each discriminant model. The selected spectral regions are also shown in **Figure 5**.

Discrimination of Pasture Forage

When discriminating the pasture forages, the model built using the PCA scores led to 78.9% of correct classification for the training set and to 76.0% of correct predictions of the external sample set, whereas 100% of samples were correctly classified in the calibration and in the validation steps, using either the whole averaged spectra or the variables selected by the GA (**Table 5**).

Discrimination of the AT to Pasture

The three AT categories (2 h/day vs. 4 h/day vs. 6 h/day) were poorly discriminated using the PCA scores as predictors, with only 38.5 and 39.8% of correct classifications for the training and the test sets, respectively. The results improved when using the whole averaged spectra as predictors reaching 56.4% and 60.5% of correct classifications for the two sample sets. The application of GA led to a reduction of variables from 525 to 222, corresponding to 14 spectral regions, but the model accuracies were not improved, with 58.4 and 60.3% of samples correctly predicted in the training and test sets, respectively.

When grouping the 2 h/day samples and the 4 h/day samples in the same category and comparing them to the 6 h/day samples, the discriminant model obtained using the PCA scores led to 55.1% of correct classification for the training set and to 55.0% of correct predictions for the test set. The use of the averaged spectra as predictors, improved the results to 70.1 and 70.8%, respectively. Using the GA-selected spectral variables led to similar results: 72.0 and 71.0% of correct classifications for the training and test sets, respectively.

Slightly better accuracies were achieved when the 4 h/day samples were grouped together with the 6 h/day samples in the same category and discriminated from the 2 h/day samples. The PCA scores as predictors led to 54.7 and 56.1% of calibration and validation accuracies. The corresponding values were 71.2 and 75.6%, respectively using the whole averaged spectra as predictors, and 72.3 and 75.6% with the GA-selected variables.

Finally, the discrimination of the 2 h/day samples from the 6 h/day samples did produce the best accuracy among the AT trials when using the whole averaged spectra or the GA-selected variables as predictors. In fact, the use of the PCA scores in the building of the model led to 55.0 and 55.6% of correct classifications in the samples of the training and test sets, respectively, but the use of the whole averaged spectra as predictors led to 73.0% of correct classifications in the calibration step and 78.5% of correct prediction in the validation step. The application of GA to the spectra reduced the number of variables used as predictors, from 525 to 168, and the model correctly predicted 72.4% of the training set samples and 77.0% of the test set samples.

Discrimination of the Grazing Day

The model built using the PCA scores was not satisfactory as only 55.2% of samples were assigned to the right category (**Table 5**). The model built considering the whole averaged spectra as predictors led to an improvement of the results, with 92.8% of correct classification in the calibration step and 93.7% of correct predictions in the validation step. GAs selected 324 variables out of 525, leading to percentages of correct classification of 91.1% for the calibration step and 91.9% for the validation step.

Discrimination of the Milking Time

In this case, even the model built using the PCA scores as predictors led to good results, as the percentage of correct classifications was 91.5% for the training set samples and 91.0% for the test set samples. The use of the whole averaged spectra led to 98.5 and 98.6% of correct classifications. The GA led to a reduction of about half of the total spectral variables (from 525 to 258), and to a correct classification of 97.8% of the training and 97.1% of the test samples, respectively.

DISCUSSION

Univariate and PCA of Predicted FA Profile

Since the specific objective of this paper is the tracing of milk produced by ewes having different feeding regimes, we will briefly discuss the results of the above analysis, which set the benchmark for evaluating and interpreting the performance of the discriminant analysis. As expected, the univariate analysis of milk FA showed that increasing the amount and quality of herbage in the diet enhances the level of beneficial FA in milk (2). In fact, this study explores a wide range of diets, going from low quantity (2 h/day) of moderate quality herbage (day 7 on the grass) to high quantity (6 h/day) of high-quality herbage (day 1 on the legume, **Table 1**). In particular, the average intake of grass on the AT 2 h/day was 648 g DM (35% of total intake), with the neutral detergent fiber (NDF) level of 493 g/kg DM and a crude protein (CP) level of 119 g/kg DM on day 7 (3), whereas on the legume with AT of 6 h/day, the average intake was 1,723 g DM (62% of total intake), with the NDF level of 328 g/kg DM and CP level of 231 g/kg DM on day 1 (4). We can reasonably argue that this wide range of nutrient composition was mirrored by an even wider range of FA intake, since berseem clover has usually higher content of long-chain fatty acid (LCFA) than the Italian ryegrass, according to our laboratory data (19, 20). Although the grazed forage had a major impact on FA profile, since the

TABLE 5 | Percentage of correct classifications obtained by LDA using FT-MIR spectra as predictors.

Predictors	Pasture forage		AT		AT		AT		AT		Grazing day		Milking time	
	Grass vs. Legume		2 vs. 4 vs. 6		(2+4) vs. 6		2 vs. (4+6)		2 vs. 6		Day 1 vs. Day 7		Morning vs. Afternoon	
	CV	Pred.	CV	Pred.	CV	Pred.	CV	Pred.	CV	Pred.	CV	Pred.	CV	Pred.
PCA scores	78.9	76.0	38.5	39.8	55.1	55.0	54.7	56.1	55.0	55.6	55.2	56.4	91.5	91.0
Averaged spectra	100	100	56.4	60.5	70.1	70.8	71.2	75.6	73.0	78.5	92.6	93.7	98.5	98.6
GA selected regions	100	100	58.4	60.3	72.0	71.0	72.3	75.6	72.4	77.0	91.1	91.9	97.8	97.1
Selected spectral regions (cm^{-1})	995.4–1026.2 1261.6–1292.4 2962.9–2993.8		1006.9–1315.6 1377.3–1408.2 1435.2–1466.0 1493.0–1604.9 1701.4–1767.0 2071.7–2079.5 2256.9–2287.8 2488.4–2530.8 2581.0–2611.9 2650.4–2692.9 2743.0–2750.7 2766.2–2808.6 2835.6–2866.5 2928.2–2947.5		1018.5–1095.7 1145.8–1269.3 1504.6–1523.9 1574.1–1593.3 2245.4–2287.8 2395.8–2426.7 2662.0–2681.3 2824.1–2854.9		949.1–956.8 1030.1–1165.1 1273.1–1361.9 1388.9–1442.9 1539.3–1604.9 1701.4–1720.7 1770.8–1824.8 2268.5–2276.2 2442.1–2449.8 2476.8–2496.1 2557.8–2600.3 2777.8–2797.5 2858.8–2866.5 2916.6–2924.4		1018.51–1118.82 1134.25–1269.28 1284.71–1315.58 1388.88–1408.17 1516.2–1604.9 1701.4–1743.8 1782.4–1801.7 2245.4–2287.8 2453.7–2519.3 2638.9–2692.9 2847.2–2854.9		937.49–956.78 1041.66–1095.67 1157.40–1304.00 1342.58–1373.45 1388.88–1489.19 1539.34–1604.93 1701.38–1766.96 1817.12–2056.31 2094.89–2241.50 2303.23–2438.26 2662.02–2866.49		937.49–1084.10 1111.10–1211.41 1261.57–1431.32 1446.75–1581.78 1712.95–1801.69 1817.12–1847.98 2303.23–2322.52 2384.24–2426.68 2442.11–2449.83 2499.98–2530.85 2638.87–2658.16 2708.32–2727.61 2824.06–2959.09	
N. selected variables	27		222		102		153		168		324		258	
N. spectral regions	3		14		8		14		11		11		13	

CV, cross validation; pred, prediction based on test data set; PCA, principal component analysis; GA, genetic algorithm.

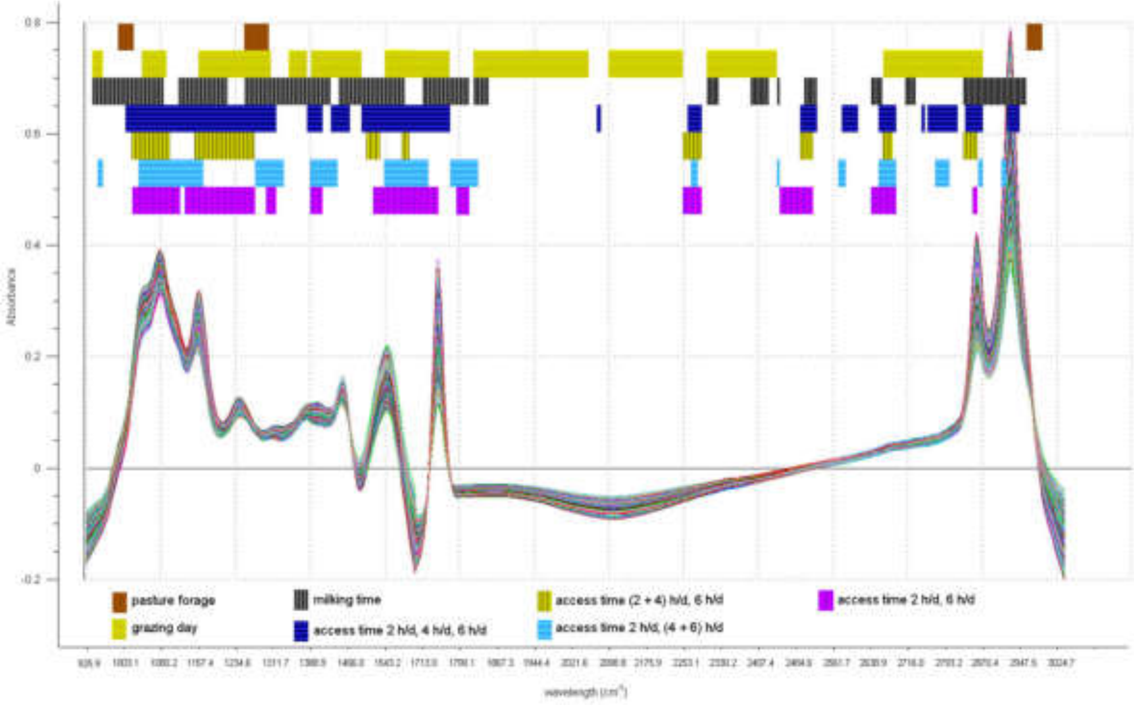

FIGURE 5 | Spectral regions selected by genetic algorithm (GA) in all the GA-linear discriminant analysis (LDA) discrimination models.

supplementation changed between experiments, we cannot rule out that the different types of supplements can have an impact on the results obtained. For this reason, we conventionally refer to the effect of pasture forage combined with the supplementation type, nested in the trials (E1 and E2) as forage/trial effect.

It is well-known that leaves contain more LCFA than stems, as observed in berseem clover by Cabiddu et al. (20). This can suggest a higher intake of LCFA on the 1st day than on the last grazing day of the grazing periods throughout the experiments.

Actually, the effect of the different forages/trials was very strong on all variables. The effect of the grazing day was evident in most of them but the effect of AT was moderate, with a higher content of short FA and n-3 in the milk obtained from the highest AT (6 h/day), being 4 h/day intermediate. This is because the intake of herbage was also intermediate in these ewes as compared to the extreme levels of AT (3, 14). Moreover, the level of linoleic acid was possibly higher in the diets of sheep with lower AT, since lupin and maize are rich in linoleic acid (21, 22). This can explain why FA tended to be higher in milk samples from low-AT ewes.

Milking time has been so far an overlooked factor of milk FA composition in sheep. Few studies refer to the effects of milking times in grazing cows (19), but the milking schedule and feeding regimes are very different from the feeding background which is under scrutiny for a useful comparison. In our conditions, the schedule of grazing allocation in the morning and of supplementary main meals in the afternoon probably favored an increase of beneficial FA in the morning milk rather than in the afternoon milk, with exception of n-3. In the prevailing n-3 FA, linolenic acid was however not affected likewise. It is possible that other n-3 FAs, such as EPA and DHA were responsible for this inconsistency. In general, the longer n-3 needs several elongation and desaturation steps which may explain for a longer lag time between the intake of precursors and appearance in milk. Other long-chain PUFA can also be contained in lupin seed (22). In general, afternoon samples were characterized by higher levels of linoleic and oleic acid which can be sourced from supplements [linoleic acid in maize and lupin and oleic acid in lupin (21, 22), and/or the fat depot mobilization (oleic acid).

Discriminant Analysis Using the FA Profile as Predictors

Pasture forages/trials were accurately discriminated using the FA profile and GA selected only six informative variables, such as C18:2 9c 12c, C18:3 9c 12c 15c, C18:2 9c 11t, PUFA, n-6, and n-3. All the above FAs, with the exception of the linoleic acid, were higher in the milk of legume-grazing ewes. Discrimination between different forage-based diets is rather uncommon using this LDA approach, but previous results of our laboratory showed that legumes tend to increase the level of C18:1 11t, C18:2 9c 11t, and occasionally C18:3 9c 12c 15c and n-6 in sheep milk (23).

In contrast, AT was rather poorly discriminated using the FA profile, for several reasons: first, this discrimination was more challenging since three categories were implied instead of two. Second, as shown by the univariate analysis, the difference between AT levels was evident only in a small number of FA, basically from *de novo* synthesis at the mammary gland level, with the exception of C4:0. Third, since there were three instead of two categories under focus, a smaller number of samples was present per category. The diet including a higher level of grazed herbage (6 h/day) was probably able to increase ruminal acetate production which is the main precursor for milk fat synthesis. However, since the supplementation partially compensated for the lower herbage intake in the ewes with the lowest AT (2 h/day), the GA-LDA of their FA profile poorly discriminated the 2 h/day from the 6 h/day samples, showing a large error (39 % of the test set samples). Interestingly, almost the same FAs were selected by GA in the four different trials that regarded AT to pasture discrimination. The common selected FAs were all the SFAs, except C14:0, C18:1 11t, UFA, and n-6. Distinguishing among mixed diets, including grazed herbage and supplements is a very challenging task, as already demonstrated by Coppa et al. (10).

The GA-LDA of the FA profile performed better when comparing milk from different grazing days. In fact, the milk collected in the first and last grazing days of the rotational scheme implemented in both studies was distinguished with 73.0% of accuracy in the test set. Interestingly, among the selected variables, some are also indicators of long AT, in particular, the short- and medium-chain FAs, such as C6:0, C8:0, C12:0, and C14:0. The long-chain FAs were selected and their classes are all probably related to the level of precursors in the herbages, such as C18:1 11t, PUFA, n-6, and n-3, being higher in the first than in the last grazing day, whereas C18:1 9c (an indicator of body fat mobilization but also present in lupin), C18:2 9c 12c (concentrated in maize grain), and SFA were all related to high supplementation of proportion in the diet and poor herbage precursor intake and uptake.

The GA-LDA of FA profile was able to classify samples collected at different milking times with good accuracy (88.8% of the test set samples). This is in line with the relevant effect of milking time in the univariate analysis. Our milking schedule was thoroughly abided with an 8 h interval between morning and afternoon milking and a 16 h interval between afternoon and morning milking. This can explain why milking time affected most of the milk FA, consistently across studies, with a few exceptions. Some of the GA-selected FA and FA classes were higher in the morning samples (C6:0, C8:0, C10:0, C14:0, C16:0, C18:2 9c 11t, SFA, PUFA, and n-3, **Table 4**), mostly mirroring the intake of herbage precursors (except for SFA). The other GA-selected FA and FA classes were higher in the afternoon samples (C18:1 9c, C18:2 9c 12c, UFA, and n-6; **Table 4**) mostly mirroring the supplementation regime and the energy balance. To our knowledge, such a discrimination approach has not been implemented so far.

Discriminant Analysis Using the FT-MIR Spectra as Predictors

Overall, the models built using FT-MIR of milk spectra (**Table 5**) gave better prediction accuracy than those based on FT-MIR of FA profile (**Table 4**). This advantage of spectra is explainable by the prediction error of FA (16), although small, which obviously does not affect the spectra. Moreover, spectra contain information that goes far beyond FA composition, being also related to other milk components and the interaction between them.

Focusing on spectra-based LDA (**Table 5**), the predictions obtained by the PCA score-LDA models were overall less accurate than those obtained by the averaged spectra-LDA models and the GA-LDA models. The difference between LDA of averaged spectra and GA-LDA of whole spectra were minimal but the models built with a selection of variables are to be preferred as they contain only the informative variables. This makes these parsimonious models more simple and less sensitive to random variability, and therefore more stable and reliable for future predictions.

Despite the benefits of LDA of milk spectra as compared with that of predicted FA profile, spectra are *per se* less interpretable than predicted FA content, unless we are able to relate the absorbance at specific wavelengths with the presence/content of FA in milk. The following sections are devoted to this aim.

In the discrimination of the pasture forage, a connection between the FAs and the spectral regions selected by GA was found, since the region from 995.4 to 1026.2 cm^{-1} is related to the absorbance of the C-H group bound to double bonds in *trans* configuration, present in C18:1 11t and C18:2 9c 11t. The latter FA was also selected by the GA-LDA of the FA profile. Actually, C18:1 11t and C18:2 9c 11t were much higher in the milk sampled from the legume than the grass-grazing ewes. In contrast, the regions from 1261.6 to 1292.4 cm^{-1} and from 2962.9 to 2993.8 cm^{-1} have no apparent connection to the selected FAs. In fact, these regions have no typical absorbance of chemical groups that differ in the FAs. For example, the last region is typical of the stretching vibrations of C-H bond in methyl groups which are present in all milk FA.

When discriminating the three AT categories, the GA selected only three regions that could contain absorbance peaks due to vibrations of chemical bonds present in FAs, and in particular, to the bending and stretching vibrations of C-H bond in methylene groups, present in saturated carbon chains; these regions are 1435.2 to 1466.0 cm^{-1}, 2835.6 to 2866.5 cm^{-1}, and 2928.2 to 2947.5 cm^{-1}. This is partially in line with the selection by GA of C4:0, C6:0, C8:0, C10:0, C12:0, C16:0, and C18:0 in the LDA based on the FA profile.

Similar spectral regions were selected for distinguishing the 2 h/day from the 4 and 6 h/day samples: from 1388.9 to 1442.9 cm^{-1}, from 2858.8 to 2866.5 cm^{-1}, and from 2916.6 to 2924.4 cm^{-1}. These regions contain wavelengths on which the C-H bond in methylene groups absorbs the infrared beam light.

Instead, none of the spectral regions selected to discriminate the 6 h/day from the 2 and 4 h/day samples and the 2 h/day from the 6 h/day samples are related to any chemical bond that differs in the types of FAs and classes of FAs present in milk.

When discriminating for the grazing day, GA selected 11 spectral regions, two of which are related to saturated carbon chains, and consequently to the amount of sum of SFAs, which was also selected by the GA-LDA of FA profile.

The spectral regions selected by GA for the discrimination of different milking times contain absorbance peaks related to the bending vibrations of the C-H bond in *trans*-configuration double C=C bond (937.5 to 1084.1 cm^{-1}) and to the bending and stretching vibrations of the C-H bond in methylene groups (from 1446.7 to 1581.8 cm^{-1} and from 2824.1 to 2959.1 cm^{-1}, respectively). The first region could be therefore related to the amount of C18:2 9c 11t (one of the FAs selected by GA in the LDA of FA profile), whereas the latter two regions could be related to the amount of other selected FA, such as SFA and the individual SFAs, such as C6:0, C8:0, C10:0, C14:0, and C16:0. All the other selected spectral regions are not directly related to any selected fatty acid.

To sum up the discussion on the GA-LDA of FA and spectra, it is worthy to note that in the discrimination of the pasture forages/trials, AT, grazing days, and milking times in the GA-LDA based on FA, some of the selected FAs were common (C18:1 9c, C18:2 9c 12c, PUFA, n-6, and n-3, **Table 4**). Likewise, in the GA-LDA based on FT-MIR spectra, some of the selected regions were common (**Figure 5**). This recalls the gradient of precursor and nutrient intake that was explored in this study. In fact, the above FAs are indicators of herbage intake, and C18:1 9c, in particular, can also be sensitive to energy balance. Milk from ewes grazing only for 2 h/day were in fact the ones that show lower contents of some beneficial FAs, such as n-3, but also a higher content of oleic acid, possibly related to a higher desaturation of C18:0 at the fat tissue level.

CONCLUSIONS

The comparison between the performance of the multivariate models confirms that the models using the GA-selected variables are to be preferred, as only the informative variables are retained, making the predictions more robust and hence reliable to be implemented to external data sets. The discrimination performance of GA-LDA as expected was better when the spectra were used instead of the milk FA content, estimated on the basis of previously validated calibrations, although the difference in accuracy between the approaches varied among targeted comparisons.

Individual milk samples from ewes under a rotational PTG of *Lolium multiflorum* and *Trifolium alexandrinum* were well-discriminated using the GA-LDA of their FA profile and even better applied using the same statistic to their FT-MIR spectra.

However, GA-LDA based only on FT-MIR spectra discriminated accurately individual milk samples collected in the first grazing day from those collected in the last grazing day and those collected in the morning from those collected in the afternoon milking.

In contrast, neither the GA-LDA of FA nor GA-LDA of spectra were able to accurately disentangle samples obtained from ewes having 2, 4, or 6 h/day AT to pasture, although the error was limited to c.a. 25% of samples with GA-LDA of spectra, if only the extreme AT milk were compared. This is in line with univariate analysis results which showed differences only for a few FA, between milk sourced from ewes with 2 and 6 h/day of AT to pasture.

These findings overall suggest that the best GA implemented in this study (GA-LDA of FT-MIR spectra) provides encouraging results for discriminating morning vs. afternoon milk samples and for tracing individual sheep milk back to sheep feeding regimen, with reference to the grazed forage and the grazing day, which can be regarded as an indicator of quality/amount of herbage eaten in rotationally stocked sheep. On the contrary, results are not yet fully satisfactory when discriminating mixed diets of ewes, part-time grazing with AT to pasture differing by 2 or 4 h/day.

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 were reviewed and approved by Agris Ethical committee.

AUTHOR CONTRIBUTIONS

GM, AC, MD, and VG conceived the idea and performed the experimental design. MS helped for the field experiment. II carried out the milk chemical analysis. MA and MC carried out the simulation in the implementation of milk fatty acid analysis and spectral by chemometric approach. GM supervised the project. All authors discussed the results and contributed to the final manuscript.

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The Effect of Grazing Intensity and Sward Heterogeneity on the Movement Behavior of Suckler Cows on Semi-natural Grassland

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Extensively grazed semi-natural grasslands contribute to a wide range of ecosystem services, including the preservation of biodiversity and provision of livestock feed. Depending on the grazing intensity, cattle are set in motion to fulfill their nutritional needs. In this way, they influence the vegetation composition, while at the same time the foraging behavior is affected by the vegetation. A better understanding of the relationship between grazing intensity and animal behavior is an essential component for strategies to improve the value of semi-natural grasslands and for gaining insights for the development of smart farming technologies. The long-term cattle grazing experiment "FORBIOBEN" with its replicated three paddock-scale (1 ha) grazing intensities [moderate (M), lenient (L), very lenient (VL)] was used to investigate the movement behavior of suckler cows during four grazing periods between 2017 and 2020. For this, pregnant suckler cows (Fleckvieh) were equipped with Vectronics GPS Plus (VECTRONIC Aerospace GmbH, Berlin) collars, which recorded the position of the animals at defined time intervals. The main outcomes were that with an increase in the grazing intensity, the herbage on offer declined and, consequently the herbage allowance. However, the spatial heterogeneity of the herbage on offer decreased with increasing grazing intensity (M < VL) which means that the amount of available herbage was lower but more evenly distributed under moderate grazing. Further, there was a tendency that the moderate grazing intensity was associated with the highest effort of walking compared to lenient and very lenient grazing in three out of four grazing periods. We found a strong ($p < 0.001$) negative correlation among walking distance vs. herbage variability across all treatments × periods. Consequently, the grazing intensity itself was not a good predictor of walking distances which were mainly a result of the available herbage, its distribution or heterogeneity. Future smart farming livestock management systems will, therefore, likely require interfaces with the grassland growth rates and heterogeneity benchmarks if decisions based on livestock movement should be reliable.

Keywords: herbage allowance, GPS tracking, precision livestock farming, walking distance, spatial distribution

INTRODUCTION

Grassland is the largest terrestrial biome, covering ∼3.2 billion ha worldwide (1) and a large part of this area is used by grazing herbivores. Depending on the environmental conditions, the animal species and grazing method, these grazing herbivores influence the sward while their performance, on the other hand, is influenced by the sward properties (2, 3). Extensification of grassland leads to a shift towards a more diverse botanical composition and increased plant species richness (4). For instance, extensively grazed semi-natural grasslands host a great number of plant species, which is why they essentially contribute to the biodiversity of agricultural landscapes (5, 6). The vegetation often develops into a heterogenous pattern of different sward height classes of tall and short patches (7) which results from the so-called patch grazing (8). Patch grazing is characterized by a pronounced spatial heterogeneity in forage intake (9) with intensive and extensive grassland utilization occurring in close proximity within the same pasture. Several studies in semi-natural grassland found that the productivity (10), soil nutrient contents (10, 11), and the vegetation composition (4, 12) are driven by these temporally stable patches (7) rather than by the pasture-scale grazing intensity. The extent of patch grazing is controlled by the pasture stocking rate, i.e., the herbage allowance per grazing animal. It has been shown that under low stocking rates, animals tended to graze only on short grass patches even at the end of the grazing season (13). This indicates that the cattle regularly return to the same spots of high-quality herbage. Assuming that the productivity of these patches is maintained, the effort for foraging is low and the walking distances should mainly depend on the spatial distribution of these patches. With a more restrictive herbage allowance, i.e., higher grazing pressure, the animal has to visit more places every day to fulfill its energy demand because less herbage is available per patch so that more movement is required. On the other hand, a higher herbage allowance per animal does not always result in less movement since in a patchy grassland the foraging areas are spatially distributed (7).

Hejcmanová et al. (14) investigated behavioral patterns under extensive and intensive continuous grazing (fewer vs. more cattle per pasture) and found a clear trend towards longer grazing time under intensive grazing. However, in a study of Dumont et al. (13), the walking distances per grazing event were not affected by the stocking rate and group size. Thus, it remains an open question to what extent the grazing intensity and, hence, the availability or distribution of herbage control the activity of grazing cattle in semi-natural grassland ecosystems. Such information is needed if any decision support tools in future smart farming systems will be based on the spatial animal movement.

Using GPS (Global Positioning System) collars to track the spatial behavior of grazing cattle is a well-established method to investigate the drivers of animal behavior. Since 1978 GPS is operational and since 1984 civilian use is allowed. The University of Kentucky began to use GPS collars for cattle tracking in the 1990s to be able to integrate spatial information into cattle management procedures (15). Using GPS collars in studies of animal movement has many benefits: individuals can be tracked over a long-term period with predefined time intervals and automatically recorded geographical positions (16), which is very helpful information on large pastures and rangelands (17). In addition, accurate and efficient information on grazing behavior can be provided by the use of GPS for monitoring of grazing animals (18). Animal-related GPS recordings in combination with a geographic information system (GIS) can provide information on spatial interrelations of animal behavior and the vegetation (19). In recent years, several studies have investigated the potential of GPS tracking data to deduce behavioral patterns of grazing cattle. Homburger et al. (20, 21), both based on investigations in heterogeneous subalpine pastures, recommended to differentiate only grazing and resting when using GPS tracking. Walking is mainly correlated with grazing because cattle always walk several steps between bites while walking without grazing is a relatively rare activity (22). In the study by Homburger et al. (20), only 6.7% of movement was accounted for by walking without grazing as assessed by visual observations. In another study (17) it was shown that the time budgets of the main cattle behavior (grazing, resting, walking) were not influenced by the grazing management. However, the walking distances were affected in that study and also in that by Baudracco et al. (23), where cows on a pasture with lower herbage allowance spent more time walking. Consequently, assessing movement patterns in terms of walking distances will provide a reliable indicator for the effort of the grazing cattle to fulfill dietetic demands under conditions of varying herbage allowances. Moreover, such assessments can help to identify the driving forces of livestock movement, including the role of sward characteristics. The study presented here was conducted in a multi-year grazing experiment with livestock cattle on semi-natural grasslands under three different grazing intensities, defined by different target sward heights (moderate: 6 cm, lenient: 12 cm, very lenient: 18 cm) resulting in decreasing stocking rates (moderate to very lenient). The grazing experiment was established in 2002 under the EU framework 5 research project "FORBIOBEN" (3). The aim of "FORBIOBEN" with its three paddock scale grazing intensities is to represent the entire gradient of grassland extensification. Over three seasons (2017, 2019, 2020), cattle were equipped with GPS collars with the aim to disentangle interactions between the grazing intensity and cattle movement by taking into account both herbage allowance and the spatial variability of the herbage on offer. We hypothesized that (i) cattle activity increased with lower herbage allowance because the area, size and stability of tall patches increase with decreasing grazing intensity (7), and foraging resources are the most obvious drivers of grazer distribution at pasture (8), we further hypothesized that (ii) the spatial distribution of cattle during activity (grazing) peaks is more even under moderate compared to lenient grazing intensity.

MATERIALS AND METHODS

Experimental Site, Setup and Weather Conditions

The present study investigated the movement behavior of suckler cows in response to three different grazing intensities. It was carried out over four periods between spring 2017 and spring 2020 as part of the grassland experiment "FORBIOBEN," which is located at the experimental farm of the University of Göttingen in Relliehausen, Solling Uplands, Lower Saxony, Germany (51°46'55.9 "N, 9°42'11.9"E), 250 m above sea level. The vegetation is a moderately species-rich semi-natural grassland classified as *Lolio-Cynosuretum*. The three most important grasses in 2017 were *Festuca rubra*, *Lolium perenne* and *Cynosurus cristatus*, while the three most important dicot species were *Taraxacum officinale*, *Trifolium pratense,* and *Galium mollugo*. In 2020 this changed slightly towards *F. rubra, Dactylis glomerata* and *L. perenne* and for the dicots to *T. officinale, Lotus corniculatus,* and *Galium mollugo*.

The longtime climatic averages (yearly) of the German weather service 'Deutscher Wetterdienst' reference period (1991–2020), measured approximately 21 km apart, were: precipitation: 764 mm, temperature: 9.8 °C, sunshine hours: 1500 (24). Weather conditions in the investigated periods are summarized in **Table 1**. The grazing experiment "FORBIOBEN" was established in 2002 (3) and is maintained in its current state since 2005. It compares three intensities of cattle grazing described by different target vegetation heights, hereafter M: moderate grazing (6 cm), L: lenient grazing (12 cm) and VL: very lenient grazing (18 cm target vegetation height). The three grazing intensities are replicated in a randomized block design of three paddocks (1 ha each) per grazing intensity. The general framework of the "FORBIOBEN" experiment is extensive grassland management as no fertilizer, pesticide or any sward improvement measure is applied. Within this framework, the different grazing intensities represent the following strategies. Moderate grazing is aiming at reasonable agronomic performance; lenient grazing does not make full use of the herbage, leaving remaining herbage for biodiversity targets, and very lenient grazing is representing the minimum grazing intensity that is required to keep the grazing land open, i.e., maintain the open character of the grassland. The management is a continuous grazing system with a put-and-take approach. In this system, cattle are added to the paddocks when the target vegetation height is exceeded and removed when the vegetation height falls below the target.

Animals

During each stocking season (April/May – September/October), up to 27 pregnant, non-lactating Fleckvieh suckler cows grazed in all three grazing intensities. Usually, the target sward height of 6 cm in M is reached faster in spring, so that this treatment can be stocked earlier. The VL treatment was stocked when the target height of the L treatment was reached, to prevent natural succession of the grassland. Outside the grazing period, from November to April, the animals are in winter housing. Calving takes place in November and December; mating is in February and March. Cows return to pasture in mid-April, after weaning. Animals that were removed from the experimental paddocks because sward heights fell below the target values grazed an area adjacent to the experimental paddocks. During the investigated periods, the cows were randomly assigned to groups and distributed among the paddocks. Average stocking densities of the different grazing intensities during the investigation were, moderate grazing: 4.6 LU ha^{-1}, lenient grazing: 3.8 LU ha^{-1}, very lenient grazing: 2.7 LU ha^{-1} (LU: livestock unit, 500 kg live weight). A detailed overview is given in **Table 2**. The respective stocking rates under moderate, lenient and very lenient grazing, calculated as (LU × days on pasture) per year and pasture area, were 1.4, 0.5, and 0.4 LU ha^{-1}a^{-1} in 2017; 0.9, 0.6 and 0.4 LU ha^{-1}a^{-1} in 2019; and 0.7, 0.4 and 0.2 LU ha^{-1}a^{-1} in 2020.

TABLE 1 | Weather conditions (TM: mean daily temperature (°C) and precipitation sum (mm) during the four investigated periods recorded by the meteorological station in Bevern 51°51′10.8" N 9°29′42.0" E coordinated by the German Weather Service 'Deutscher Wetterdienst' (DWD), 21 km from the experimental site.

Period	TM (°C)	Radiation (W m^2)	Precipitation sum (mm)
2017	16.2	19,950.6	52.1
2019 spring	16.1	19,722.2	77.0
2019 autumn	12.6	13,004.0	16.0
2020	17.3	17,875.0	79.6

Collecting Data

The duration of the investigated periods differed in response to the weather conditions and, hence, the herbage growth (**Table 2**). Each period lasted for 28, 35, 17, and 32 days in 2017, 2019 spring, 2019 autumn and 2020, respectively. To avoid bias from acclimatization to the collars and increased movement associated with paddock changes, the data collected on the first and last day of each period were excluded. The dates shown in **Table 2** omit these days and correspond to the actual daily data used.

At the beginning of each period, one cow per grazing intensity and replicate was equipped with a Vectronics GPS Plus (VECTRONIC Aerospace GmbH, Berlin) collar (weight: 1.36 kg), attached to the neck of a randomly chosen cow per paddock, corresponding to a total of nine GPS collars. In the periods 2019 spring and 2020 two collars, and in 2019 autumn one collar were found not to have recorded data when the collars were removed. The collars are equipped with internal devices for GPS localization and an activity sensor (three-way accelerometer). Every 128 s (2017 and 2019 spring), or every 60 s (2019 autumn and 2020), the GPS sensors in the collar recorded a signal about the location of the animal within the pasture. Each GPS data point was recorded with date, time, distance, speed, absolute and relative angle between two successive path segments. In addition, the activity sensor in the collar recorded data in 64-s intervals. For each interval, it measured the proportion of time that the head tilt angle of the animal exceeded 15°, i.e., the time that the head was not lowered. At the end of the respective grazing period, the collars were

TABLE 2 | Overview of the grazing management and treatments during the investigated periods and annual stocking rates.

Period (duration)	GI	Age years ± sd	LW kg ± sd	SD (LU ha^{-1})	SR (LU ha^{-1} a^{-1})
2017 (18.05–14.06)	M	4.8 ± 1.6	666.3 ± 73.3	5.3	1.4
	L	5.6 ± 2.8	638.7 ± 96.2	3.8	0.5
	VL	5.4 ± 1.2	658.3 ± 86.0	2.6	0.4
2019 spring (24.05–27.06)	M	6.0 ± 2.5	684.8 ± 97.0	5.5	0.9
	L	5.7 ± 2.4	667.0 ± 101.0	4.0	0.6
	VL	5.2 ± 2.2	638.0 ± 57.4	2.6	0.4
2019 autumn (06.09–22.09)	M	5.1 ± 2.7	749.3 ± 105.0	4.5	0.9
	L	6.2 ± 2.6	795.1 ± 60.8	4.8	0.6
	VL	5.5 ± 2.1	748.7 ± 91.2	3.0	0.4
2020 (11.06–12.07)	M	3.4 ± 1.3	620.0 ± 69.7	2.5	0.7
	L	6.0 ± 2.9	626.7 ± 80.6	2.5	0.4
	VL	7.8 ± 1.2	673.5 ± 44.6	2.7	0.2

GI, grazing intensity; LW, live weight; SD, stocking density; LU, livestock unit; SR, stocking rates.

removed to retrieve data and analyzed to measure the activity in terms of walking distance.

Walking distance (m) per animal was measured at two temporal scales, per day and also per hour within day. Geographic coordinates were available in the Universal Transverse Mercator coordinate system (UTM) format. To calculate the distance between two sequential positions, the Pythagorean theorem was used. The results were summed for hourly and daily (24-h) periods.

Data obtained from the activity sensor of the collar in spring 2019 were used to assess the relationship between walking distance per hour and the duration of grazing in minutes per hour, following Homburger et al. (21). Measurement intervals during which the activity sensor reported a lowered head at least half of the time were classified as grazing. This classification was validated by visual observations during 2016.

Sward Herbage Measurements and Sward Characteristics

To determine the grassland herbage on offer, a double sampling approach was conducted from early April to October. For this, the compressed sward height (CSH) was measured every 2 weeks using a rising plate meter of 30 cm diameter and 200 g plate weight (25) at 50 places randomly distributed in each paddock. Approximately every 4–8 weeks, the standing herbage dry matter was determined at six to eight random points per paddock. Biomass was cut manually at 1 cm above the soil surface in a 30-cm diameter ring after first measuring CSH at this location. This procedure was conducted in order to calibrate the relationship between CSH and grassland herbage mass based on linear regression models (26, 27). The herbage biomass samples were oven-dried at 60°C for 48 h to obtain the dry matter weight. Based on the relationship between CSH and standing herbage dry matter, the available herbage on offer (herbage mass) was modeled for every other date and CSH measurement without calibration sampling so that 50 herbage values were available per paddock on each date of CSH measurements. Herbage biomass prediction from CSH was reasonable ($RMSE = 70.4\,g\,m^{-2}$ and mean $R^2_{adj} = 0.63$ averaged over all periods). The derived herbage on offer per CSH sampling point was used to calculate the spatial heterogeneity of the herbage on offer by calculating the standard deviation within paddock (SD herbage).

Botanical composition in ten 1-m^2 quadrats was assessed in accordance with the method of Scimone et al. (28) with average proportions between 2017 and 2020 of 59.7 ± 9.6, 59.2 ± 13.5, and 53.7 ± 10.9% grasses and of dicotyledonous species of 26.1 ± 5.7, 27.8 ± 6.8 and 25.7 ± 6.2 (± SD) in M, L and VL, respectively. Further studies showed that within grazing intensities, the botanical composition differed between short and tall patches as a consequence of modified resource availability for light and soil nutrients (4). Tonn et al. (11) observed larger phytodiversity in short patches compared to tall ones, and Perotti et al. (29) found that species in tall patches had higher competitiveness and the ones in short patches higher stress tolerance according to the competitor, stress tolerator, ruderal (CSR) theory after Grime (30).

The *in vitro* organic matter digestibility as assessed using near-infrared reflectance spectroscopy in ten continuous observation plots of 1 m^2 size per paddock were 78.5 ± 7.4, 76.2 ± 6.2 and 74.6 ± 6.0% (mean ± SD) on average over 2017 to 2020 in M, L and VL, respectively. No patch-specific forage quality data was assessed in the present study. We know, however, from the beginning of the grazing experiment, that tall and short patches differ in the stem-to-leaf ratio toward the end of the growing season (27) with consequences for forage quality (3). Pavlu et al. (31) indicated differences in patch-specific forage quality and a recent study by Ebeling et al. (10) on the same site 12 years after extensive grazing revealed that the short patches were less productive and likely remained in a vegetative state as a consequence of selective grazing.

Data Analysis

Statistical analyses were carried out with the software R (32). Linear mixed effects models were calculated for each target variable using the package “nlme” (33). For this, every period was analyzed separately. Outliers were eliminated if present by considering values ranging 1.5-fold above the 75th or below the 25th percentile of the interquartile range (34). For all analyses, $\sim <5\%$ of the data were excluded as outliers. Normality of the residuals was checked by visual inspection of quantile–quantile plots. Variance homogeneity was evaluated by plots of residuals vs. fitted values and residuals vs. predictor values (35). Multiple contrast tests according to Tukey's test for significant influencing factor levels were followed using the "emmeans" package (36) after analysis of variances.

The daily distance was regressed on the fixed effects of grazing intensity and date as well as their interaction. The cow nested in block was modeled as a random effect in order to account for correlation between measurements on the same object. Then model reduction was performed from the global model using the MuMIn package (37). The model with the lowest AICc was chosen as the final model.

To assess the diurnal patterns within days, models with fixed effects of grazing intensity, hour per day and their interaction and the random effect of the block and cow nested in block were generated. The dates per period were treated as replicates and the interaction between hour and date was consequently not considered. The hourly walking distance was log-transformed before analysis in order to improve normality of residuals.

The average period-wise herbage allowance was determined in order to assess the strength of competition for forage resources which may drive the walking distances in pastures (23). For this, the herbage allowance was regressed on the fixed effect of grazing intensity and the random effect of block. The herbage allowance was square-root transformed before analysis.

To quantify the extent of spatial clustering within period and grazing intensity treatment, each paddock was rasterized into 400 5×5 m squares. GPS locations were split into two groups: "active time" included all animal locations during the activity peaks in the morning and afternoon, as determined from the analysis of walking distance per hour. "Other time" included all other animal locations. For each of these sets, the duration (min) spent within each grid cell was calculated. These values were then used to determine the Camargo Index of Evenness across all cells within paddock and period (38) for both groups. The Camargo index allows to assess spatial patterns and the relative distribution of GPS locations within each paddock. Values near zero indicate a patchy distribution and values near one a homogenous distribution (38). This index is, thus, a metric for the requirement of searching to fulfill the herbage intake in relation to the grazing intensity. The Camargo Index was then analyzed in models with the grazing intensity as fixed and block as random effect separately for each period. For other time, the approach was similar.

The relationship between the activity of time spent grazing, (grazing time in min hour^{-1}, based on the activity sensor measurements) and the hourly walked distance was analyzed in an analysis of covariance with the walking distance per hour as covariate, the grazing intensity and the interaction of both as fixed and the block as random effect. Variance adjustments were allowed per date in that model. A significance level of $p \leq 0.05$ was chosen throughout.

All spatial maps were plotted with QGIS (3.10.12 "A Coruña").

RESULTS

Average Daily Walking Distances Within Each Grazing Period

Differences of the daily walking distances between grazing intensities were mostly significant but depended on the grazing period (**Tables 3**, **4**). While in 2017 and autumn of 2019, the daily walking distances were affected by the grazing intensity (**Table 3**), no effects were found in 2020 and spring of 2019 (although $p < 0.1$). In most periods, walking distances were largest for grazing intensity M (not in 2020), while they were lowest for grazing intensity L in most periods (not in 2017) and those of VL tended to range between them (**Table 4**). The daily distances varied between 2,592 m (2017 grazing intensity L) and 3,929 m (2020 grazing intensity VL).

Average Hourly Walking Distances Within Each Grazing Period

The interaction between hour per day and the grazing intensity affected the hourly walking distance in all periods (**Table 3**). A strong diurnal pattern became evident with a shift in the activity peaks during the autumn 2019 period compared with the other periods (**Figure 1**). The main activity was recorded in the hours 5, 6, 7 a.m. and 7, 8, 9 p.m. (spring and summer periods). In autumn, the activity peaks were narrower, comprising the hours 7, 8 a.m. and 5, 6, 7, 8 p.m. (**Figure 1**). These time periods were considered as "active time" when the Camargo Index was calculated. On average, they encompassed 40% (M), 39% (L) and 39% (VL) of daily walking distances. The main periods of inactivity occurred during night time and between the activity peaks (**Figure 1**).

The hourly walking distance and the grazing time (spring 2019) were positively related, with the slope depending on the grazing intensity treatment (**Figure 2**) as indicated by the significant interaction between distance × grazing intensity.

Herbage on Offer, Spatial Heterogeneity of Herbage on Offer and Herbage Allowance

The average herbage on offer during each period was affected by the grazing intensity (**Tables 3**, **4**) with a general increase of available herbage from grazing intensity M, over L to VL, but also a visual decline in the available herbage from 2017 until 2020 (**Table 4**). The values for each measured date are provided in the supplements (**Supplementary Figure 1**). The herbage allowance was affected by the grazing intensity in all periods (**Table 3**) and generally increased in the order M < L < VL (**Table 4**).

There were only significant effects of the grazing intensity on the SD herbage mass in spring of 2019 and 2020 (**Table 3**) with a clearly lower variability within grazing intensity treatment M compared with L and VL in that period (**Table 4**). A general trend for increases in SD herbage mass in the rank order $M < L \leq VL$, however, became clear for all periods.

TABLE 3 | Output of linear mixed effects models for the analyzed parameters of interest during each grazing period.

Period	Variable	Fixed and interaction effects	*F*-value	*P*-value
2017	Daily walking distance	Grazing intensity	30.6	$P < 0.01$
	Hourly walking distance	Grazing intensity	0.2	n.s.
		Hour	104.7	$P < 0.001$
		Grazing intensity × hour	3.7	$P < 0.001$
	Herbage on offer	Grazing intensity	10.3	$P < 0.001$
	SD herbage	Grazing intensity	4.8	$P < 0.1$
	Herbage allowance	Grazing intensity	118.8	$P < 0.001$
	Camargo active time	Grazing intensity	7.8	<0.05
	Camargo other time	Grazing intensity	22.5	<0.01
2019 spring	Daily walking distance	Grazing intensity	5.6	$P < 0.1$
	Hourly walking distance	Grazing intensity	1.7	n.s.
		Hour	71.6	$P < 0.001$
		Grazing intensity × hour	5.0	$P < 0.001$
	Herbage on offer	Grazing intensity	75.8	$P < 0.001$
	SD herbage	Grazing intensity	39.2	$P < 0.01$
	Herbage allowance	Grazing intensity	493.7	$P < 0.001$
	Camargo active time	Grazing intensity	5.1	n.s.
	Camargo other time	Grazing intensity	7	n.s.
	Grazing time	Distance	4,064	$P < 0.001$
		Grazing intensity	7.5	$P < 0.001$
		Distance × Grazing intensity	38.7	$P < 0.001$
2019 autumn	Daily walking distance	Grazing intensity	58	$P < 0.01$
	Hourly walking distance	Grazing intensity	1.7	n.s.
		Hour	60.7	$P < 0.001$
		Grazing intensity × hour	2.5	$P < 0.001$
	Herbage on offer	Grazing intensity	74.8	$P < 0.001$
	SD herbage	Grazing intensity	3.4	n.s.
	Herbage allowance	Grazing intensity	8.4	$P < 0.05$
	Camargo active time	Grazing intensity	18.3	<0.05
	Camargo other time	Grazing intensity	17.5	<0.05
2020	Daily walking distance	Grazing intensity	n.s.	n.s.
	Hourly walking distance	Grazing intensity	6.3	n.s.
		Hour	107.1	$P < 0.001$
		Grazing intensity × hour	5.5	$P < 0.001$
	Herbage on offer	Grazing intensity	29.6	$P < 0.001$
	SD herbage	Grazing intensity	11.1	$P < 0.05$
	Herbage allowance	Grazing intensity	15.6	$P < 0.01$
	Camargo active	Grazing intensity	1.7	n.s.
	Camargo other time	Grazing intensity	24.7	<0.05

Shown are F- and p-values.

Spatial Distribution in Relation to Grazing Intensity and Period

The Camargo Index was determined for the "active time," identified as the hours of peak activity according to **Figure 1**, and for the remaining time (other time) within each period. The Camargo index for the active time was affected by the grazing intensity only in 2017 and 2019 autumn (**Table 3**), and declined from M to L and VL, indicating a more even distribution within the paddock in grazing intensity M during these periods (**Figure 3**). This was also confirmed for the Camargo index of the other time periods (**Figure 4**) which were affected by the grazing intensity in all periods except of spring 2019 (**Table 3**).

The distribution of spatial points between pastures within each period is given in **Figure 5**. Time (s d^{-1}) spent in each 5 × 5 m grid cell was categorized into five percentiles, visualized as density maps.

TABLE 4 | Estimated means ± se (standard error) of linear mixed effect models for every period.

Period	GI	Individual daily distance (m)	HO (g DM m^{-2} ± se)	SD Herbage (g DM m^{-2} ± se)	HA (kg DM LU^{-1} ± se)
2017	M	3,642 ± 173 b	235 ± 19.1 a	81.3 ± 8.41	455 ± 51.6 a
	L	2,958 ± 173 a	319 ± 19.1 ab	108.8 ± 8.41	854 ± 70.7 b
	VL	2,901 ± 173 a	355 ± 19.1 b	119.2 ± 8.41	1,421 ± 93.4 c
2019 spring	M	3,542 ± 201	107 ± 7.1 a	56.6 ± 4.31 a	178 ± 14.9 a
	L	2,592 ± 201	203 ± 7.1 b	89.6 ± 4.31 b	539 ± 25.8 b
	VL	3,108 ± 142	219 ± 7.1 b	96.4 ± 4.31 b	902 ± 27.9 c
2019 autumn	M	3,773 ± 92.7 b	99.5 ± 7.64 a	95.2 ± 5.5	265 ± 59.8 a
	L	3,329 ± 91.4 a	196.8 ± 7.64 b	106.4 ± 5.5	339 ± 67.6 ab
	VL	3,653 ± 91.4 b	215.1 ± 7.64 b	115.4 ± 5.5	695 ± 96.9 b
2020	M	3,680 ± 448	80.9 ± 8.96 a	48.9 ± 3.5 a	358 ± 38.4 a
	L	3,701 ± 402	156.1 ± 8.96 b	63.7 ± 3.5 ab	621 ± 50.7 b
	VL	3,929 ± 448	172.2 ± 8.96 b	72.0 ± 3.5 b	670 ± 43.0 b

Lowercase letters: means with different letters are significantly different between GI within year ($p < 0.05$). GI, grazing intensity; HO, herbage on offer; SD Herbage, standard deviation of herbage on offer; HA, herbage allowance.

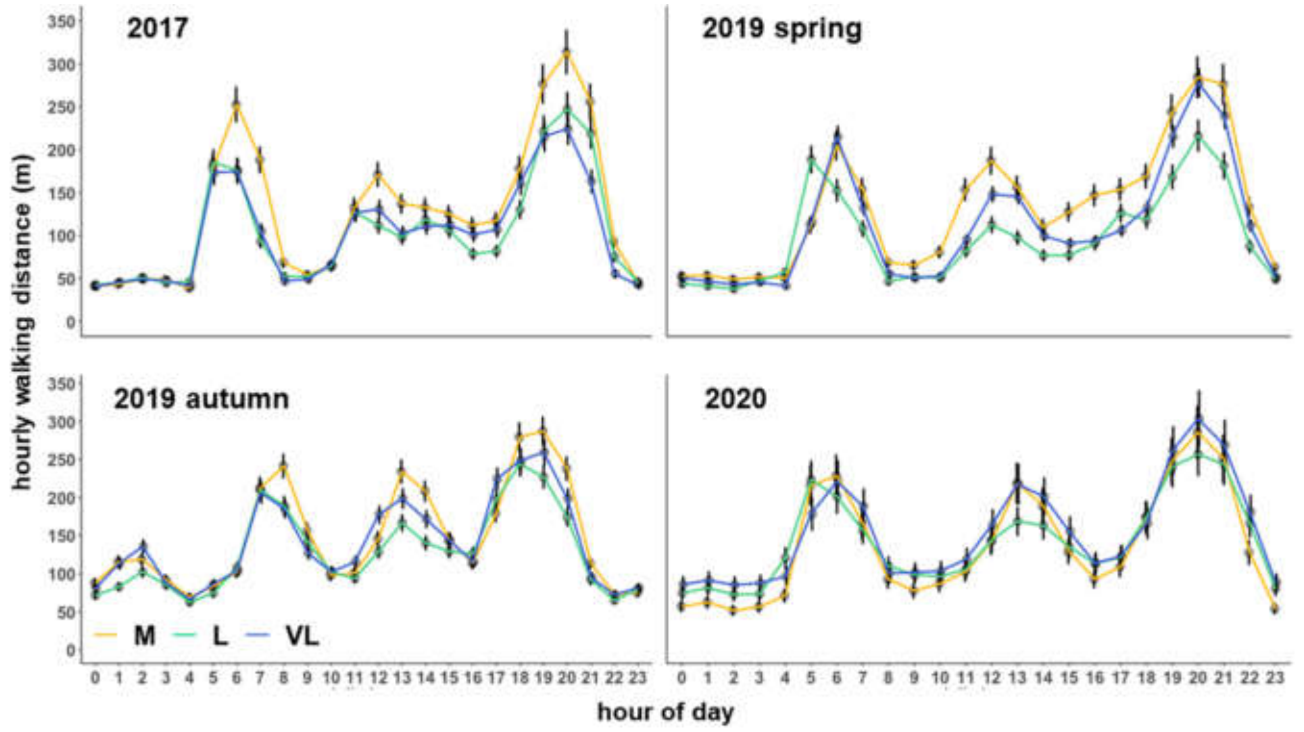

FIGURE 1 | Estimated means (±SE) of the average hourly walking distance (m) as influenced by the grazing period, grazing intensity and hour per day. M, moderate; L, lenient; VL, very lenient grazing intensity.

DISCUSSION

While there are many studies on the effects of cattle grazing in different grazing intensities on outcomes for herbage quality (13, 39), biodiversity (3, 40–42), sward botanical composition (43) or productivity (3, 40, 41), the current study is the first to quantify the relationship between cattle movement and grazing intensity, taking into account herbage availability. We hypothesized that (i) cattle activity increased with lower herbage allowance. We further hypothesized that (ii) the spatial distribution of cattle during activity (grazing) peaks is more even under moderate compared to lenient grazing intensity.

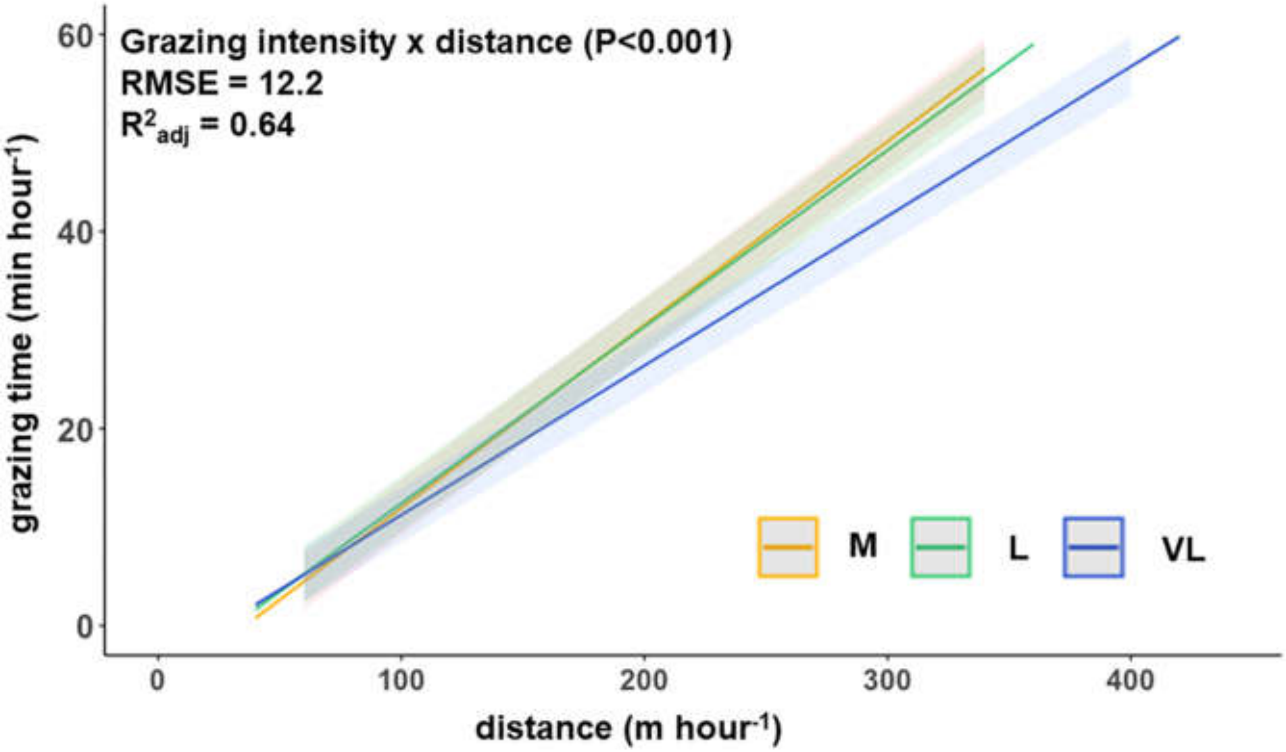

FIGURE 2 | Functional relationship between the hourly walking distance and grazing time per hour (spring 2019) for the three grazing intensities (model prediction). M, moderate; L, lenient; VL, very lenient in spring 2019.

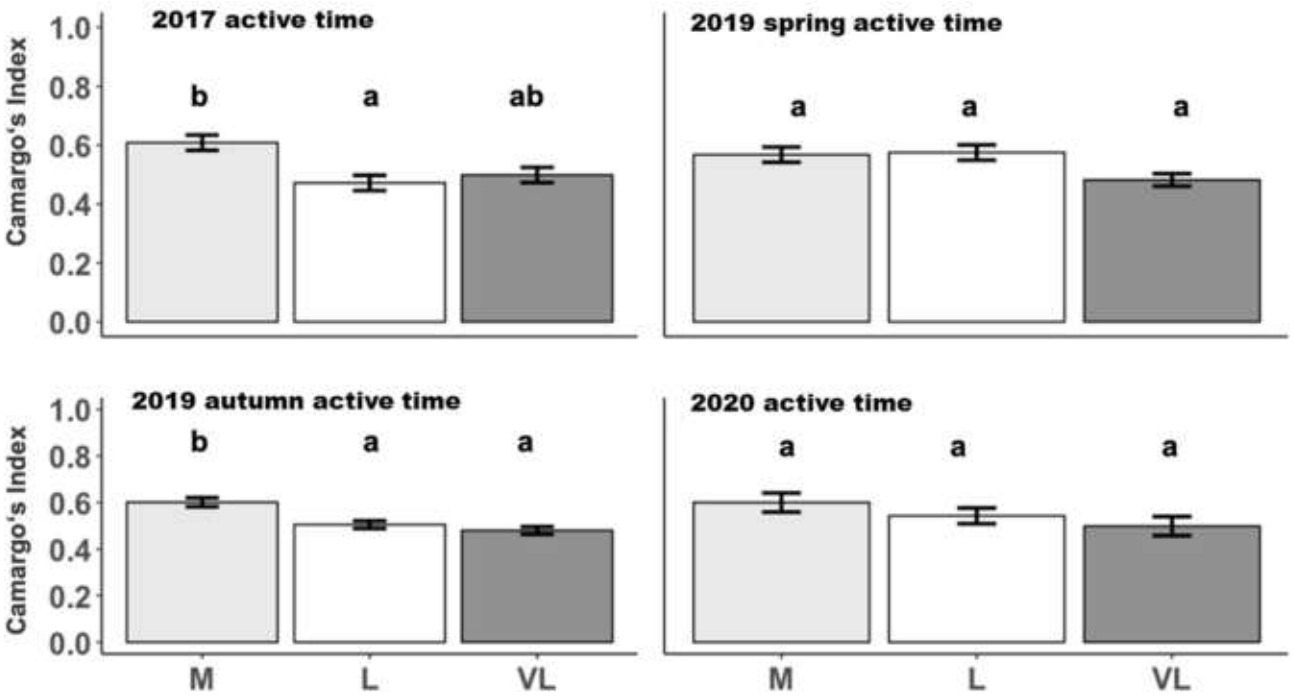

FIGURE 3 | Estimated means (±SE) of the Camargo Index during active time as influenced by the grazing period and grazing intensity. M, moderate; L, lenient; VL, very lenient stocking rate. Identical lowercase letters indicate that means are not different at $p < 0.05$.

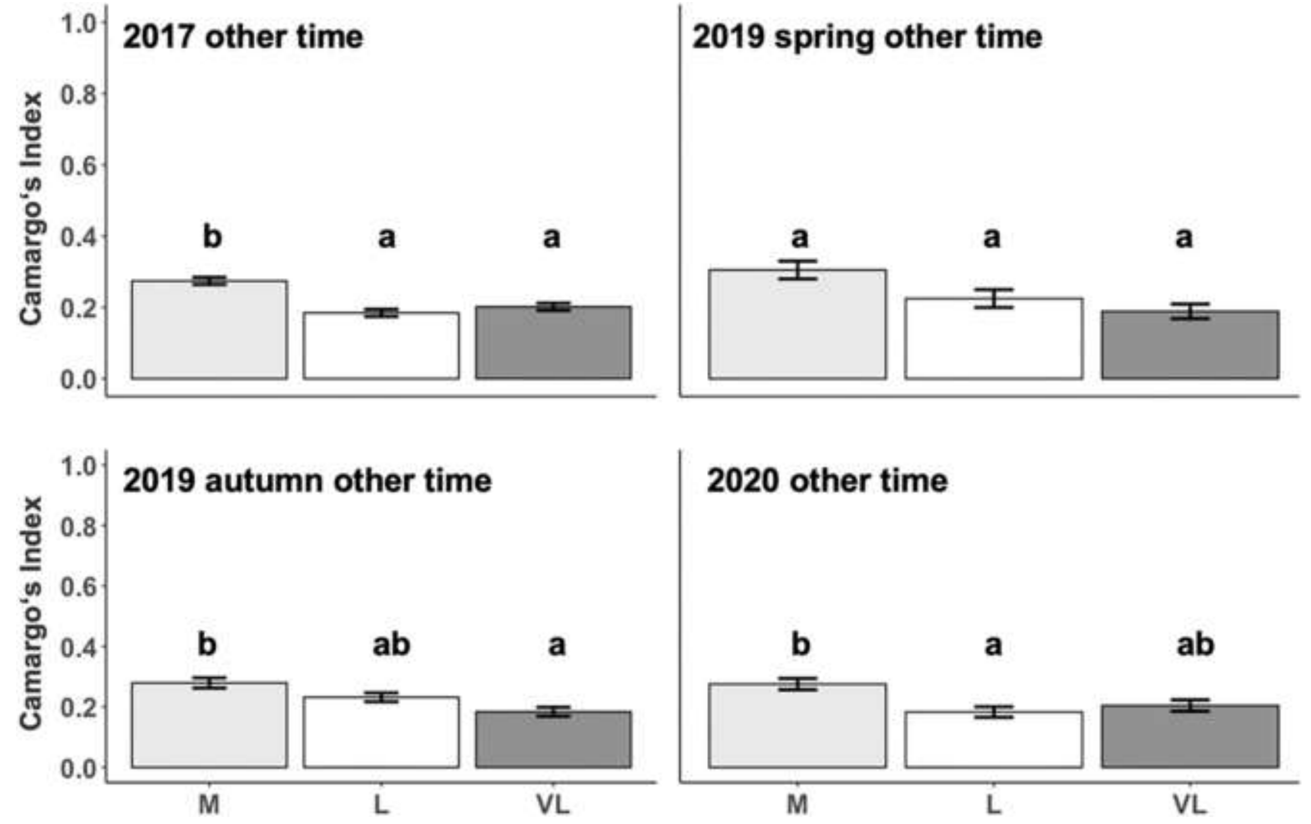

FIGURE 4 | Estimated means (±SE) of the Camargo Index during other time as influenced by the grazing period and grazing intensity. M, moderate; L, lenient; VL, very lenient stocking rate. Identical Lowercase letters indicate that means are not different at $p < 0.05$ within periods.

Variation in Herbage Availability and Patterns of Walking Distances in Relation to Grazing Intensity

With an increase in the grazing intensity, the herbage on offer and consequently also the herbage allowance declined (rank order: M < L < VL). However, the spatial heterogeneity of the herbage on offer decreased with increasing grazing intensity (M < VL) which means that the amount of available herbage was lower but more evenly distributed under the moderate grazing treatment M. Increases in the stocking rate and a decline in herbage allowance per individual will cause an increase in the effort of walking on pastures of similar botanical composition (22) – especially under low-input conditions when grassland growth rates are low. Except for the last period, moderate grazing intensity tended to be associated with the greatest effort in walking compared with the other grazing intensities, an effect which became clearly significant in 2017 (**Table 4**). Hejcmanová et al. (14) investigated behavioral patterns under extensive and intensive continuous grazing and found a clear trend towards longer grazing durations under intensive management. Generally, this larger effort in walking under moderate than under lenient grazing arose from longer durations of the two or three main peak activity phases per 24-h period (**Figure 3**). However, walking distances were also higher under very lenient than under lenient grazing in some periods (**Table 4**). Based on the flatter slope between grazing time and walking (**Figure 2**), this could be attributed to an increased effort in searching of foraging sites.

The mean daily walked distances in the present study ranged between 2,592 and 3,929 m. These values are in accordance with Baker (44), who described a minimum daily activity of 3,000 m on pasture. In a study by Draganova et al. (45), pregnant suckler cows walked between 2,700 and 3,300 m daily on pastures of 8–12 ha in size. Earlier reports state that the daily walking effort of cattle ranges between 2,000 and 6,000 m (22). Consequently, the daily effort in walking is in line with previously reported values (**Table 4**).

Spatial Patterns of Movement

In order to differentiate between potential reasons for differences in movement between grazing intensities, we investigated the spatial patterns of movement. As the Camargo index during the active time tended to decrease from M toward VL (**Figure 3**), we suggest that the larger variability of distribution of the short patch foraging sites is responsible for a stronger clustering in VL. The more even distribution of the animals across the paddocks in M was likely caused by the lower herbage on offer in that treatment

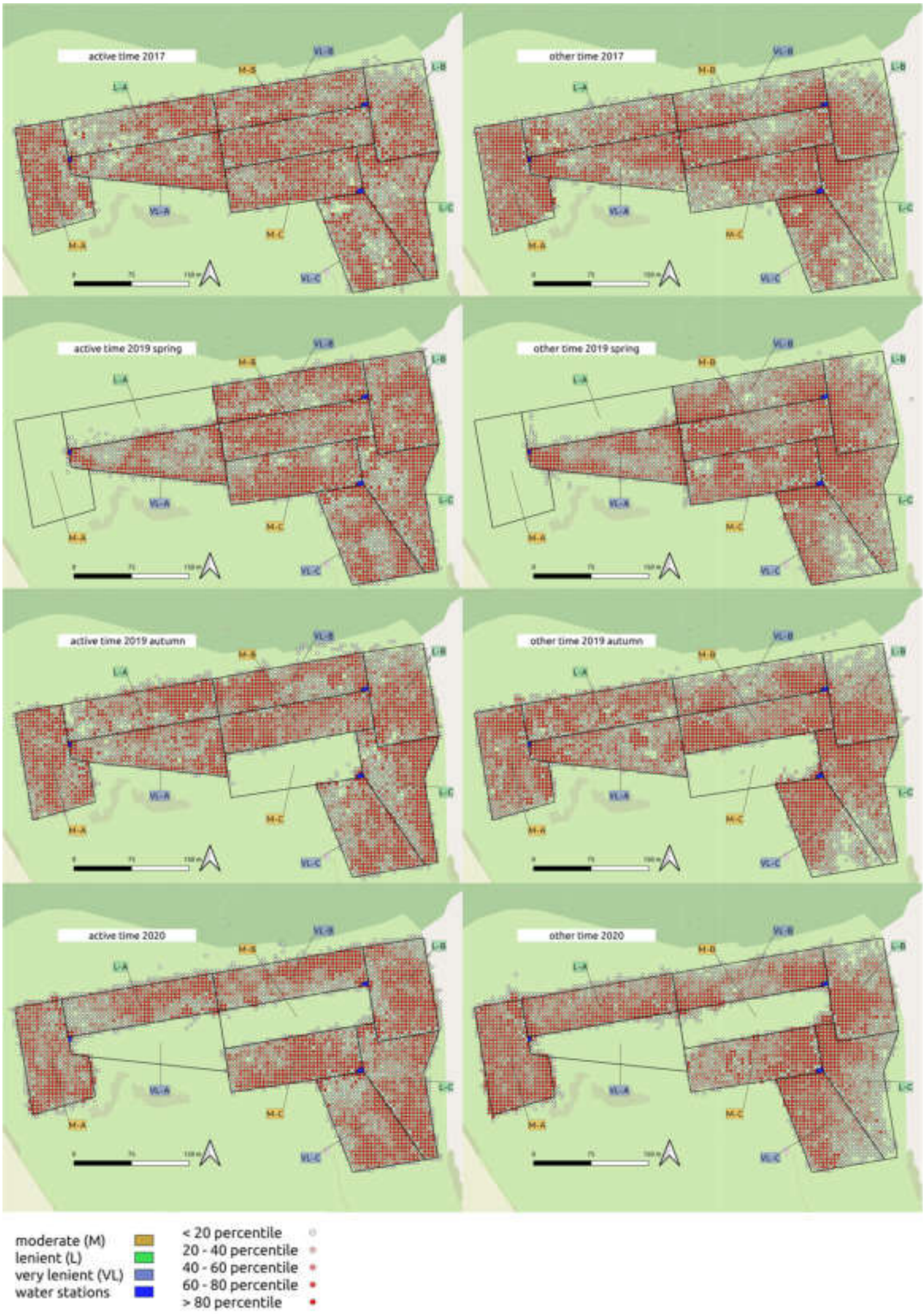

FIGURE 5 | Density maps of cattle location during active time/other time within 5 × 5 m gridcells on the experimental site.

and the resulting need to enlarge the grazing area to fulfill the dietetic demand. As described by Perotti et al. (29), in a study on the same experimental site in 2017, the botanical composition differed between short and tall patches. As indicated by Tonn et al. (7), the distribution of the patch classes is mediated by the grazing intensity with larger proportions of short areas under the moderate grazing intensity.

Heterogeneity/Homogeneity Based on the Standard Deviation of Herbage Mass

It is well established that cattle prefer leafy and digestible vegetation (46) and search actively for it. Cattle are known to develop a spatial memory of the grazing land (47). The pattern of patches seems to be the landmap of the cattle to find preferred forage spots which are repeatedly visited (48). This behavior maximizes the foraging efficiency in terms of forage intake per unit of walking distance (49). However, we found a significantly negative relationship when regressing the walking distance on the standard deviation (SD) of herbage mass as indicator of spatial heterogeneity ($P < 0.001$) (not shown). One has to take into account that the standard deviation of the herbage on offer may be misleading in terms of the actual variability in the spatial distribution of herbage as it is sensitive to the range of values (SD herbage will increase with greater herbage on offer values). Under very lenient grazing, tall avoided areas with large herbage on offer are close to shortly grazed patches with little herbage on offer (7). In contrast to this, under moderate grazing the overall amount of herbage on offer is lower and so is the SD herbage. The very lenient grazing intensity has, thus, a larger amount of unpreferred tall herbage while the moderate treatment has more valuable herbage sources at a lower amount, which both lead to a homogeneous distribution. However, both treatments have the same coefficient of variation (CV) in terms of herbage on offer (not shown). According to Pavlu et al. (31), patches differ in their forage quality and we found a decline of the paddock-scale *in vitro* digestibility from M to VL. When a pasture is stocked with less cattle (as in most cases during our study in VL compared with M) one grazing patch will provide forage resources for a longer duration. Visual cues associated with disparate feed qualities are used by cattle for more efficient forage intake (50), providing evidence for the spatial memory of the grazing livestock. On the contrary, more effort in walking in the moderate grazing treatment is likely a cause of the lower productivity of short patches (10) which requires to enlarge particular grazing areas per individual under higher stocking density in line with Gibb et al. (51). The negative relationship between SD Herbage and walking effort, however, supports our assumption of two different reasons for increased movement. In M, the grazing stations (short patches) provide forage and were evenly distributed but triggered the cattle to enlarge the grazing area during grazing to fulfill the dietetic demand. In VL, the homogeneously distributed tall and mature herbage drove the movement of the cattle to find preferred forage spots.

Limitations of the Current Study and Variations Among the Periods

In the present study, only one cow per paddock was equipped with a GPS collar which might not fully reflect the potential effect of the group of grazing animals and individual differences on the grazing behavior. Yet, there is indication for the validity of the findings for the following reasons: the experimental setup provides true replication of the grazing intensity treatments at the paddock-level. Among years, the individuals changed between the grazing treatments. In addition, members of a group of animals usually graze simultaneously (52) while only for the resting time and the time spent for ruminating there is a higher variability among different animals within a group (53). However, we suggest that future studies should look into herd dynamics in greater detail to understand effects of the stocking density on the effort for walking.

The put-and-take system aims at maintaining sward heights close to the target values by adapting stocking densities to current herbage growth rates, resulting in a gradient of stocking rates across the whole grazing system. The precision with which these aims can be achieved at a given moment strongly depends on the variability of paddock-specific dynamics in grass growth. Sward measurements during the periods showed that the mean measured CSH in grazing intensity M was mainly close to the intended sward height of 6 cm. Measured sward heights under L and VL were close to each other despite different target sward heights (**Figure 6**). The target sward height of VL of 18 cm was not achieved during our investigation period, which means that the grazing intensities L and VL differ mainly in their herbage allowance but not in the total herbage on offer.

In the periods of 2019 autumn and 2020, the stocking densities between the grazing intensity treatments were nearly the same. Comparable stocking densities result when the actual herbage on offer requires some adjustment in the number of cows stocked per paddock in order to allow for at least 14 days of grazing, which is the rhythm of sward height measurements in the experiment. However, the treatment M is usually stocked earlier in the season so that the annual stocking rates differ clearly between treatments. Nevertheless, it cannot be excluded that the lack of differences in the walking effort between the grazing intensity treatments in 2020 resulted from the fact that the stocking densities among the treatments were the same during that period, even though herbage allowance differed. However, in a study by Dumont et al. (13), the group sizes did not affect the walking distances of individuals. Further research is necessary to prove this point.

Spatial patterns are usually analyzed in larger scale paddocks which give the livestock a higher probability of performing distinct behavioral patterns at specific places (54). Preliminary work had shown that the mean deviation of the GPS signals of the cattle collars used in the present study, were in a range between 0.6 and 1.9 m. As the collars were set to record values every 128 seconds in 2017 and 2019 spring, or every 60 seconds in 2019 autumn and 2020, this noise adds up to the hourly distances of c. 40 m recorded for the nighttime hours.

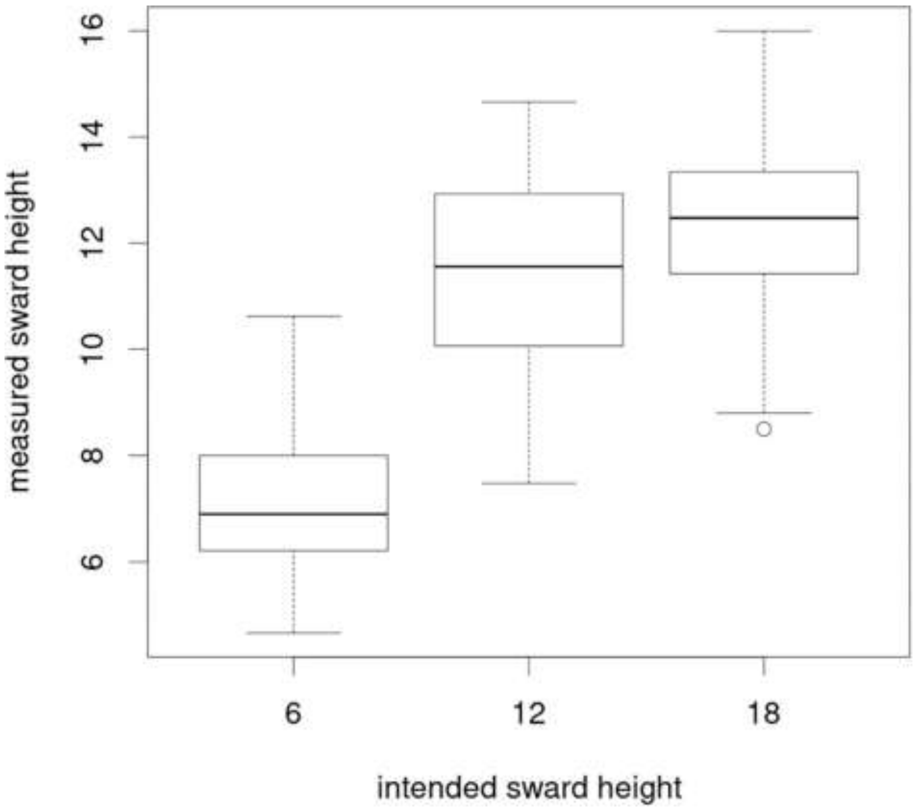

FIGURE 6 | Measured (mean of 50 measurements per paddock taken every 2 weeks) against realized sward heights per grazing intensity across all study periods.

CONCLUSION

Our hypotheses could be confirmed with the present study: (i) cattle activity increased with lower herbage allowance and (ii) the spatial distribution of cattle during active time (grazing peaks) is more even under moderate compared to lenient grazing intensity. However, in our study, cows increased their walking efforts under both the most intensive and also the least intensive grazing treatment. Thus, the herbage availability in terms of herbage allowance and also the spatial distribution (i.e., heterogeneity) of the sward have to be taken into account since all these are drivers for cattle motion. This is relevant information in order to design decision support tools in future precision livestock farming, aiming at a better balance of biodiversity and production targets of grazing systems.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study is in accordance with the German legal and ethical requirements of appropriate animal procedures. The consultation of the Institutional Animal Welfare Body is documented under no. E5/20 by the Animal Welfare Officer of the University of Goettingen.

AUTHOR CONTRIBUTIONS

JI, BT, and MK: initiation and supervision of research. DH, MK, and JI: conceptualization. JI and BT: funding acquisition. JH, MK, and BT: data acquisition incl. field measurements. DH, MK, BT, and JH: data analysis. DH (lead), MK, and NG: visualization and writing. NG, MK, JI, and BT: manuscript revision. 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.2021.639096/full#supplementary-material

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