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Higher forage:concentrate ratio and space availability may favor positive behaviors in dairy cows

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ABSTRACT

A novel livestock management system (LMS) for dairy cows mainly based on a high forage:concentrate ratio, no silage, and large outdoor paddocks, has been introduced in Italy during the last five years. It was proved that such system, further than improving the quality of milk, benefits the health status of cows. The goal of the present research was to compare the behavioral responses of a group of animals kept with the LMS and outdoor paddocks of 200 m^2, and cows reared in a traditional semi-intensive manner and outdoor paddocks of 100 m^2. The study was carried out on Italian Friesian cows analyzing the feeding and social behaviors and the locomotor activity. The statistical analysis of locomotor activity showed that the HFC group spent lower time lying down and standing and higher time walking; they also showed a longer rumination time and spent less time drinking. Furthermore, the HFC group displayed longer allogrooming and social rubbing times. Results suggest that the novel LMS could be the basis for an improvement of the cows' welfare.

Introduction

A novel livestock management system (LMS) for dairy cows, mainly based on a high forage:concentrate ratio, no silage, and large outdoor paddocks, has been introduced in Italy during the last five years. It includes several rules aimed to increase animal welfare and to improve the quality of milk as described by Musco et al. (2020). The protocol encompasses a diet forage:concentrate ratio of at least 70:30 on dry matter basis (DM) and forage with at least five different herbs, no silage at all, and free access to outdoor paddocks all along the day, with enough space to warrant freedom of movement and physical activity at the best. The novel LMS benefits cow's health, improving the animal oxidative status, as observed by the blood metabolic profile in which lower levels of reactive oxygen metabolites, higher levels of antioxidant potential, and anti-reactive oxygen metabolites were reported (Musco et al., 2020). Moreover, this LMS enhances the quality of milk, making it able to positively affect the inflammatory state, oxidative stress, and mitochondrial function in rats (Cavaliere et al., 2018; Trinchese et al., 2019).

Apart from physiological improvements, the novel LMS has not been tested for behavioral effects, while it is known that livestock management could affect behavior at several levels. For example, the feeding behavior can change according to feed quality, such as the physical and chemical composition of the diet and the dry matter intake (Miller-Cushon and DeVries, 2019). Concentrates are consumed quickly when offered alone, while they are consumed slowly using frequent meals of small quantities (Beauchemin, 2018). The diet composition can also affect dairy cows' drinking behavior. Clean water is generally accepted as essential to prevent adverse effects on animal health and performance (Beede and Collier, 1986; Murphy, 1992; LeJeune et al., 2001). Cows satisfy their water requirements even from the in-

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take of water contained in feed and from water originating from the metabolic oxidation of body tissues, whereby the dietary content is an important determinant of water intake (Khelil-Arfa et al., 2012). Diets low in dry matter provide more sources of water (Murphy, 1992), which consequently reduces water intake through drinking.

Beyond feeding behaviors, the LMS also affects locomotor activity. Given the choice between outdoor paddocks and indoor barns, cows chose to lie outdoor (Ketelaar-de Lauwere et al., 1999). Cows engage in a broader range of stationary behaviors when housed in pasture, including lying down on the side (Krohn and Munksgaard, 1993). The aptitude to adopt these positions may help to explain the cows' preference for lying down on pasture compared to the more restrictive environment of free stalls. Additionally, the ability of the cow to change from standing to lying down could be changed by comparing the access to outdoor paddock vs. indoor housing conditions (Lidfors, 1989). Conversely, periods in which cows are confined to the indoors after an outdoor period may lead to a difference in the locomotor activity displayed (Shepley et al., 2020).

The LMS can also affect social behaviors, including positive interactions that mirror friendly behaviors, as well as competitive-type interactions. Numerous studies underlined the importance of allogrooming (Wasilewski, 2003) as the most common form of social behavior (Wasilewski, 2003; Boissy et al., 2007). Allogrooming can be considered a reliable indicator of affiliative bonds among group members in cattle (Sato et al., 1993; Val-Laillet et al., 2009) and plays a role in reducing the external parasite load (Sato et al., 1993). Takeda et al. (2003) and Harris et al. (2007) have found preferential grooming between individuals in studies on feral free-ranging cows but such a preference has not been observed among intensively housed cattle (Endres and Barberg, 2007).

Allogrooming may be related to social dominance since subordinate cows groom dominant cows more often than vice versa (Fraser et al., 1990; Sato et al., 1993; Val-Laillet et al., 2009), although some authors reported opposite results (Mulleder et al., 2003). Competitive interactions between cows have been well studied and consist of multiple forms of aggressive behavior, such as shuffling, pushing, and butting (DeVries et al., 2004; von Keyserlingk et al., 2008). Many factors may influence the occurrence of these behaviors, but the housing condition plays a decisive role (Tresoldi et al., 2015). In particular, the way in which animals are housed could affect social interactions, with indoor housing providing less space and more opportunities for cattle to compete for resources. Indeed, it has been shown that reducing space availability or increasing stocking density can increase competition for feed (Val-Laillet et al., 2009; Proudfoot et al., 2009) and lying stalls (Fregonesi et al., 2007). Moreover, diet composition can affect feeding behaviors, with high forage content requiring greater time to eat (Grant and Ferraretto, 2018).

Overall, it appears that the LMS can affect the behavioral outcomes of livestock in several ways according to diet composition and space provided to cows. Therefore, in the present study, we compared the behavior of a group of animals kept with the novel

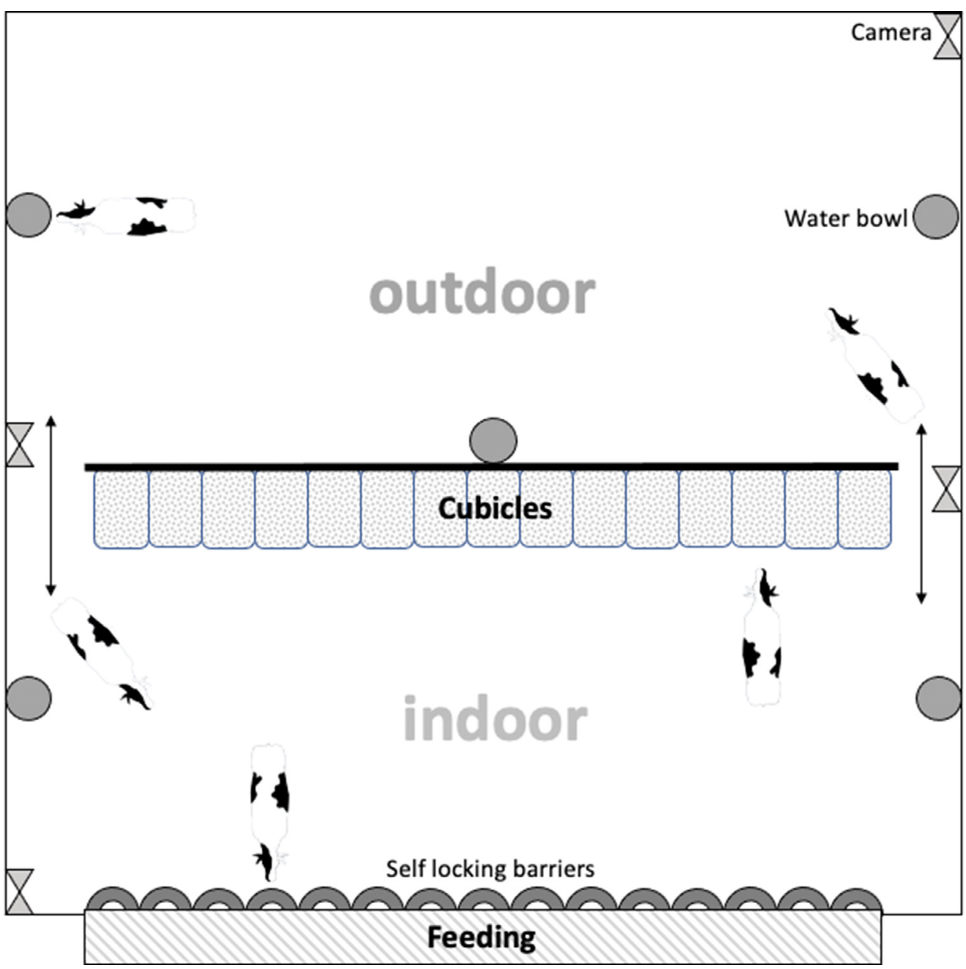

Fig 1. Plan of the barn for the LFC group. The plan for the HFC group is the same apart for the outdoor space which is the double.

Table 1
Diet ingredients, forage:concentrate ratio, and DM intake.

Diet ingredients	LFC		HFC	
	kg as fed	kg DM	kg as fed	kg DM
Corn silage	24.0	7.20	-	-
Mixed hay*	-	-	7.6	6.46
Alfalfa hay	5.0	4.25	9.8	8.33
Wheat bran	1.3	1.13	1.2	1.04
Corn meal	4.0	3.48	3.1	2.68
Triticale	1.9	1.65	1.0	0.87
Faba bean	-	-	2.0	1.74
Sunflower panel	1.7	1.48	-	-
Soybean e.s.	2.0	1.74	-	-
Forage:Concentrate ratio. *DM basis*		55:45		70:30

**Vicia sativa. Avena sativa. Lolium multiflorum. Trifolium alexandrinum. Trifolium squarrosum.*
LFC: lower forage:concentrate ratio; HFC: higher forage:concentrate ratio.

Table 2
Dry matter (%), chemical composition (g/kg DM) and nutritive value (MJ/kg DM) of the two diets.

Chemical composition	Group LFC	Group HFC
DM	53.6	85.6
CP	147.0	141.0
EE	29.7	18.2
NDF	351.0	468.0
ADF	212.0	367.0
ADL	80.2	107.3
Starch	129.0	93.8
Ash	57.1	77.0
NEl	6.3	5.8

DM: dry matter. CP: crude protein. EE: ether extract. NDF: neutral detergent fibre. ADF: acid detergent fibre. ADL: acid detergent lignin. NEl: net energy for the lactation.
LFC: lower forage:concentrate ratio; HFC: higher forage:concentrate ratio.

LMS (Rubino, 2014) and cows kept in a traditional semi-intensive manner. To this scope, locomotor activity, feeding, and social behaviors were analyzed to assess whether the rules provided by this novel LMS were able to affect animal behavior in a significant way. Besides the advantageous effect of the novel LMS on cow's health (Musco et al., 2020), we hypotesized that this system could be indicative of an improvement of dairy cows' welfare.

Materials and Methods

Animals and diets

The study was carried out on Italian Friesian cows, on a farm located in a hilly area of Centre Italy (Segni, Rome, Italy; longitude 13°00E, latitude 41°410N, altitude 668 m above sea level). The farm produces two types of commercial milk (lower forage milk and higher forage milk) from animals fed as semi-intensive (forage:concentrate 55:45 DM basis) or the feeding strategy described above (i.e. forage:concentrate ratio of at least 70:30 DM basis and forage with at least five different herbs; no silage at all), respectively. We compared the behavioral outcomes of cows kept with the traditional and the novel LMS (Rubino et al., 2014). For the trial, two groups (high forage, HFC and low forage, LFC) of 15 dairy cows each were taken into consideration; the groups were homogenous for parities (HFC: 3.56 ± 0.87; LFC: 3.45 ± 0.59), days in milk (HFC: 86 ± 48.0; LFC: 91 ± 53.5 days) and average daily milk yield (25.6 ± 3.2 and 26.1 ± 2.0 kg/day for HFC and LFC, respectively). They were fed two diets (3.5 kg DM/100 kg of live weight) as total mixed ration of which ingredients and forage:concentrate ratio are reported in Table 1. According to the feeding strategy, the HFC diet included five herbs: *Vicia sativa*, *Avena sativa*, *Lolium multiflorum*, *Trifolium alexandrinum*, and *Trifolium squarrosum*.

Samples of both diets were collected and analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and ash according to AOAC (2005) procedures. Structural carbohydrate fractions were also determined as described by Van Soest et al. (1991). Starch content was analyzed with polarimetric detection (Polax L, Atago – Tokyo, Japan) as indicated by Martillotti et al. (1987). The nutritive value was calculated as reported by NRC (2001). The chemical composition and the nutritive value of diets are reported in Table 2.

The groups were housed in barns with outdoor access of 100 and 200 m^2, for LFC and HFC respectively, with five automatic water bowls each. Apart the outdoor space, the indoor space (concrete solid floor) and the outdoor areas (ground) characteristics were the same (6 m^2 /head). The indoor area was enriched with individual metal tubes partitioned cubicles, one for each animal. The bedding material of cubicles was straw based directly on the base of the stall. The feed was provided indoor warranting a linear space of 75 cm/head. Self locking barriers allowed each animal to eat correctly (Figure 1).

Refusals were weighed daily, and group feed intake was calculated as the difference between offered and residual feed. For three weeks, a 24 h recording was performed every monday by using four AP-320S C&Xanadu cameras, thus, three 24 h recordings were obtained. During the recording, apart from people normally involved in milking and feeding, nobody had access to the animals to avoid interfering with their normal behavioral repertoire. Recordings for each group were watched by an expert observer and analyzed for the behaviors detailed in Table 3. For each animal, the duration of the above-mentioned behavioral repertoire was measured over 24 hours. A second operator codified half of the videos and the interobserver reliability was very high in all observed behaviors, ranging from 88 to 96%. Milk yield was registered the last three days of the trial. Representative individual milk samples (300 mL, obtained pondering milk yield at the two daily milkings, at 0500 hours and 1600 hours) were collected each day and analysed for fat, protein, and lactose by using the MilkoScan FT 6000 (Foss Electric A/S, Hillerød, Denmark).

Statistical analyses

The data were collected 3 times once a week and the averages values from the three samplings were used for the statistic comparisons. The Kolmogorov-Smirnov Test reported a not normal distribution for three sets of data (standing, $p=0.01$; drinking, $p=0.03$; allogrooming, $p=0.04$), thus we chose a non-parametric approach by using the Mann-Whitney U test. The p-values were adjusted according to the multiple testing (13 pairwise comparisons according to the number of behaviors monitored).

Milk data were analysed by one-way ANOVA (JMP software version 11; SAS Institute, Cary, NC, USA).

Results

During the trial, non-significant differences in milk yield (16.2±3.3 vs. 14.8±3.7 for LFC and HFC, respectively) and dry matter intake (19.6±1.8 vs. 21.4±1.2 kg/DM) were observed. Also, milk composition did not differ between the groups (data not shown). Concerning the behavioural repertoire the statistical analysis reported several differences among groups (Table 4).

Table 3
Behavioral repertoire explored during the trial.

Behavioral categories	Behaviors	Description
Locomotory activity	Lying	Positioned with either flank in contact with the ground
	Standing	Positioned with all four feet on the ground
	Walking	Moving with 2-3 feet on the ground in a four-beat gait in activities unrelated to other behaviors
	Trotting	Moving with 2 feet on the ground in a two-beat gait in activities unrelated to other behaviors
	Sleeping	Lying down with head resting against the side of the body
Feeding behaviors	Eating	Head through the head gate at the feed bunk. The head can be up or down in the bunk. Feed intake, chewing or ruminating need not be observed
	Rumination	Masticating away from the feed bunk
	Drinking	The time spent at the drinking trough while the cows were clearly engaged in water ingestion.
Social behaviors	Selfgrooming	Two cows non-aggressively pushing head/body against each other without intent to mount or groom
	Allogrooming	Cow licking /being licked by another cow
	Social rubbing	Rubbing head-on/being rubbed on by the head of another cow
	Play	Two cows non-aggressively pushing head/body against each other without intent to mount or groom
	Submission/avoidance	Moves away from the aggressive behavior of another cow

Shepley et al., (2020)

Table 4
Statistic values. LFC: lower forage:concentrate ratio; HFC: higher forage:concentrate ratio.

Behavioral categories	Behaviors	HFC median	LFC median	Mann-Whitney U	Z	p
Locomotory activity	Lying	67	74	43	-2.89	0.039
	Standing	52	58	20.5	-3.84	0.002
	Walking	29	26	45	-2.81	0.05
	Trotting	40	36	49.5	-2.63	0.1
	Sleeping	301	385	1	-4.63	<0.001
Feeding behaviors	Eating	200	180	53	-2.47	0.169
	Rumination	437	365	0	-4.67	<0.001
	Drinking	24	33	11	-4.23	<0.001
Social behaviors	Selfgrooming	59	66	64	-2.02	0.585
	Allogrooming	44	38	16.5	-4.00	<0.001
	Social rubbing	54	43	0	-4.68	<0.001
	Play	25	30	51.5	-2.55	0.13
	Submission/avoidance	37	43	58	-2.27	0.299

Particularly, the locomotory activity showed that the HFC group performed significantly lower lying down (U=43, z=-2.89, p=0.039), standing (U=20.5, z=-3.84; p=0.002), and sleeping times (U=1, z=-4.63, p<0.001), while spending more time walking (U=45, z=-2.81, p=0.05) (Figure 2).

Concerning the feeding behaviors, the HFC group showed a significantly longer rumination (U=0, z=-4.67, p<0.001), and shorter drinking (U=11, z=-4.23, p<0.001) times (Figure 2).

About social behaviors, the allogrooming (U=16.5, z=-4.00, p<0.001) and the social rubbing (U=0, z=-4.68, p<0.001) were significantly longer in the HFC group (Figure 4).

Discussion

The results of the present study showed that the behavioral repertoire of dairy cows was differently affected by the novel LMS (Rubino, 2014) as compared with traditional livestock rearing.

We observed that the HFC group was more dynamic, spending more time walking and less time in stationary behaviors (i.e., lying down, standing, and sleeping). The time spent in locomotory behaviors is influenced by the access to the outdoor (Charlton et al., 2013) and paddocks outdoors provide more space for the cows to move freely, thus increasing leg condition and improving the cow's locomotory ability (Hernandez-Mendo et al., 2007). Indeed, exercise has a positive effect on the welfare of dairy cattle (Crump et al., 2019). Thus, the novel LMS, by favoring locomotory behaviors, may improve the welfare of dairy cows. It should be underlined that, in compliance with the rules of the novel LMS, more outdoor space has been reserved for the HFC group; therefore, our data do not allow assessing the relative influence of diet or space in conditioning locomotory activity. The novel LMS affected cows feeding behaviors differently from the traditional one, with the HFC group showing a longer rumination time, while spending less time drinking. This is not surprising since it is well known that diets containing high forage content require greater time to eat (Grant and Ferraretto, 2018). Notwithstanding the higher moisture content of HFC diet, these results agree with previous observation reporting that the proportion of concentrate is positively correlated with the amount of water intake (Khelil-Arfa et al., 2012). Enhanced rumination times raise salivary secretion (Maekawa et al., 2002; Jiang et al., 2017) and less salivary secretion is associated with concentrate, rather than with forage (Beauchemin et al., 2008). Consequently, the feeding management of the novel LMS (Rubino, 2014), could help to reduce the risk of Sub-Acute Ruminal Acidosis by promoting rumination in dairy cows (Beauchemin et al., 2018).

HFC group showed higher allogrooming and social rubbing times. In a study comparing the social behaviors of dairy heifers housed in either a free-stall barn or allowed to access pastures, it was shown that the animals exhibited a 4-fold few social interactions in pastures (Tresoldi et al., 2015). This outcome leads the authors to conclude that the lower space was responsible for both the increased positive (i.e., allogrooming) and negative (i.e., agonistic interactions) social interaction. On the other hand, an increase in space allowance reduces the social interactions between cows, thereby additional outdoor spaces foresee a decline in social interactions (Smid et al., 2020). Therefore, the larger space available for the HFC group in our study should have given theoretically less op-

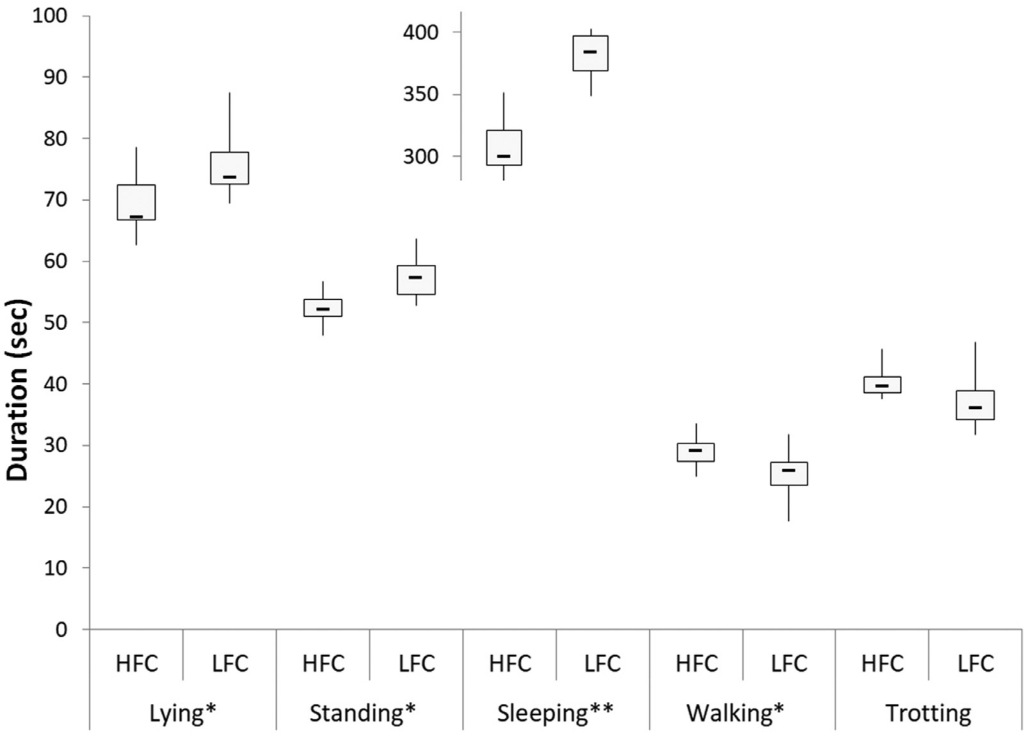

Fig 2. Graphical representation of locomotory behaviors. Bold horizontal lines: medians; gray boxes: quartiles; thin vertical lines: minimum and maximum values. Sleeping is on a different scale. LFC: lower forage:concentrate ratio; HFC: higher forage:concentrate ratio. $^{*}p \leq 0.05$; $^{**}p < 0.001$

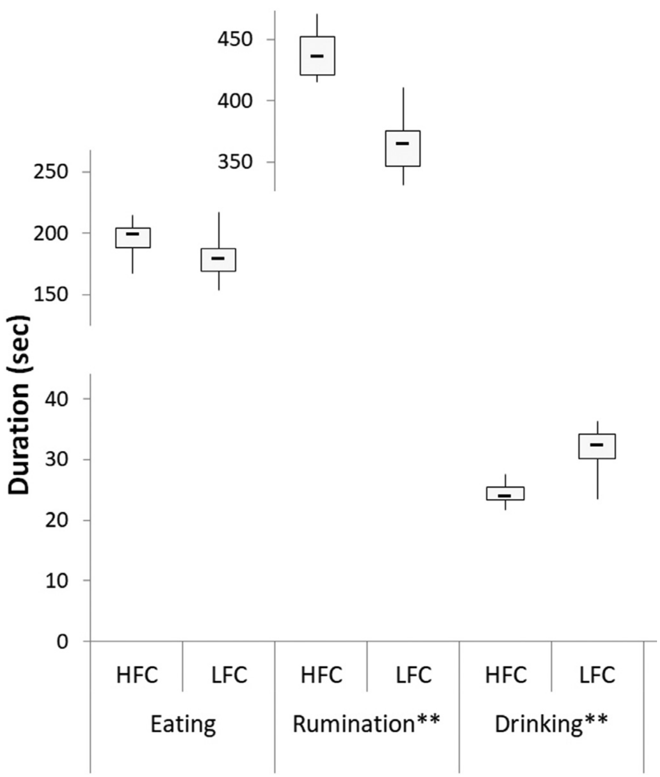

Fig 3. Graphical representation of feeding behaviors. Bold horizontal lines: medians; gray boxes: quartiles; thin vertical lines: minimum and maximum values. Rumination and drinking are on different scales. LFC: lower forage:concentrate ratio; HFC: higher forage:concentrate ratio. $^{**}p < 0.001$

portunity for social contacts with respect to the LFC group. Instead, positive social interactions were longer lasting in the HFC group, while no statistical differences were encountered for negative interactions. These observations make more likely an effect of the diet rather than the space provided to cows. Allogrooming is an important behavioral pattern, with functional significance for the formation and maintenance of social bonds and it reflects friendly interactions between ungulates (Boissy et al., 2007). Social grooming is also used as a coping strategy aimed to reduce social tension and might have a cohesion role in balancing positive and negative social interactions (Val-Laillet et al., 2009). Therefore, it seems that the novel LMS improves the social interaction in dairy cows and that this effect is more likely related to the diet.

The livestock system often cares about production and economics, but behavioral implications underpinning animal welfare are often poorly explored. The results of this study showed that the novel LMS for dairy cows significantly affected animal behavior. In addition, this study showed the positive effect of a higher forage:concentrates ratio in the diet. Moreover, beneficial outcomes were also observed on locomotor activity and affiliative social behaviors.

In conclusion, this study underlines the beneficial effects of the LMS and suggests that behavioral studies should be included when exploring new feeding and/or livestock strategies. Moreover, these results could help to improve animal welfare guidelines by taking into account alimentary and social needs in LMS.

While providing new insights on husbandry practices, this study presents some limitations. It was performed on a single group of cows in each treatment and thus it should be interpreted with caution. Further research, e.g., by extending the range of farms adopting the novel LMS, will be necessary to better understand the impact of such management on the behavior in dairy cows and to validate our findings.

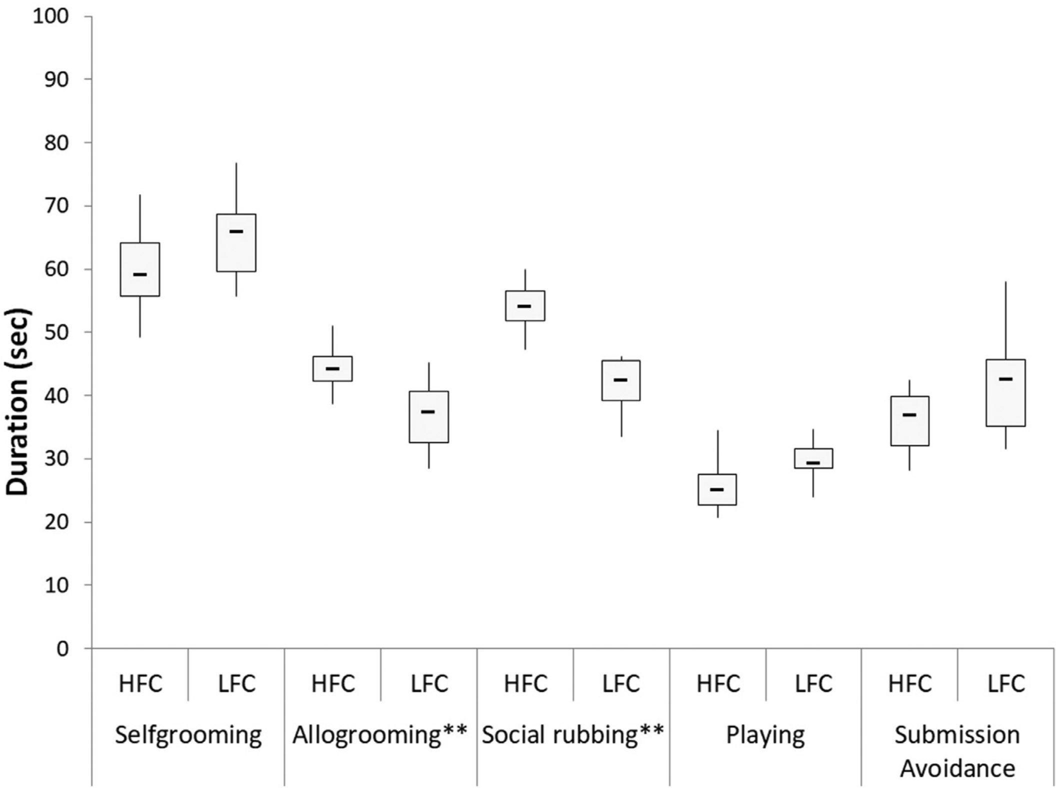

Fig 4. Graphical representation of social behaviors. Bold horizontal lines: medians; gray boxes: quartiles; thin vertical lines: minimum and maximum values. LFC: lower forage:concentrate ratio; HFC: higher forage:concentrate ratio. $^{**}p<0.001$

Ethical Statement

We have not manipulated the animals but only recorded their behaviors. According to the definition of "animal experiments" by the currently operating Italian law, our observational study on dairy cows behaviors is not considered an animal experiment and did not require any special permission.

Declaration of Competing Interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Vincenzo Mastellone: Methodology, Investigation. **Nadia Musco:** Methodology, Data curation, Writing – original draft, Writing – review & editing. **Federico Infascelli:** Conceptualization, Writing – original draft, Supervision. **Anna Scandurra:** Validation. **Biagio D'Aniello:** Validation, Writing – original draft. **Maria Elena Pero:** Formal analysis. **Piera Iommelli:** Formal analysis. **Raffaella Tudisco:** Methodology, Formal analysis, Investigation. **Pietro Lombardi:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision.

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