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Valutazione del repertorio comportamentale di bovine di razza Pezzata Rossa Italiana durante la permanenza in un recinto esterno alla struttura di stabulazione e gli effetti sulla produzione di latte

Evaluation of the ethogram of Italian Simmental dairy cows during the activity in an outdoor fence and the effects on milk production

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Enjoy the butterflies,
enjoy being naive,
enjoy the nerves, the pressure.
If you want to stand on the top from day one,
then there's nothing else to look forward to.
Enjoy the process of making a name for yourself
and meeting some great people along the way.
There's a lot of worldly people who you can laugh with,
learn from them, enjoy some moments with so,
embrace the good ones.
Stay focused.
Don't veer too far off your path.
Keep trying to build and grow and learn from yourself.
But don't forget what got you here.
Bring friends along.
Bring family along.
They are also excited to be on this journey as well.

Daniel Ricciardo

INDEX

RIASSUNTO

Il benessere e la salute degli animali possono essere migliorati anche fornendo loro la capacità di svolgere un adeguato esercizio fisico in un ambiente più naturale. L'intensificazione della produzione di latte, destinato al consumo e alla trasformazione, ha portato ad un aumento dell'uso di sistemi di stabulazione al chiuso più restrittivi, più comunemente sistemi a stabulazione libera, e una costante riduzione di quelli a stabulazione fissa, con un conseguente abbandono dell'uso del pascolo. Il pascolo permette alle bovine di esprimere comportamenti specie-specifici e di prevenire varie malattie o dismetabolie, così come rilevato dal Farm Animal Welfare Council nel 2009. Poiché la disponibilità di superficie da adibire a pascolo in Italia è piuttosto scarsa, una possibile alternativa potrebbe essere l'utilizzazione da parte delle bovine di aree o paddock esterni alla stalla per favorire la ginnastica funzionale.

L'obiettivo del presente studio è stato quello di osservare il repertorio comportamentale di 10 bovine in lattazione di razza Pezzata Rossa Italiana, distinte in due gruppi sperimentali omogenei, che in due periodi diversi (U2A: dal 30 gennaio al 12 febbraio 2023 e U2B: dal 13 al 26 febbraio 2023) hanno svolto per 2 ore attività fisica in un recinto esterno, di superficie pari a 1500 m^2. È stato inoltre valutato se lo spostamento nell'area esterna adiacente potesse influenzare la produzione di latte e la sua composizione. Le bovine selezionate erano dotate di un sistema di attivometria convalidato (Lely Astronaut A4, Maassluis, Paesi Bassi), presente nell'allevamento di bovine da latte in cui è stato condotto lo studio (La Fattoria di Pavia, Udine, Italia).

I comportamenti espressi dalle bovine sono stati monitorati quotidianamente grazie all'installazione di sei telecamere a circuito chiuso, ma anche grazie all'uso del sistema di attivometria installato nel software del robot di mungitura che registrava il livello di attività delle bovine su base giornaliera. Al termine della prova sono state osservate in totale 240 ore di registrazione. I campioni di latte per ogni bovina sono stati raccolti prima dell'inizio della prova e al termine di ogni periodo sperimentale di 2 settimane ciascuno. I campioni sono stati poi analizzati in laboratorio presso il Dipartimento DAFNAE dell'Università di Padova.

I risultati del presente studio indicano che lo spostamento delle bovine in un recinto esterno a quello di stabulazione ha degli effetti positivi sia sul comportamento degli animali sia sulla produzione di latte. L'espressione comportamentale è un fattore fortemente influenzato dall'individuo, che in questo modo esprime il proprio temperamento. Le 5 bovine del gruppo U2A hanno espresso con maggiore durata comportamenti di movimento (camminare e correre) e di esplorazione dell'ambiente circostante. Le bovine del gruppo U2B, invece, hanno trascorso più tempo a pascolare (anche se l'erba era stata sfalciata prima dell'inizio della prova) e a

interagire tra conspecifici. L'attività di ruminazione durante le uscite di due ore è stata mediamente di circa 11 minuti superiore rispetto a quando sono rimaste nella stalla, ma hanno parzialmente recuperato (in media 4 minuti) nelle due ore successive al rientro in stalla. Nessuna correlazione è stata evidenziata tra il tempo di ruminazione monitorato dal robot di mungitura e le osservazioni in vivo.

Il tempo trascorso a mangiare in corsia di alimentazione e l'assunzione di mangime concentrato sono stati fortemente influenzati dall'individuo. L'attività all'aperto ha diminuito il tempo trascorso a mangiare in corsia di alimentazione per le bovine del gruppo U2B, in quanto hanno trascorso più tempo a pascolare nel recinto esterno, mentre lo ha aumentato per le bovine U2A. Inoltre, l'attività all'aperto ha aumentato la produzione di latte (2.62 ± 0.43 kg in più per la bovina 970; 1.68 ± 0.43 kg per la bovina 990) e il numero di mungiture (0.18 ± 0.18 volte per la bovina 970; 0.27 ± 0.18 volte per la bovina 990; 0.15 ± 0.18 volte per la bovina 992) per le bovine U2B. Sorprendentemente, le bovine che hanno pascolato in quantità maggiore durante le uscite e che hanno ingerito una minore quantità di mangime concentrato sono state quelle che hanno registrato un maggiore aumento della produzione di latte e un più alto contenuto di proteine e di caseina, nel latte stesso.

Infine, come atteso il contenuto di urea è risultato più elevato nel latte prodotto dalle bovine durante le uscite poiché hanno ingerito una maggiore quantità di erba fresca durante la permanenza nel recinto esterno, rispetto a quando sono rimaste in stalla.

Dai risultati di questa prova sperimentale emerge come l'attività fisica svolta per due ore al giorno in un recinto esterno alla stalla nei primi mesi dell'anno può essere efficace per gli animali, che riducono il grado di stress a cui sono sottoposti, con effetti positivi anche sulla produzione e sulla qualità del latte. Tuttavia, ulteriori ricerche dovranno essere per poter confermare questi risultati valutando ad esempio il maggior carico di lavoro per l'allevatore legato alla movimentazione degli animali, che potrebbe essere ridotto adottando soluzioni tecnologiche che prevedono aperture e chiusure automatiche dei cancelli. La disponibilità di superficie esterna potrebbe diventare un fattore limitante per stalle di grandi dimensioni così come i sistemi di mungitura automatica (robot), che rispetto alla tradizionale mungitura due volte al giorno possono causare alcuni problemi nella gestione delle uscite. Maggiore attenzione alla presenza di superfici ombreggiate e alla disponibilità di acqua nel recinto esterno dovrà essere inoltre posta quando le uscite avvengono nel periodo estivo.

ABSTRACT

Animal welfare and health can be improved by providing adequate exercise in a more natural environment. The intensification of milk production for human consumption and cheesemaking has led to an increase in use of more restrictive indoor housing systems, most commonly free housing systems and a constant reduction in fixed housing system with a consequent abandonment of the pasture. Grazing allows cows to express species-specific behaviours and prevent various disease or metabolic disorders, as noted by the Farm Animal Welfare Council in year 2009. Since the availability of surface area for grazing in Italy is rather scarce, a possible alternative could be the use of areas or paddocks outside the barn to encourage functional exercise.

The aim of this study was to observe the behavioural patterns of 10 Italian Simmental lactating dairy cows, divided into two homogeneous experimental groups, which in two different periods (U2A: from 30th January to 12th February2023; and U2B: from 13th to 26th February 2023) carried out physical activity for 2 hours in an outdoor fence (1500 m^2). It was also evaluated whether moving to the adjacent outdoor area could affect their milk production and composition. The selected cows were equipped with a validated monitoring system of animal activity (Lely Astronaut A4, Maassluis, Netherlands), present in the dairy farm where the study was carried out (La Fattoria di Pavia, Udine, Italy).

The behaviours expressed by the cows were monitored daily using 6 closed circuit cameras but also through the use of the detection system installed in the milking robot software (AMS) which recorded the activity level of the cows on a daily basis. At the end of the trial, a total of 240 hours of recording was observed. Milk samples were collected for each cow before the beginning of the trial and at the end of each experimental period (2 weeks). The samples were then analysed in laboratory of the DAFNAE Department (University of Padua).

The results of the present study indicate that moving cows to an outdoor fence has positive effects on both animal behaviour and milk production. Behaviour is a factor strongly influenced by the individual, who in this way expresses the temperament. The 5 cows in the U2A group exhibited movement behaviours (walking and running) and environmental exploration for a longer duration. The cows in the U2B group spent more time grazing even if the grass has been cut before the beginning of the trial and interacting with conspecifics. Rumination activity during the 2-hours outings was on average about 11 minutes higher then when they stayed inside the barn, but they partially recovered (on average 4 minutes) in the two hours following their return to the barn. No correlation was found between the rumination time monitored by the AMS and the observations.

The time spent eating in the feeding lane and the intake of compound feed is affected by the cow. Outdoor activity decreased the time spent eating in the feeding lane for the cows of U2B group, as they spent more time grazing on the outside while it increased for the U2A cows. Furthermore, outdoor activity increased milk production (2.62 ± 0.43 kg for cow 970; 1.68 ± 0.43 kg more for cow 990) and the number of milkings (0.18 ± 0.18 times for cow 970; 0.27 ± 0.18 times for cow 990; 0.15 ± 0.18 times for cow 992) for U2B cows. Surprisingly, a higher increase in milk production and a higher protein and casein contents of milk were observed by the cows that spent more time grazing and ingested a lower amount of compound feed. Finally, as expected, the urea content was higher in the milk produced by the cows during the outings because they ingested a greater quantity of fresh grass during their stay in the external fence, compared to when they remained in the stable.

The findings of this experiment underscore that engaging in physical activity for two hours daily in an open space adjacent to the stable, particularly in the initial months of the year, proves beneficial for the animals. This practice demonstrates the potential to enhance their welfare without detrimental impacts on milk production and quality. Nevertheless, additional investigations are imperative to validate these results. Factors such as the increased workload for the farmer associated with animal movement should be explored further, with consideration given to potential technological solutions like automated gate systems. The extension of outdoor space may pose challenges for larger stables and automatic milking systems (robots), introducing potential complications compared to the conventional twice-daily milking routine. Additionally, careful attention should be directed towards ensuring the presence of shaded areas and access to water in the external enclosure, particularly during summer outings.

ABBREVIATIONS

AA = Afternoon Milking

AMS = Automatic Milking System

CCTV camera = Closed Circuit Television camera

CTR = Control Group

CTRL = housing continuously without any access to the outdoors

DIM = Days In Milk

EX = Exercise paddock

G1 = First group of cows (986-997-978-946-881)

G2 = Second group of cows (990-995-992-970-948)

MA = Morning Milking

NWA = Non-Walking Activity Group

PAST = housing with free-choice pasture access during the dry period

PROD = Production pasture

TMR = Total Mixed Ratio

U2 = Treatment Group (exit for 2 hours in the exercise paddock)

U2A = Treatment Group A (from 30th January to 12th February2023)

U2B = Treatment Group B (from 13th to 26th February 2023)

WA = Walking Activity Group

1. INTRODUCTION

1.1. Dairy cows' welfare and well-being

In recent years, consumer concern about animal welfare and well-being has been increasing dramatically. Pasture-based systems certainly have positive effects on welfare and health of dairy cows, including the ability to express their natural ethogram, such as socialization and environmental exploration, and reduce the incidence of some pathologies, especially lameness.

The growing global demand for milk has led to the need to increase the feed efficiency of high-producing cows. Intensification in the dairy industry has been characterized by an increase of indoor confinement and cows reach almost twice their milk production in the last 50 years, needing larger amount of high-energy feed. Indoor confinement has also had unforeseen consequences, such as the increase of a variety of health problems, the reduction of behavioural expression and the decrease in fitness resulting in a shorter lifespan (Shepley et al., 2020a). This contrasts with what Hughes (1976) states about animal welfare and well-being defining it as the state of physical and mental health in which animals are in harmony with the environment.

For this reason, particular attention is invested to allow the access for animals to areas outside the barn to ensure adequate exercise with direct effects on their health and well-being (Welfare Quality ®, 2009). In the welfare assessment of dairy cows, it is suggested to give the possibility for adult cows to access to an outside paddock with a total surface area of 4-5 m^2/cow or to a pasture provided with adequate shelter for at least 60 days (CReNBA, 2018).

Recently, the Royal Society for the Prevention Cruelty to Animal published a document regarding the welfare standards for the dairy cows where it states that cows should have access to pasture for at least 4 hours per day, and ideally 6 hours per day. The guidelines suggest giving 1 acre for 12 cows (RSPCA, 2021). Pasture-based systems are a sharp contrast to these indoor housing systems. Research shows that pasture and outdoor access have positive influences on cows, including the expression of more natural behaviours such as socialization and environmental exploration (Loberg et al., 2004) and it also reduces the incidence of health issues such as lameness (Hernandez-Mendo et al., 2007).

However, this is not a feasible option for all producers because of different reasons including geographic location, land availability and forage quality (Shepley et al., 2020a). Besides, suitable grazing surfaces are not always available to farmers and alternative solutions must be investigated, such as the possibility of providing access to areas outside the barn during the day. The sizes of the external surface and the length of stay of the cows during the day should be investigated to ensure good movement opportunities for the animals.

The indoor housing systems (tie-stall or free-stall), the characteristics of the outdoor area (size, type of flooring or grass, presence of trees or shaded surfaces), the duration of time on the paddock and the frequency of access, the type of outdoor access (free or controlled by the farmer), the milking system (manual or automatic system) and other factors affect the benefits of the external access of the cows, as reported by Shepley et al. (2020a).

1.1.1 Relationship between cow welfare and locomotion activity

One of the "Five Freedoms" proposed by the Farm Animal Welfare Council in Great Britain is that animals should be free to express normal behaviour (Kilgour, 2012), preventing animal from health issues and from a reduced lifespan (Robbins et al., 2019).

Cows at pasture spend a large proportion of their time engaged in three main behaviours: grazing, ruminating, and resting and these make up 90-95% of an animal's day. Generally grazing, which is performed during the hours of daylight, is the most common followed by ruminating and resting. The grater part of rumination occurs while animals are lying rather than standing and ruminating and resting occur mostly at night than the day. Peaks of grazing activity are presented during sunrise and sunset. Besides, cows at pasture systems propose walking behaviour frequently (Kilgour, 2012).

Access to pasture in fact can solve lameness problems (Shepley et al., 2020a), offering comfortable lying and locomotion options for dairy cows (Alsaaod et al., 2022).

Cow lameness (hoof lesions, limb lesions, or locomotor deficiency) is a key factor for reduced performance on many farms, impacting both animal welfare and livestock productivity. The timely detection of lameness is important for providing effective and inexpensive treatment and for preventing future ailments. Painful disorders in the locomotor system results in the animal modifying its gait and posture to minimise pain, which is observed as impaired motion, or non-standard gair or posture. Lameness restricts locomotion and movement and leads to reduced milk production, lower fertility, higher culling rates (Qiao et al., 2021), and raising the "*days open*" (Shepley et al., 2020a). As Qiao et al. (2021) reported, lameness is typically found in between 10% and 30% of the herd, depending on the production system (pastures and barns) and farm management.

Cow behaviour mainly refers to the animals' interaction with the environment and the way they express themselves, so it a valuable indicator in assessing the health and welfare of animals (Qiao et al., 2021), which is in fact compromised in indoor housed cows (Alsaaod et al., 2022). Qiao et al. (2021) reported that the main bovine activity can be categorized into grazing, ruminating, lying, or resting, and walking (as presented by Kilgour, 2020), but also exploring, grooming, mounting, standing, and aggressive behaviour (Table 1). Measuring and assessing the behaviour of livestock is important as it can be used to indicate their pain feeling, lameness, and welfare status. When animals are ill, their behaviour changes include a decrease in exploratory activity, reproductive activity, food and water intake, grooming, and other social behaviours. Hence, monitoring and analysing changes in behavioural activity could provide useful information for timely management decisions to optimise animal performance and welfare (Qiao et al., 2021). Lying is one of the most important behavioural parameters of dairy cows and it can be used as indicator of cow and barnl comfort. Inadequate lying and prolonged standing time increase the likelihood of lameness developing in dairy cow (Alsaaod et al., 2022).

Table 1. Main cow behaviour descriptions (Qiao et al., 2021).

Behaviour	**Description**
Grazing	Head is placed in or over feed or pasture, while cow searches, chew or sorts the feed (silage) or pasture
Ruminating	The cow regurgitates feed, or swallows masticated feed and regurgitates it
Lying	The cow lies in any position except flat on its side
Walking	The position of the body and four legs changes, with the head and neck not moving
Exploring	Head is in close proximity to or in contact with the ground, using the nose to detect smells or food
Grooming	Turns head towards abdomen with a stretched neck, using their tongue to groom the body
Mounting	Animal climbs on any part of the body or head of another animal
Standing	The cow stands on all four legs with its head erect and without swinging its head from side to side
Aggressive behaviour	Causes actual or potential harm (e.g., threat) to other animals

When outdoor access is involved, the duration of its application (e.g., number of h/d) and the frequency of access (e.g., number of d/wk) can make a considerable difference on the efficiency of increasing locomotor activity. The housing and the stage of lactation have an influence on the duration of the application of exercise treatment. Positive benefits are also related to both duration and frequency of access. Higher frequency of access to the outdoors at shorter duration has the potential to be more conductive to the improvement of issues that may require longer healing times. When outdoor access is provided continuously (pasture system) there is a general increase of the benefits because pasture provides more spaces and more opportunity for the cow to move freely, compared to exercise yards. Grazing is the main behaviour which is presented on pasture, and it is strictly correlated to the walking behaviour (Shepley et al., 2020a).

In the study proposed by Shepley et al. (2020b), the number of steps walked by cows was found to be higher when cows could access to pasture more often, indicating a grater motivation for movement and resulting in higher locomotor activity. Also, the increase in locomotor activity for those cows that were found to access pasture more often may have stemmed from frustration behaviours from the indoor housing areas. According to Keeling and Jensen (2002), frustration is the likely by-product of inhibiting an animal's ability to perform the behaviours that it is motivated to perform, such as movement on pasture in the current study. It is plausible to envisage that cows that visit pasture more frequently may have increased frustration at the level of restriction the indoor housing areas impose on their movement opportunity. So, the increasing step activity is the product of this frustration (Shepley et al., 2020b).

1.1.2 Daily activity and time budget of dairy cows

Essentially, the 24h time budget represents the net response of a cow to her environment. Deviations in any herd from these benchmarked behavioural routines represent departures from natural behaviour and can serve as a basis for estimating the performance and economic loss due to poor management strategies.

Table 2 illustrates a simplified daily time budget for lactating dairy cows in free-stall environment. It shows how cows need at least 12 to 14 h to rest/lay. It is demonstrated that cows have a very strong motivation to rest, and that this motivation to rest increases as the length of rest deprivation becomes grater. Cows have a definite requirement for resting (lying down) that they attempt to achieve, even if it means giving up some feeding time.

A key concept is that feeding and resting behaviour are linked in dairy cow, in fact if resting behaviour is somehow reduced, feeding behaviour decreases as well (Grant, 2007).

Table 2. Typical daily time budget for lactating cows in free-stall environment (Grant, 2007).

Activity	Time devoted to activity per day
Eating	3 to 5 h (9 to 14 meals/d)
Lying/resting	12 to 14 h
Social interactions	2 to 3 h
Ruminating	7 to 10 h
Drinking	30 min
Outside pen (milking, travel time)	2.5 to 3.5 h

If access to pasture is guarantee, over the whole 24 h period cows spend 7 to 13 h grazing. Generally, the amount of grazing during the daylight is greater than that during the dark. The average duration of rumination over 24 h range from 4 to 10 h and the greater part of this behaviour is performed lying down and at night than during the day. During the hours of daylight resting time range from 2 to 3.5 h (tending to rest in standing position rather than lying) and over 24 h the average time spent resting range from 4 to 10 h. Walking behaviour over 24 h range from 0.2 to 3 h and the greater amount of walking occur during the hours of daylight (Kilgour, 2012).

Arachichige et al. (2013) investigated time budgets of dairy cows offered a restricted pasture allowance, approximately 14 kg DM/cow/day. Lying behaviour was expressed of an average of 10.4 h, grazing for 5.2 h, and rumination for 8.7 h. A part of grazing behaviour, results obtained about lying and rumination don't deviate from what Grant, 2007 represent for cows housed in free stall.

Another study, conducted by Alsaaod et al. (2022), aimed to measure the locomotion activity of healthy dairy cows kept on traditional mountain summer pastures, using validated accelerometers, compared to cows kept on typical modern cubicle housing systems. The study reports that cows at mountain pasture spent 528.1 min (8.8 h) lying, 836.7 min (13.9 h) standing and 75.6 min (1.3 h) walking during the 24-hour time budget. Mountain pastured cows have a higher locomotor activity level in comparison to cubicle housed cows and lying markedly less than 12 hours per day seems to represent the normal behaviour of pastured cows (based on 48 hours measurements).

Tucker et al. (2019) also reports that at pasture the average of lying behaviour range from 6.1 to 12,1 h per day, instead free stall housed cows who lay/rest from 8.7 to 13.5 h per day.

An obvious biological important behaviour that occurs when cattle are lying down is sleeping. Research in this area is affected and limited by our ability to quantify different types of sleeping. As previously reported, the other main activity that cows engage in while lying down is rumination. The lying time of cows is affected by many housing and management factors and a reduction on lying time can have deleterious consequences for animal welfare (Tucker et al., 2019).

1.1.3 Effects of physical activity on milk production and milk composition

Current housed indoor cows have limited series of walking activity except for dairy farm with sufficient size of pasture or outdoor yard (Dong-Hyun et al., 2018). Lack of access to pasture can be a threat to welfare and health of dairy cows because of documented positive effects of grazing such as improved leg health, lower prevalence of mastitis and a decrease of stereotypes and aggression in the herd (Kilgour, 2012).

Walking activity is essential to sustain life-supporting actions such as feed searching and eating, avoidance of aggressive herd mates, seeking shelter, reproductive behaviour and so on. However, is known that walking constitutes a major activity for cows that increases nutritional requirements and reduces milk yield. Lack of exercise in fact results in decreased reproduction and health. The problem is that preparations for daily movement or walking activity of cows have a contrary tendency with farmer's economic or labour-saving management, although most farmers know and admit that cows are good at walking exercise steadily (Dong-Hyun et al., 2018).

The study carried out by Dong-Hyun et al. (2018) aimed to investigate the effect of short-distance walking activity on milk production in lactating dairy cows. No significant differences in milk yield and composition were found between Walking Activity group (WA) and Non-Walking Activity (NWA) group, even though it is known that walking might influence production because walking is an activity that requires the expenditure of energy. Least Square meansobtained from the analysis of variance (ANOVA) are reported in Table 3.

No significant effects on milk yield were found also in the trial carried out by Neave et al. (2021), when cows walked greater distances. In this trial only grazing time and ruminating time increased.

Table 3. Milk production and milk composition in lactating dairy cows (Dong-Hyun et al., 2018).

Item	NWA[2]	WA[3]	SEM	p-value
Milk yield (kg/d)	33.65	32.06	7.097	0.204
FPCM (kg/d)[1]	34.47	33.81	4.778	0.472
Milk fat (%)	3.94	3.86	0.504	0.591
Milk protein (%)	3.22	3.30	0.275	0.292
Lactose (%)	4.83	4.74	0.237	0.164
Total solids (%)	12.7	12.6	0.719	0.674

[1] Fat and protein corrected milk (FPCM) was calculated by milk yield x 0.337 + 0.116 x Fat % + 0.06 x Protein %.

[2] NWA: Non-Walking Activity

[3] WA: Walking Activity

Other trials have shown that not only milk yield could be influenced by long walks, but also milk Somatic Cells Count (SCC) is also affected, and this effect may precipitate important economic consequences when the price of milk is set in consideration of SCC. The aim of the study carried out by Coulon et al., 1998, was to analyse the effect of repeated walking on milk yield, chemical composition, and SCC in lactating cows. The trial resulted in lowered milk yield, altered milk composition, and raised SCC, but these consequences are highly dependent on the initial characteristics of the cow (e.g., infection status and udder conformation). Coulon and Pradel (1997) reported also that these effects are influenced when dairy cows walked more than 12.8 km, instead in the study of D'Hour et al. (1994) where milk yield and chemical composition were only reduced when distances greater than 6.4 km were walked.

On the contrary, milk yield based was higher for cows on an exercise paddock than for cows on pasture at night. Kismul et al. (2019), demonstrated that milk yield proved to be 1.4 kg/d higher for exercise paddock cows than production pasture cows.

As previously said, cows require 12 to 14 hours per day of rest. Benefits of resting include potentially grater milk synthesis due to greater blood flow through the udder, grater blood flow to the gravid uterus during late lactation, increased rumination effectiveness, less stress on the hoof and less lameness, less fatigue stress, and grater feed intake. Each additional one hour of resting time translates into 2 to 3.5 more pounds (0.91 to 1.59 kg) of milk per cow daily, as reported by Grant (2007).

1.1.4 - Behavioural observation: approaches and technologies

Approaches

The behaviour expressed by cow is a phenotype and it includes all the processes by which animal organisms respond to internal factors and external stimuli. It represents the set of reactions expressed by the individual in response to stimuli from the environment with which it interacts and to which it must adapt. A behaviour is defined as "*normal*" when the set of activities carried out have as their objective the achievement of a particular end or the satisfaction of a need (primary or secondary need). On the contrary, a behaviour is defined as "*abnormal*" when the set of activities proposed by the animal do not aim to a specific end or need.

The study of behaviour must meet the criteria of scientific inquiry. Therefore, it is important to classify behaviours into two main categories: "*state*" behaviours (to rest, rumination, to eat) and "*event*" behaviours (defecation, urination, negative interaction), as their duration is different.

Behaviours are studied in relation to their units of measurement:

- *Duration*: more used for "state" behaviours (sec, min, or h). It represents the interval time in which a behavioural module is proposed.
- *Frequency*: more used for "event" behaviours. It represents the number of times a behaviour occurs in the unit of time.
- *Latency*: it represents the time between two specific events or the time interval between a specific event and the occurrence of the behaviour (sec, min, or h).
- *Intensity*: studied defining different levels for each species-specific behaviour.

To be able to observe the species-specific behaviours the study must be set up before it starts. It is important to define how many and which animals should participate to the trial and then to define which behaviours are important for the study observing the temporal and spatial distribution. Also, behaviours must be categorized, and the observation methods need to be defined:

- *Direct observation*: carried out in site, in vivo.
- *Indirect observation*: carried out using a video recording system.

In the end, behaviour can be observed:

- *Ad libitum*: general observation of all behaviours without limits and predetermined patterns.
- *In continuous*: continuous observation of certain behaviours as often as they occur.
- *Timed samples*: one/zero, or instantaneous, or scan sampling (Birolo, 2023).

Technologies

In order to base welfare assessment of dairy cows on real-time measurements, integration of valid and reliable precision livestock farming (PLF) technologies is needed (Stygar et al., 2021).

The primary goal of a sensor system is to improve animal management as the dairy farming has become a highly specialized activity, but it can be used to obtain more data regarding behavioural observations.

In the systematic literature review conducted by Stygar et al. (2021), the highest validation rate was found for system based on accelerometers, while the lower rates were obtained for camera, load cells, miscellaneous milk sensos, and boluses. Validated traits concerned animal activity, feeding and drinking behaviour, physical condition, and health of animals. Non-active behaviour (lying and standing) and rumination were the most often validated as high performance tools. Regarding active behaviour (e.g., walking), lower performance of tools was reported. Also, tools used for physical condition (e.g., body condition scoring) and health evaluation (e.g., mastitis detection) were classified in lower performance group. The precision and accuracy of feeding and drinking assessment varied depending on measured trait and used sensor. Regarding relevance for animal-based welfare assessment, several validated technologies had application for good health (e.g., milk quality sensors) and good feeding (e.g., load cells, accelerometers). Accelerometers-based systems have also practical relevance to assess good housing.

One of the most used sensor systems used is the identification and localizations of animals, as reported by Gastaldo (2016), and it can be divided into:

- *Signal readers*: these are systems based on electromagnetic waves, useful for analysing animal behaviour.
- *Image analysers*: these are system based on the analysis of images taken by cameras with recognition of framed objects (animals). They are particularly useful for specific analyses of certain animal behaviours (rest, activity, positioning in different functional zones) and for automatic assessments of body weight, Body Condition Score, and podiatric injuries.

Other sensor systems refer to physiological and productive parameters and can be divided into:

- *Detectors of vital functions*: among these the most popular are activity monitoring systems (foot, collar, ear, etc.), recently implemented with detectors of animal posture (standing or lying down). Recently, rumination sensors are commercially offered for which they boast promising performance.
- *Detectors of milk characteristics*: among the various devices, the most interesting and promising is undoubtedly the system based on near-infrared spectroscopy (NIRs), which provides a wide variety of important indicators (Gastaldo, 2016).

As previously mentioned, monitoring animal behaviour is critical for measuring animal welfare as well as successful herd management, particularly in extensive grazing systems. In this regard, ongoing automated behaviour analysis is a critical task since farmer-to-animal interaction is likely to be less frequent than in indoor breeding systems. The combined systems, which have GPS (Global Positioning System) and accelerometers, allows monitoring of the activities of grazing animals in a more complete way, compared to systems that use only one type of sensor. GPS collars can send the positions of the animals in real time. The data acquired with the accelerometer can be downloaded at the end of the tests, when the objective of the studies is to detect specific behaviours (Mancuso et al., 2023).

Recent progress towards cattle behaviour monitoring and analysis can be classified into three different categories: the first category only focuses on behaviour detection, the second category is long-term behaviour monitoring and detection, and the final category is automatic behavioural changes detection and quantification based on long-term behaviour monitoring. The most used method is the second category. Therefore, to collect behavioural phenotypic information, temporal or spatial features (e.g., velocity, acceleration, speed, shape, and contour) can be extracted from sensor data for behaviour recognition. After the extraction of features, machine learning methods can be applied to identify the cow behaviours.

Sensors that can provide information about animal behaviour can be classified as contact and non-contact ones:

- *Contact sensors*: they are usually fitted on the animal, and they mainly collect individual animal data through senso fixed on cow and recognise behaviours according to animal posture (standing or lying), behavioural activity (walking, resting, grazing, and ruminating) and geolocation. For example, ear tag, collars, and GPS.

- *Non-contact sensors*: they can continuously operate without operator involvement. They are non-invasive and non-stressful methods. Moreover, they can be adapted to different animals, in both indoor and outdoor situations, using the animals' natural features (e.g., shape, colour and movement) for monitoring their behaviours. For example, cameras.

In terms of behavioural analysis, environmental conditions are prone to be ignored. Actually, environmental conditions such as temperature, humanity, and carbon dioxide density affect the cattle's activity and motion behaviours. On the other hand, the majority of the abovementioned behaviour recognition methods require high-definition videos, which may limit their practicability in complex environments such as the low image quality of farm cameras, night, and rainy days (Qiao et al., 2021).

Zambelis et al. (2019) suggest that to optimize behaviour detection and reduce inconsistencies, precision technologies based on automated measures for activity must be developed and validated for specific housing systems.

Behavioural activity used to measure animal welfare are:

- *Locomotion*: helpful for identifying cow fertility which is characterized by an increase in walking activity.

- *Feeding*: a good indication of cow well-being since unwell cows eat less.

- *Rumination*: a crucial phase of the digestive process that is distinguished by a continual rhythmic chewing activity. Chewing action contributes to rumen pH remaining at a level optimal for microbial activity.

- *Lying*: cows can be monitored for limb abnormalities by lying for lengthy periods of time in the absence of movement (Mancuso et al., 2023).

Activity monitors, such as those created for use with automatic milking systems (AMS), have been developed to collect individual behavioural data automatically and remotely. Each cow wears a collar transponder for identification by the AMS, which can collect data on individual activity and rumination. Most AMS have sensors and software integrated into the system capable of recording various cow behaviour. Further, AMS activity monitors have been developed primarily to detect oestrus and signs of illness, emphasizing certain types of motion. (Elischer et al., 2013).

In the study carried out by Elischer et al. (2013), the aim was to validate the accuracy of outputs from AMS activity and rumination monitors compared with live cow behavioural observations and previously validated pedometers. It resulted in moderate to strong correlation between live observations and pedometers in all categories of behaviour: active, walking, standing, and lying. The activity registered with AMS sensor was accurate at reporting how much a cow moved. However, it provided no information about lying or standing behaviours, which should be quantified to have a comprehensive understanding of an individual cow's health and comfort in the environment. On the other hand, the rumination provided information on the amount of time an individual spent ruminating during the day.

1.1.5 Milk production and milk composition recordings

Milk production and milk composition can be monitored daily in every single farm which use Automatic Milking Systems as they are constructed with a near-infrared (NIR) spectroscopic sensing system. Milking robot systems are calibrated to determine the three major milk constituents (fat, protein, and lactose), but also somatic cell count (SCC) and milk urea nitrogen. NIR spectroscopic systems can be used to assess milk quality in real time in an AMS. The system can provide dairy farmers with information on milk quality and physiological condition of an individual cow and, therefore, give them feedback control for optimizing dairy farm management. By using the system, dairy farmers will be able to produce high-quality milk and precision dairy farming will be realized (Kawasaki et al., 2008).

These variable can be also monitored during the monthly veterinary control (functional control). During milking, the quantity of milk produced is recorded and a sample is taken from each individual to analyse its composition in terms of protein, fat and lactose content, covering also the somatic cells and pH, cheese index, casein content, urea. All these elements are useful to control the lactating animals in terms of both production and health (I.Z.S.Ve., 2022).

2. AIM

The present study aimed to evaluate the feasibility and effects of moving lactating Italian Simmental dairy cows from their housing facilities to an adjacent outdoor area of about 1500 m^2, for 2 hours per day, on behavioural expression, locomotor activity, and milk production and milk composition.

To achieve this aim, the behavioural repertoire proposed by cows during the outdoor activity was monitored, using a video recording system, and individual milk samples were collected and then analysed in laboratory.

The video recordings were analysed at the end of the experiment. Two hundred and forty hours of recording were observed in order to register each behaviour cows proposed during the outdoor activity.

3. MATERIAL AND METHODS

All procedures were performed according to the Directive 2010/63/UE of the European Parliament on the protection of animals used for scientific purposes. All procedures have been approved by the Ethical Committee for the care and use of experimental animals at the University of Padua (OPBA 36/2023 n° 203860 of the 16^{th} of October 2023).

3.1. Environmental description

The experiment was carried out at the dairy farm “La Fattoria di Pavia” located in Pavia di Udine (Udine, Italy). The free stall was equipped with the automatic milking system (Lely Astronaut A4, Lely, Maassluis, Netherlands).

The trial started with the first exit of the cows on January 30, 2023, and ended on February 25, 2023. The trial lasted four weeks.

An electric fence was installed near the barn to ensure the performance of the cows' activity. The total area of the pen was 1500 m^2, again defined according to the guidelines of the Royal Society for the Prevention of Cruelty to Animals (RSPCA). The grass in the outdoor area was mowed before the start of the trial to limit fresh grass intake by the cows, as the purpose of the trial was to evaluate the performance of physical activity. The enclosure area was mostly rectangular in shape, and it was totally uncovered. Tree vegetation was present in half of the outside fence.

A tank containing clean water, filled every day, was placed in the side opposite the entrance, in order to promote locomotor activity of cows (Figure 1).

Access to the pen was provided six days a week, Monday through Saturday, from 3 p.m. to 5 p.m. Cows were able to access the outdoor fence thanks to the installation of a corridor that connected the housing facilities to the pen itself. All the procedures were under the control of the staff.

All the animals received TMR (Total Mixed Ratio) based on grass, as reported in Table 4. The TMR was distributed once a day, early in the morning, and compound feed was available to cows during the access to the AMS (Automatic Milking System), and its ingestion was about 4kg/cow/day. The TMR was distributed making use of the mixer wagon, and the ration was brought closer to the feeder through the Lely Juno robot (Lely, Maassluis, Netherlands), which ensured that each cow could be able to take the ration they needed for optimal rumen health and improved growth.

Table 4. Diet composition of TMR.

INGREDIENTS	Kg (TQ)
Bended Alfalfa	11.0
Alfalfa hay	6.5
Mixed hay	5.0
Barley meal	3.0
Compound feed*	3.0
Water	3.0
Total	**31.5**

* Chemical composition (%TQ): crude protein 17%; lipids 3.80%; crude fiber 7.20%; ash 5.70%; sodium 0.34%.

Figure 1. Cows inside the outdoor fence near the tank containing clear water.

3.2. Animals and experimental design

Before the study started, ten lactating Italian Simmental dual-purpose cows were selected and then divided into two experimental groups of 5 cows each. The criteria used to identify the cows for the conduction of this trial were:

- Lactation number: mean of 3 ± 1.41.

- Lactation phase (or Days in Milk, DIM): mean of 129.20 ± 18.83 days.
- Temperament (docility).
- Health status and medical history (absence of mastitis and lameness).

These characteristics were defined in order to determine two experimental groups that were as homogeneous as possible (Table 5).

Cows were able to access the external area for one week before the trial started. This period was needed for them to adapt to the new environment.

Only two cows (cow numbers 881 and 948) manifested lameness issues during the study, and it was treated with a corrective trim of the problem area without applying a hoof block to the other claw.

Table 5. Characteristics of each cow who took part in this trial, based on the functional control of the 13th of January 2023.

Cow Number	**Group**	**Milk production (kg)**	**DIM (29th Jan. 2023)**	**Lactation number**	**Mastitis recurrence**
986	U2A	37.6	122	2	NO
997	U2A	42.8	129	2	NO
978	U2A	33.0	160	2	NO
946	U2A	33.2	130	5	NO
881	U2A	31.2	152	5	YES
990	U2B	32.0	160	2	NO
995	U2B	32.0	164	2	NO
992	U2B	27.4	174	2	NO
970	U2B	40.4	125	3	NO
948	U2B	37.6	136	5	YES

Milk production: U2A mean of 35.56 ± 4.68 kg; U2B mean of 33.88 ± 5.13 kg.

DIM: U2A mean of 138.60 ± 16.43 days; U2B mean of 151.80 ± 20.47 days.

The experimental design used in this trial followed a Latin square design (2x2). As the trial lasted four weeks, it was subdivided into two periods of two weeks each. Cows were divided into two groups following the same Latin square design: G1 and G2. In each group, cows were also subdivided into two experimental treatments: CTR (control) and U2. CTR treatment cows stayed inside the housing facilities when cows of U2 treatment went out for 2 hours in the afternoon (from 3 p.m. to 5 p.m.). For a matter of simplicity, the group of cows that went out in the first period of the trial, from 30th January 2023 to 12th February 2023, was also called U2A, whereas the group going out in the second period (13th February 2023-26th February 2023) was called U2B, as mentioned in Table 5. The experimental design used in this study is reported in Table 6.

During the trial, cows went out six day a week, from Monday to Saturday. Climate conditions did not affect the exits.

Table 6. Latin square design (2x2) used in this experimental trial.

	TREATMENT	
PERIOD	**G1**	**G2**
30th January 2023-12th February 2023	U2 (U2A)	CTR
13th February 2023-26th February 2023	CTR	U2 (U2B)

CTR: inside the housing facilities for 24 hours.

U2: went out for 2 hours per 6 days a week (from 3 p.m. to 5 p.m.); U2A: out from 30th January to 12th February; U2B: out from 13th February to 26th February

3.3. Experimental controls

Average temperature and wind data for each experimental period were recorded during the trial, assuming that weather conditions may affect the behavioural manifestation of cows (Figure 2a, 2b).

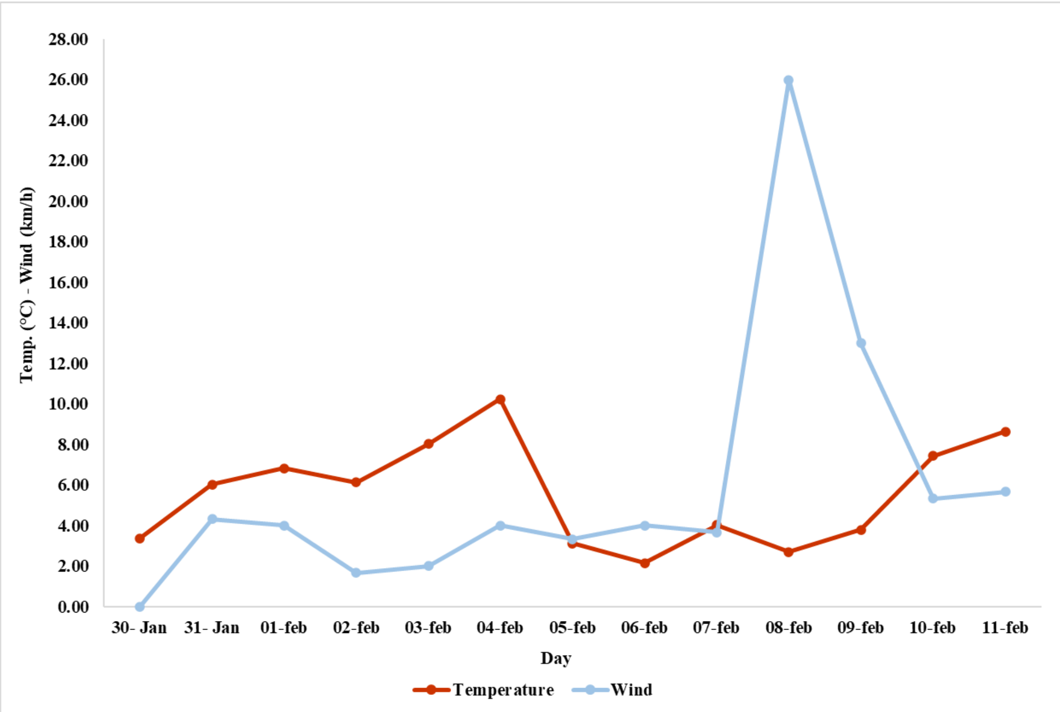

Figure 2a. Temperature and wind data recorded during the first experimental period.

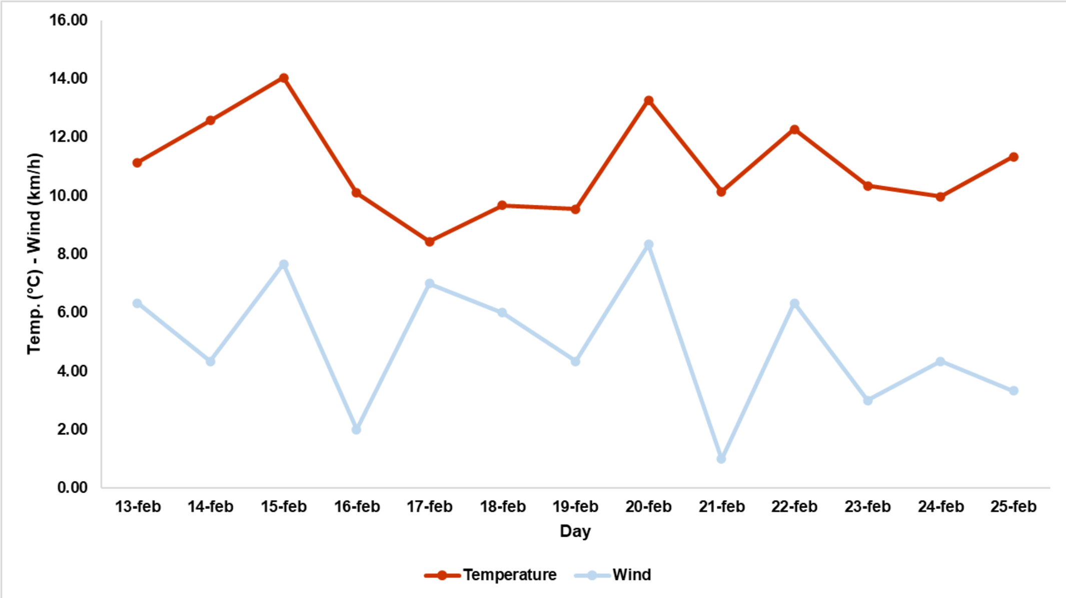

Figure 2b. Temperature and wind data recorded during second experimental period.

A sample of the TMR based on grass was taken at the beginning and at the end of the experiment, 30th January 2023, and 27th February 2023, respectively. Each sample was analysed using NIRs method at the laboratory LabCNX of the University of Padua (MAPS Department) to define the chemical composition and verify if there was any effect on animals' behaviour and milk composition (Table 7).

Table 7. TMR Chemical composition (%DM).

UNIFEED	DM	ASH	CP	EE	NDF	ADF	ADL	AIA	STARCH
30th January 2023	73.21	9.56	15.12	1.52	41.26	27.52	5.02	0.92	18.53
27th February 2023	74.95	9.45	16.81	1.27	36.26	24.39	5.13	0.5	19.2
Mean	**74.08**	**9.51**	**15.97**	**1.40**	**38.76**	**25.96**	**5.08**	**0.71**	**18.87**

Milk samples were collected from the 10 cows under test before the beginning of the trial on the 30th of January 2023, at the end of the first experimental period on the 13th of February 2023, and at the end of the second experimental period, so at the end of the experimental trial, on 27th of February 2023. Fifty ml of milk from each cow was collected directly from the AMS, with the addition of the preservative (Bronopol Tecnico, Fagron Italia, Italy) at a concentration of 0.03% (w/v) (Figure 3).

The samples were then delivered to the milk laboratory of the DAFNAE Department of the University of Padua to determine the chemical composition using MilkoScan methodology (MilkoScan FT2 infrared analyser, Foss Electric A/S, Hillerød, Denmark). The second sample, on the other hand, was also analysed by the farm veterinarian as the sampling coincided with the functional control, which occur monthly.

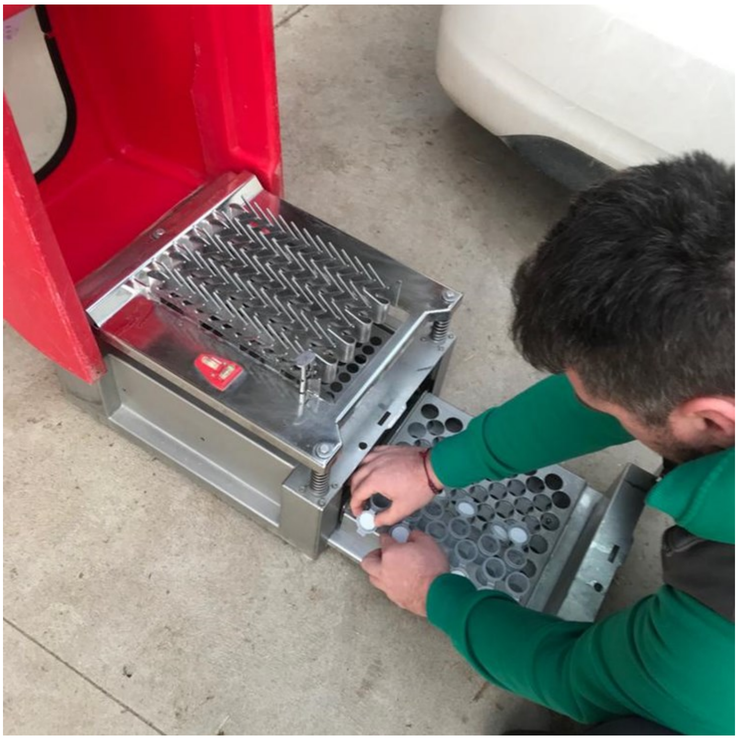

Figure 3. Sampling of the 50 ml of milk from the AMS.

As the use of cow activity monitoring system on the livestock farm makes it possible to verify the behavioural pattern of animals, based on the simultaneous detection of the duration of different activities such as rumination, standing, rest, and locomotor activity, and allows managing or reporting events and health issues of cows (oestrus, lameness or metabolic disease), the data recorded by the AMS Astronaut A4 installed in the dairy cow farm were downloaded.

Contacts with Lely technicians were necessary to obtain the data necessary to obtain the information useful for the purposes of the experiment. The Qwes system is standard in Lely Astronaut (AMS), and it converts data into actionable information to manage the health of the cows, giving a real-time overview of each animal in terms of milk production, stage, and lactation history. This gives a good overview of their activity and lets the farmer know when the intervention is needed, providing disease, and avoiding production losses. Changes in rumination are the first sign of potential problems. The Qwes system monitors this activity and alerts the farmer if such changes occur. By linking this data with milk production information provided by Lely Astronaut, it is possible to monitor overall effects on cow performance and herd appetite. The Qwes system can detect also heat detection functionality. Data are constantly sent to the AMS by the installation of individual collars to the neck of cows (Figure 4).

The registration of the activities continued even when cows where in the outside area as the antenna covered an area of 500 x 250 meters.

Figure 4. AMS and Collars used at the farm "La Fattoria di Pavia".

3.4. Behavioural observations

The behavioural patterns were indirectly observed. In order to collect the behaviours manifested by the animals during the trial, a video recording system (H264 DVR) was used, and 6 CCTV cameras (Figure 5) were installed, covering the total surface of the outside fence. As the enclosure area had a surface of about 1500 m^2 (60 x 25 m), the cameras (6) were installed on the two long sides at a distance of 30 meters from each other.

A handmade steel protection was applied to protect the cameras from bad weather conditions, avoiding restricting the view of the cameras (Figure 6).

The six CCTV cameras were installed on the 25th of January 2023, after an initial inspection on the 11th of January 2023, and then uninstalled at the end of the trial on the 27th of February 2023.

The registrations were taken from Monday to Saturday during the four weeks of the trial. The daily recording was used to start at 3.00 p.m., when the cows used to go into the outside fence, and stopped at 5.00 p.m., after two hours of outdoor activity. At the end of the study, a total of 240 hours were analysed using the Playback Software program (Figure 7), which made it possible to observe the behavioural pattern exhibited by each cow every single minute and to note it on an Excel spreadsheet using a binary method where 0 represents the absence of the expression of a specific behaviour and 1 represents the presence of a specific behaviour for each min of observation. To recognise each cow in the external area, a coloured paper circle (green, red, blue, yellow, and black) was applied to the side of the cow.

During the trial, the CCTV cameras did not register for three days: 7 February 2023, 8 February 2023, and 9 February 2023.

Figure 5. CCTV cameras used in the experimental trial.

Figure 6. Handmade steel protection used in the experimental trial.

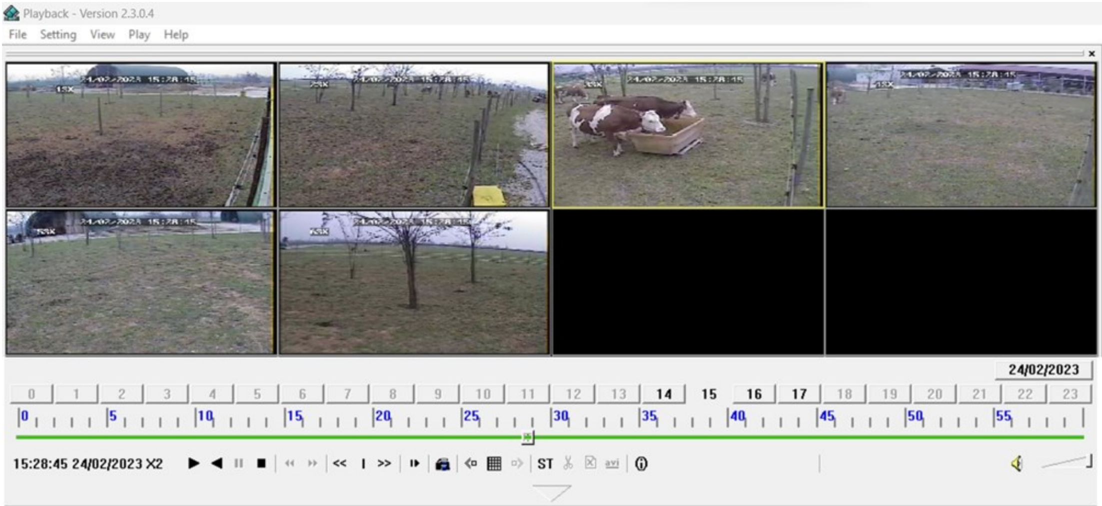

Figure 7. Visual of the *Playback Software* used to analyse the behaviour exhibited by the cows.

The observed behaviours were classified into the following categories:

- *Activity*: walking, running, standing, lying, environmental interaction, positive interaction, negative interaction, playing, sexual behaviour, selfgrooming, allogrooming, stereotypy.
- *Eating*: grazing, drinking, and rumination.
- *Excretion*: defecation and urination.
- *Nonvisible*.

The one-min individual recordings were then summed to obtain the amount of time (min) that each cow spent daily in the external fence (i.e., in the two hours spent outdoor). A total of 105 individual records for each behaviour were thus obtained for analyses.

Also, data that were recorded daily by the AMS were used for comparisons with the observed behavioural patterns. It could be possible to extrapolate data about:

- Minutes of rumination (min/hour).
- Minutes of eating behaviour (min/day).
- Total ingestion of compound feed (kg/day)
- Daily milk production (kg/day)
- Numbers of milkings (n°/day)

To better understand the differences between rumination behaviour inside the stall and in the outdoor area, two time slots were considered for the rumination: from 15:00 to 17:00, which is the same period that U2 cows spent in the outdoor fence, also recorded by the video cameras, and from 17:00 to 19:00, when both U2 and CTR cows were inside the stall.

Considering the second time slot allowed to catch possible differences between CTR and U2 thesis when both groups of animals were in the same environment (i.e., within the stall). In the first period (30th January 2023-12th February 2023), U2 cows were the animals belonging to G1; in the second period (13th February 2023 - 26th February 2023), U2 cows belonged to G2 (see also Table 6). The AMS dataset included a total of 270 individual daily records, 27 records/cows.

The AMS quantified the eating behaviour by looking at the head movements of the cows, therefore, a few false positive or false negative information could have been recorded.

The data of individual milk samples collected at the end of each experimental period (13th February 2023 and 27th February 2023), were analysed for quality components using the MilkoScanTM system (see Paragraph 3.2). MilkoScan FT2 (Foss Electric A/S, Hillerod, Denmark) is a milk analyser based on Fourier Transform Infrared (FTIR) technology. It provides a multitude of parameters for quality control. FTIR spectroscopy is already globally used to routinely assess milk composition in milk recording programs (ICAR, 2016).

In this thesis, individual milk samples were analysed for fat, protein, lactose, and casein percentages and for urea (mg/dl). All milk samples were analysed using the following methods: percent fat content according to the Weibull-Stoldt method and Soxhlet extraction (VDLUFA, 2003); percent protein content according to the method 991.20 (nitrogen total content by Kjeldahl × 6.38; AOAC International, 1995); percent lactose content by HPLC (Schuster-Wolff-Bühring et al., 2011); percent casein content according to AOAC International (1995) method no. 927.03; urea content by ISO14637/IDF 195. These milk quality data were also considered for the subsequent statistical analyses. The dataset comprised 20 records, two records/cows, and included the protein, casein, fat, lactose percentages, and the urea content in milk (mg/dl) as traits.

3.5. Statistical analysis

In this thesis, different statistical models were used to interpret the various data collected, and all models followed a general linear model analysis (GLM procedure, SAS Institute Inc., Cary NC, 2014), assuming a normal distribution of the phenotypes.

Behavioural observation

The statistical model used to process the data obtained from direct behavioural observations is as follows:

$$y_{ijk} = \mu + T_i + C(T)_j + D(T)_k + e_{ijk}$$

where:

- y= duration of each target behaviour observed in vivo (min)
- μ= general mean of the data
- T= fixed effect of the experimental period, or thesis, considered (2 levels: U2A and U2B)
- C= fixed effect of the cow within thesis (10 levels)
- D= fixed effect of the day of observation within thesis (24 levels). The days are expressed as a categorical effect to detail the behavioural variations over time

Rumination behaviour (Lely AMS)

Rumination data were analysed considering the two time slots 15.00-17.00, and 17.00-19.00, and expressing the rumination as the sum of minutes within the respective time slot. The statistical model used to process the data obtained from the rumination behaviour detected by the activity monitoring system is as follows:

$$y_{ijk} = \mu + C_i + T_j + (C*T)_{ij} + D_k + e_{ijk}$$

where:

- y= behaviour observed (rumination) (min)
- μ= general mean of the data
- C= fixed effect of the cow (10 levels)
- T= fixed effect of the thesis (or group) in relation to the time slot considered (2 levels: 15.00-17.00, and 17.00-19.00)
- $C*T$= interaction between the two fixed effects above described

- *D*= effect of the day expressed as linear covariate starting from 1, which corresponds to the 30th of January 2023
- *e*= residual error of the data

Eating behaviour, Milk production, and numbers of milkings (Lely AMS)

The AMS allowed recording individual daily data for eating, milk production, and numbers of milking (per day), which were analysed using the following statistical model:

$$y_{ijk} = \mu + C_i + T_j + (C*T)_{ij} + D_k + e_{ijk}$$

where:

- *y*= eating behaviour (min/day), total ingestion of feed compound (kg/day), milk production (kg/day), and numbers of milkings (n°/day)u
- μ= general mean of the data
- *C*= fixed effect of the cow (10 levels)
- *T*= fixed effect of the thesis (2 levels: CTR and U2)
- *C*T*= interaction between the two fixed effects above described
- *D*= effect of the day expressed as linear covariate
- *e*= residual error of the data

Milk quality (MilkoScan)

The individual milk samples collected at the end of each experimental period (13th February 2023 and 27th February 2023) were separately processed using the MilkoScan™ system to obtain milk composition (quality) information. The statistical model used to process the milk composition (quality) data is as follows:

$$y_{ijk} = \mu + T_i + S_j + C_k + e_{ijk}$$

where:

- *y*= chemical composition of the milk: protein (%), fat (%), lactose (%), casein (%), urea (mg/dl)
- μ= general mean of the data
- *T*= fixed effect of the thesis (2 levels: CTR and U2)
- *S*= fixed effect of the sample (2 levels, corresponding to the two days of sample collection)
- *C*= fixed effect of the cow (10 levels)

e= residual error of the data

As a post-hoc analysis, for all the statistical models, the Least Square meansfor the levels of the fixed effects were then computed and compared using a Student's t-test. For behavioural analysis, linear contrasts were run among the levels of the D(T) effect, to detect a possible linear variation of the behaviours over time. For all the analyses, statistical significance was considered at a level of $P \leq 0.05$, and a tendency to significance was reported for $P \leq 0.1$.

4. RESULTS

4.1. Behavioural observation

The descriptive analysis of the ethogram expressed by the cows in this trial is represented in Figure 8. It shows the time, expressed in %, that all cows in the experimental trial spent performing a given behaviour during the 2 hours of activity in the external fence.

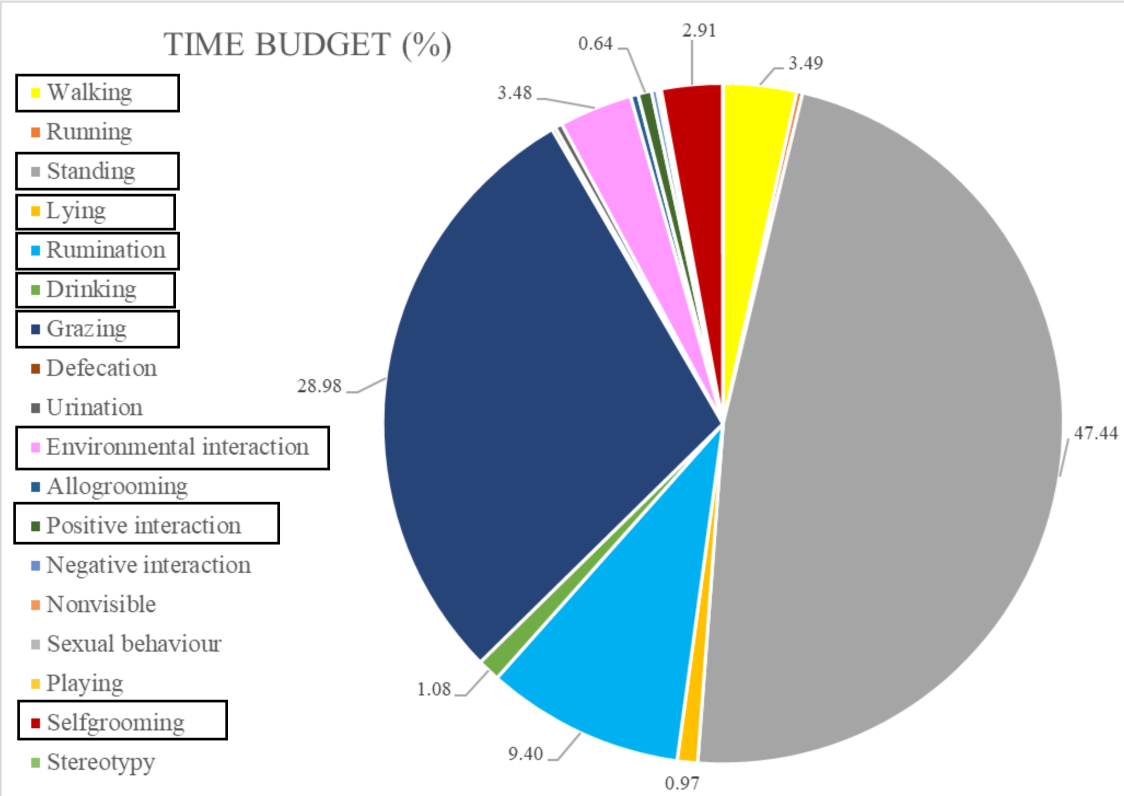

Figure 8. Time budget (%) of each behaviour expressed by cows during the 2 hours of external activity. The caption of the visible behaviours in the pie chart is enclosed within a rectangle (the other behaviours are hard to see due to the low occurrences).

For most of the time during the 2-hour outing, cows stood (47.44%). The second behaviour in terms of duration is "*grazing*" (28.98%), and the third is "*rumination*" (9.40%). The last two behaviours are closely related. Actually, the more a ruminant eats, the more it should ruminate. Cows exhibited other behaviours for brief moments during the outings.

Table 8 reported the ANOVA of the behavioural observations analysed with the first statistical model reported in paragraph 3.5, and the related fixed effect: thesis or experimental period (as they are strictly correlated), cow in the experimental period, and day in the experimental period. The F-value represents the variability of the effect on the residual variability, and the more the F-value is higher than 1, the more significant it is. The significance of the effect per each behaviour is also associated with the P-value. In the analysis of variance (ANOVA), higher F-value and lower P-value imply a strong significant effect about the considered behaviour. Instead, the coefficient of determination R^2 represents the estimation accuracy of the statistical model for that behaviour.

In this analysis there was no control group because cows were observed only outside; therefore, the so-called U2A were observed in the first period and the U2B in the second. Thus, the analysis aimed to validate the repeatability of the experimental conditions by observing the ethogram of the cows of both groups during the activity in the outdoor fence.

Walking, grazing, and standing were the most significant behaviours per effect, so there were significant differences between thesis, cows, and day of the trial. They were followed by running, environmental interaction, and positive interaction, which were significant for only two effects, so there were differences between the thesis and the days.

The other behaviours were significant for only one of the three effects, or tended to be segnificant. For example, lying behaviour was significant in relation to the cow effect, explaining that there were differences between the five cows of both treatments.

No differences betwee the two theses were found for the lasting time of defecation and urination in this experimental trial, suggesting that the activity in the external fence did not affect them.

Only one behaviour was not significant for any effect of the statistical model, and it was stereotypy. This result is relevant because the outings did not affect or maybe reduce the frequent abnormal behaviors in the housing facilities due to lower space given per animal or discomfort conditions.

Looking at the R^2, it is possible to observe that the model well explained the variability of the phenotypes for all the behaviours considered, with the lowest values of 0.316 to 0.320 for Lying, Rumination, and Drinking. Running was the behaviour better explained by the model, with an R^2 of 0.957. This high value is likely to be largely due to the Day(Thesis) effect, since this behaviour was close to zero for all the days except for the Day 8 (see Figure 10). High coefficients of determination (0.704 to 0.811) were also found for Walking, Standing, Environmental interaction, and Grazing.

Table 8. ANOVA (F-values) of the behavioural observation.

	THESIS/PERIOD	COW(THESIS)	DAY (THESIS)	R^2
BEHAVIOUR	**F-value**	**F-value**	**F-value**	
Activity				
Walking	18.90***	8.23***	5.04***	0.704
Running	179.17***	1.25	78.74***	0.957
Standing	10.48**	20.65***	3.82***	0.766
Lying	3.90°	2.18**	0.72	0.316
Environmental interaction	135.71***	0.71	8.10***	0.795
Positive interaction	30.66***	1.03	3.29***	0.572
Negative interaction	0.09	2.07*	3.77***	0.537
Playing	3.67	3.21**	1.00	0.389
Sexual behaviour	2.41	1.06	1.93*	0.384
Selfgrooming	4.23*	7.08***	2.09*	0.569
Allogrooming	0.10	1.60	1.68°	0.371
Stereotypy	1.39	1.19	1.00	
Eating				
Grazing	31.66***	27.69***	3.83***	0.811
Drinking	0.11	1.58	1.21	0.320
Rumination	5.15*	1.29	1.04	0.316
Excretion				
Defecation	0.10	0.77	2.27**	0.394
Urination	0.54	2.63*	1.16	0.365
Nonvisible	7.42**	2.00°	2.23**	0.464

°P<0.10; *P<0.05; **P<0.01; ***P<0.001

4.1.1. Period effect

Considering just the in vivo behavioural observation, animals were observed only in the external fence; therefore, no control group (followed inside) is used in this analysis. This choice is because the external observations aimed to determine which behaviours cows expressed in conditions different from housing facilities, their variation in a short period, and if different individuals expressed similar behaviours in different periods.

In this analysis, when referring to the thesis effect, or period effect, the comparison was carried out in the same experimental condition between two groups of cows consisting of 5 animals each. So, both groups were treatment (U2), but no Latin Square design was used. Both groups went out for 2 hours and for two weeks, but in two different periods: the first group from the 30th of January 2023 to the 12th of February 2023, and the second group from the 13th of February 2023 to the 25th of February 2023. These groups were defined as U2A thesis and U2B thesis, respectively.

The comparison of the behavioural aspects under the two theses is shown in Table 9. It reported the Least Square meansof the behaviours expressed as minutes daily spent (that is, in the two hours in the external fence) in each thesis.

Table 9. Least squares mean of the time (minutes per day) spent expressing each behaviour per each thesis.

	THESIS	
BEHAVIOUR (min)	**U2A**	**U2B**
Activity		
Walking	5.76[a]	3.83[b]
Running	0.84[a]	0.00[b]
Standing	68.36[a]	59.62[b]
Lying	0.11	2.18
Environmental interaction	8.87[a]	1.48[b]
Positive interaction	0.07[b]	1.45[a]
Negative interaction	0.33	0.37
Playing	0.11	-0.00
Sexual behaviour	-0.00	0.07
Selfgrooming	3.27[b]	4.35[a]
Allogrooming	0.44	0.52
Stereotypy	0.02	0.00
Eating		
Grazing	30.47[b]	44.88[a]
Drinking	1.38	1.48
Rumination	15.24[a]	10.55[b]
Excretion		
Defecation	0.22	0.25
Urination	0.40	0.48
Nonvisible	0.42[a]	0.00[b]

[a,b] Means with different superscript letters are statistically different ($P<0.05$)

Walking, running, standing, environmental interaction, positive interaction, selfgrooming, grazing, and rumination were found to be significantly different.

In particular, walking, running, standing, environmental interaction, and rumination were expressed with a significantly higher amount of time in the first thesis or period of the experimental trial. Positive interaction, selfgrooming, and grazing were manifested for a significantly longer time in the second period.

U2A cows grazed for 30.47 minutes but ruminated for 15.24 minutes; on the contrary, U2B cows grazed for 44.88 minutes and ruminated less (10.55 minutes).

It turned out that U2A cows exhibited more motion behaviours than U2B cows, which showed more eating behaviour and interaction between conspecifics.

Nonvisible behaviour was found to be statistically different due to the malfunction of the CCTV cameras during the first experimental period of the trial.

4.1.2. Day effect in the experimental period

To be able to understand how cows exhibited all the behavioural patterns during the trial, linear contrasts were studied to verify the linearity of the behaviour and its significance. The results are shown in Table 10. Also, Least Square meansof the effect of the day in the experimental period (see Figures 9-15) were carried out to explain better how the behaviours occurred over time. The results reported in Table 10 followed the ones shown in Table 9 in paragraph 4.1.1.

Table 10. Linear contrasts (P-value) of the behaviours analysed in the trial. Significant (P≤0.05) values are colored.

	LINEAR CONTRAST	
BEHAVIOUR	**U2A**	**U2B**
Activity		
Walking	<0.0001	0.0191
Running	<0.0001	1.0000
Standing	0.5539	0.0008
Lying	0.9139	0.2766
Environmental interaction	<0.0001	0.7735
Positive interaction	0.7852	0.0004
Negative interaction	0.0738	0.4544
Playing	0.5578	1.0000
Sexual behaviour	1.0000	0.0020
Selfgrooming	0.0357	0.0405
Allogrooming	0.0132	0.4004
Stereotypy	0.2363	1.0000
Eating		
Grazing	0.0558	0.0161
Drinking	0.0077	0.0790
Rumination	0.9475	0.6059
Excretion		
Defecation	0.4406	0.0412
Urination	0.7651	0.0423
Nonvisible	0.0147	1.0000

Walking and selfgrooming showed a significant linearity in both periods. U2A period showed a significant linearity for the following behaviours: running, environmental interaction, allogrooming, drinking, and nonvisible. Running, environmental interactions, and nonvisible were also statistically different in the two periods (see Table 8), and higher in U2A (see Table 9). On the other hand, significant linearity during the U2B period was found for standing, positive interaction, sexual behaviour, grazing, defecation, and urination behaviours. Among them, positive interaction and grazing were significantly higher in U2 period (see Table 9).

The Least Square meansof the behaviours found to be significant for the day(thesis) effect are reported in Figures 9 to 15). Figure 9 represents the Least Square means of the time that cows spent walking in both periods (five different cows per period) of the experimental trial during the 2-hour outings.

The highest walking time was exhibited by U2A cows on the second day of the trial, with an average of about 12 minutes, but the expression of this behaviour significantly decreased until the last day of the experimental period (see the P-value of the linear contrast in Table 10), at the end of the second week (day 13). On the contrary, U2B cows walked significantly less than U2A cows (see Table 9), but after a initial decrease after the second day of the experimental period, the time spent walking increased (about 6 minutes; see the significant linear effect in Table 10) until the last day of the trial.

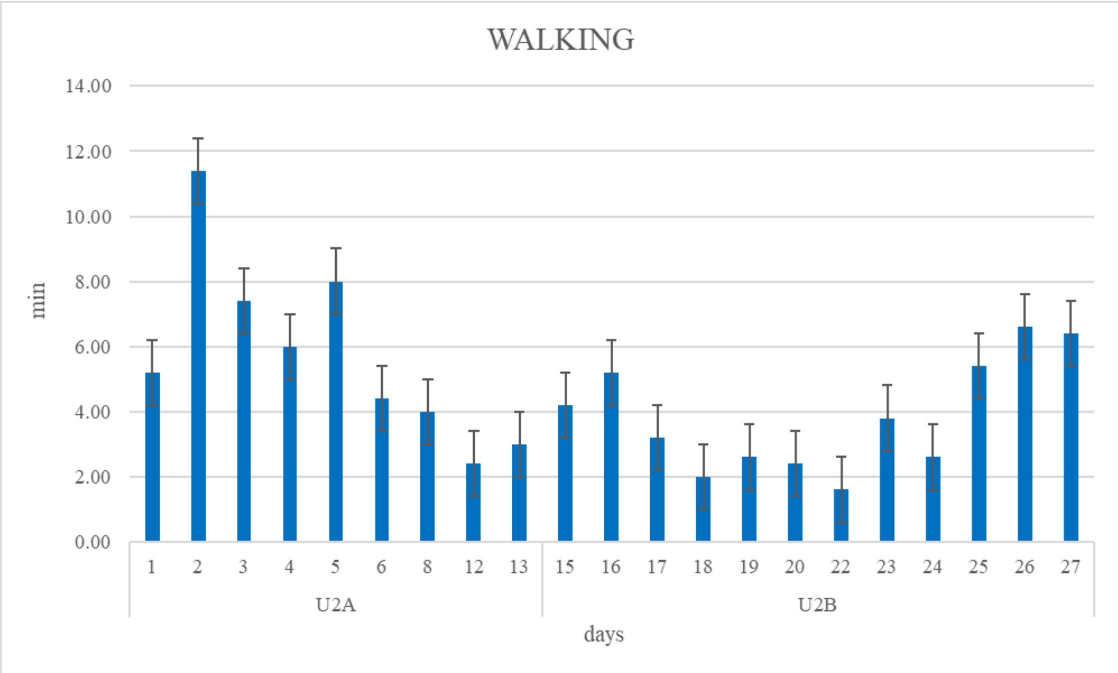

Figure 9. Least Square meansof walking behaviour considering the effect of the day in the experimental period (P<0.001).

In Figure 10 was represented the mean of the time spent running in both periods, resulting in U2A cows running for less than 1 minute in the first four days, and about 6 minutes on the eighth day of the trial. U2B cows did not run any day during the test. A significant difference in running time actually exists among the two theses (Table 9), and this strong daily variation is likely to explain the high R^2 of the model, as reported above.

Figure 11 represents the mean of the time spent standing. This behaviour is strictly correlated to walking. In fact, looking at the U2A period, in the days in which the time spent walking decreased, the time spent standing increased, with a least square mean of 68.36 minutes (Table 9) during the 2 hours of outdoor activity. Also, considering just the first day of the trial, U2A cows walked for only 5 minutes and stood about 90 minutes.

The same trend is evident in the second period. U2B cows in the first day of the trial stood for about 85 minutes, and at the end of the period, they spent less than 60 minutes standing, with a significant linear decrease as reported in Table 10.

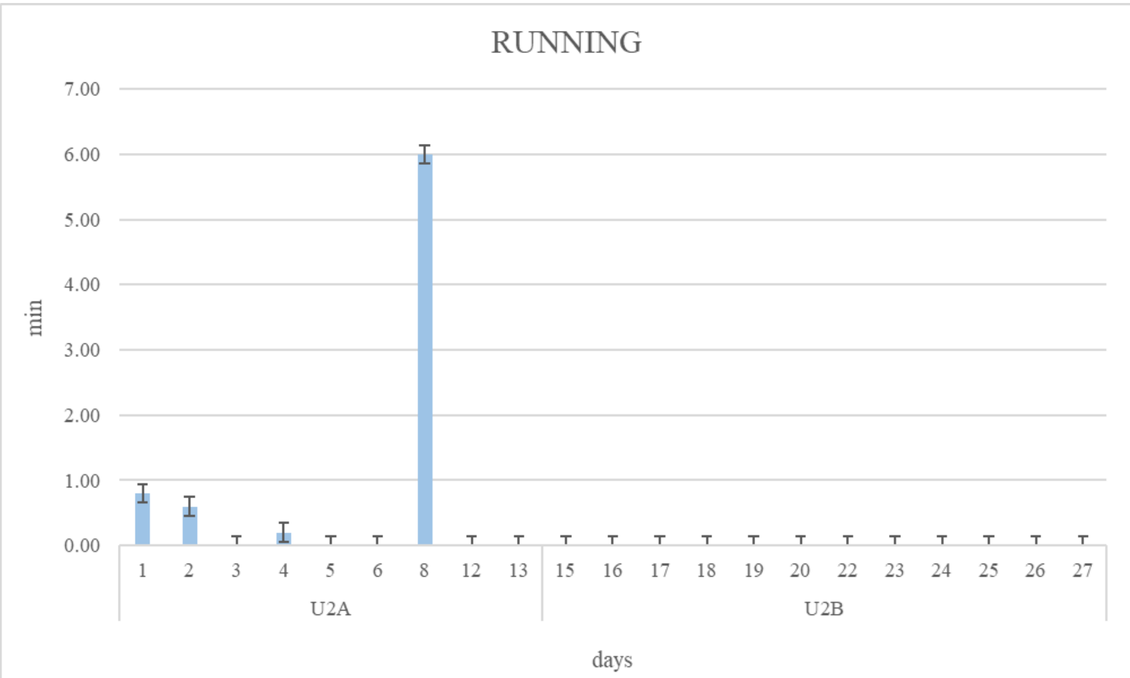

Figure 10. Least Square meansof running behaviour considering the effect of the day in the experimental period (P<0.001).

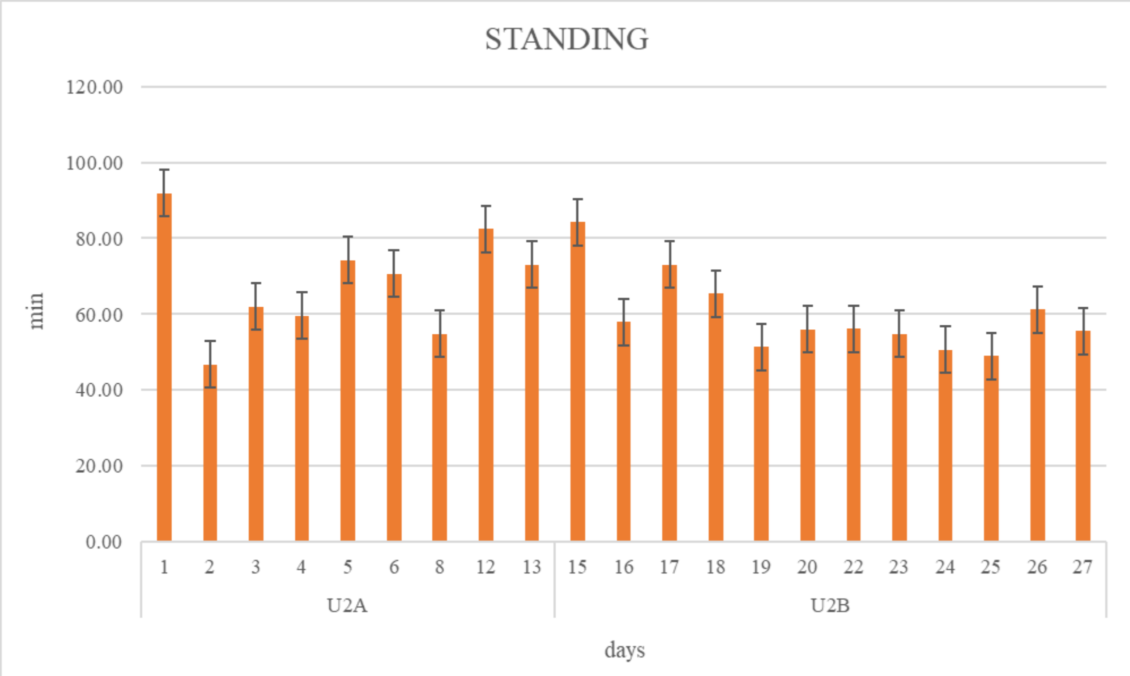

Figure 11. Least Square meansof standing behaviour considering the effect of the day in the experimental period (P<0.001).

Considering the grazing behaviour (Figure 12), a significant difference was observed between the two periods of the trial, as also reported in Table 9. U2A cows grazed less than U2B cows, which grazed for more than 50 minutes for three days of the outing period, and a least square mean of 44.88 for the whole period.

It was important to note that on the first day of the trial, cows did not graze as ot was cut before the start of the experiment. U2A cows grazed for about 30 minutes during the 2-hour outings, and U2B cows grazed for about 45 minutes (see also the Least Square meansin Table 9). Also, on the second period of the experimental trial, the time spent grazing by cows increased until the tenth day of the two weeks of the test and then decreased until the last day (see also the significant linearity of the day effect within U2B in Table 10). In the end, when cows spent more time standing, the mean of the time spent grazing was lower and vice versa.

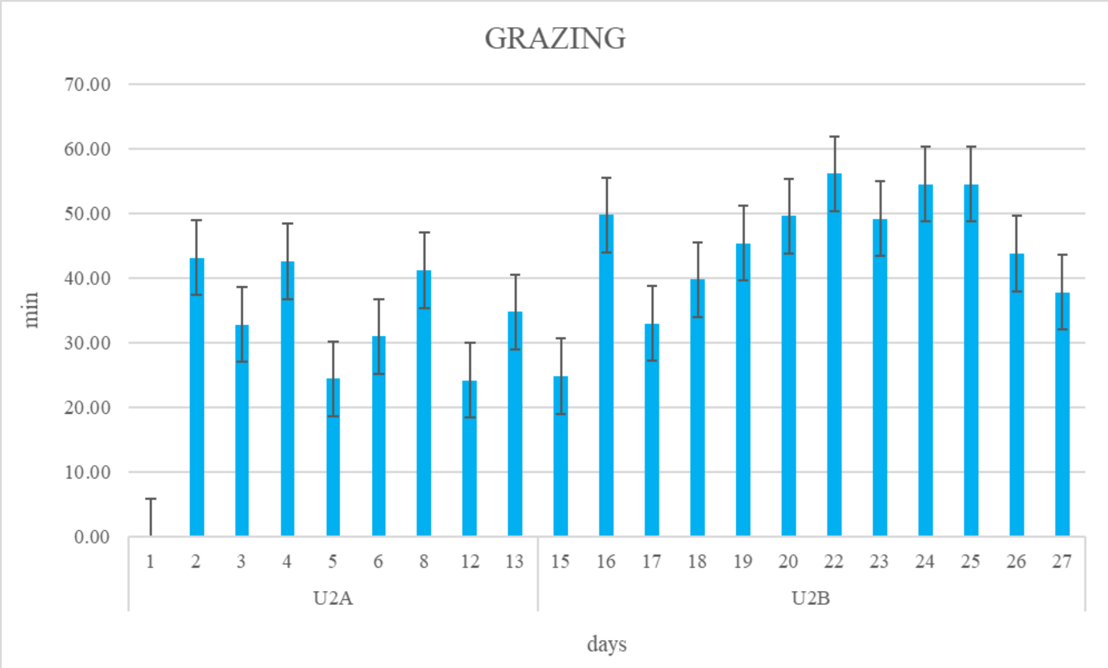

Figure 12. Least Square meansof grazing behaviour considering the effect of the day in the experimental period (P<0.001).

Comparing grazing behaviour and the environmental interaction, it was evident that on the first day of the trial, when cows did not graze, they spent about 22 minutes exploring the environment (Figure 13). In this case, U2A cows spent on average a significantly higher time (least square mean of 8.87 minutes, Table 9) exploring the environment than U2B cows, which at most explored the surrounding environment for 5 minutes.

Conversely, the cows in the first two weeks of the trial did not interact with their conspecifics. Only on the eighth day of the trial, it was carried out a mean of 6 minutes of positive interactions. On the second two weeks of the trial, cows interacted at most 4 minutes, but they expressed this behaviour daily (Figure 14).

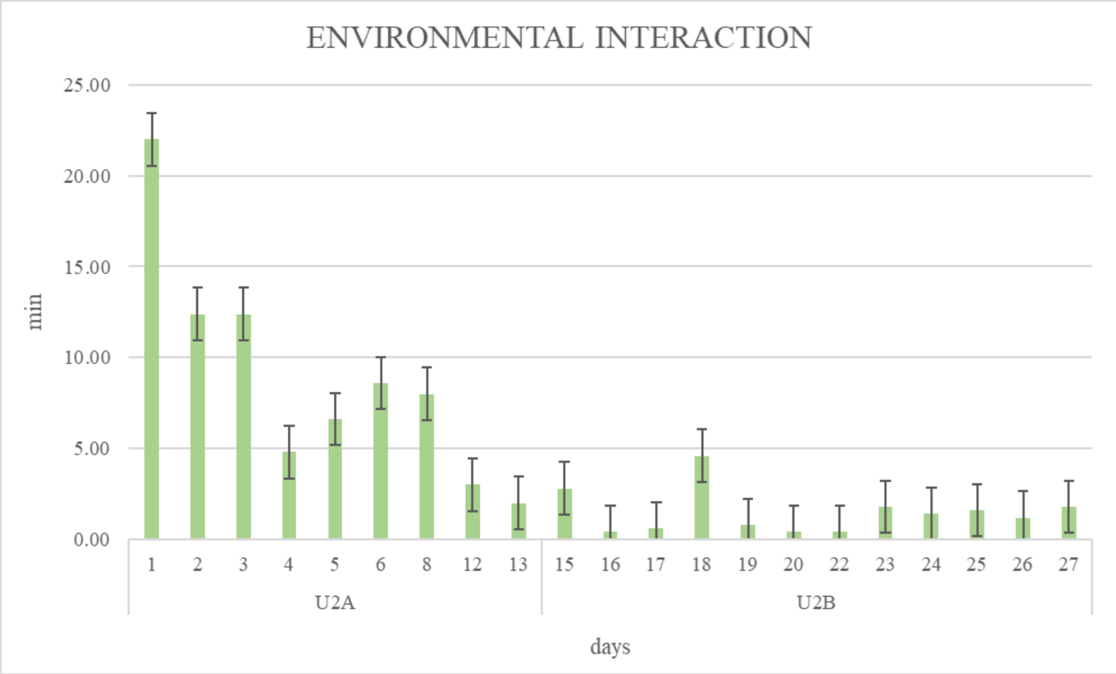

Figure 13. Least Square meansof environmental interaction considering the effect of the day in the experimental period (P<0.001).

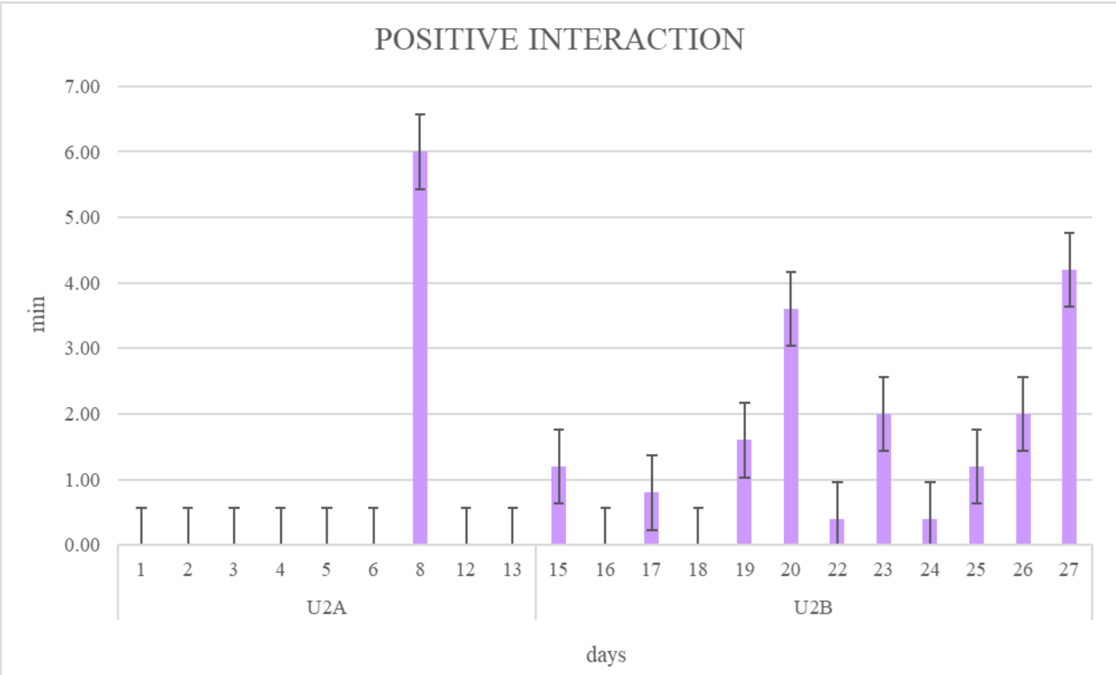

Figure 14. Least Square meansof positive interaction considering the effect of the day in the experimental period (P<0.001).

Negative interactions were exhibited in both groups at the beginning and at the end of each experimental period, assuming that stress was present at the beginning of the trial, given the adaptation to the new housing system, and at the end of the trial (the last few days) because they were close to the oestrous period (Figure 15).Cow expressed this behaviour for a shorter time during the 2-hour outings.

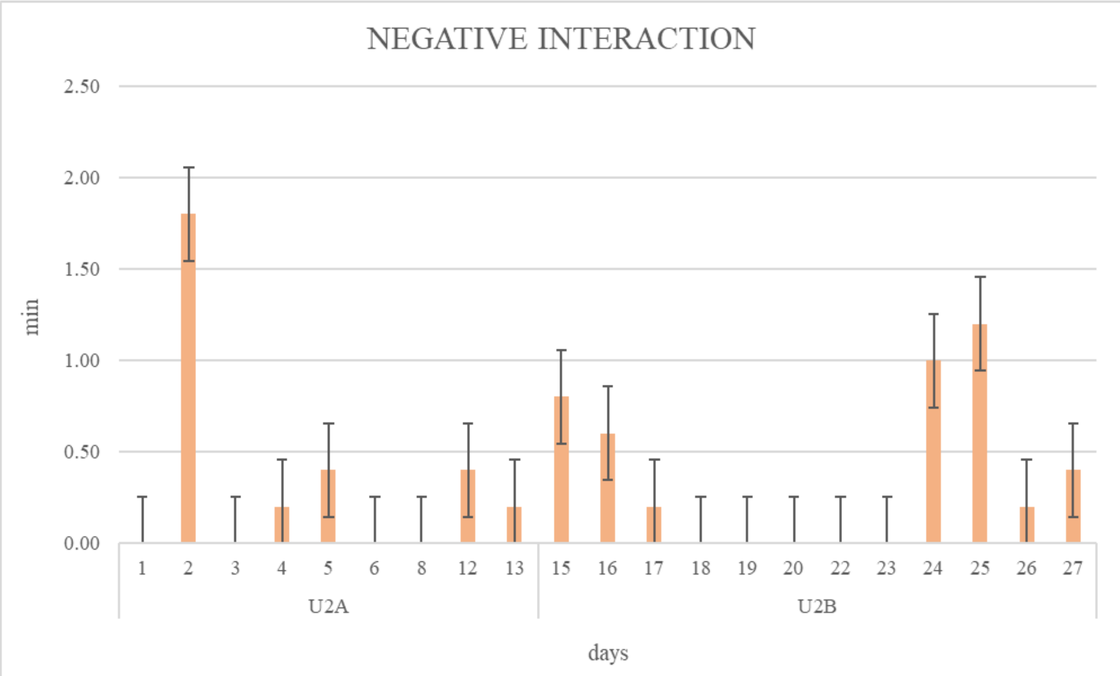

Figure 15. Least Square meansof negative interaction considering the effect of the day in the experimental period (P<0.001).

Since defecation and urination are event behaviours and not state behaviours, their presence during the activity on the external fence was very low.

4.1.3. Cow effect

In this experimental trial, the cow effect was significant for different behaviours. It meant that each cow expressed individual differences of these behaviours during the outings. So, differences were not just present between the two periods or thesis but were also present between the cows themselves.

Figure 16 shows how long each cow of both groups walked during the two weeks of the experimental period. For example, cow 986 spent a mean of 10 minutes walking during the 2 hours of activity on the external fence in the first experimental period; cows 970 and 990 spent about 3 minutes walking instead.

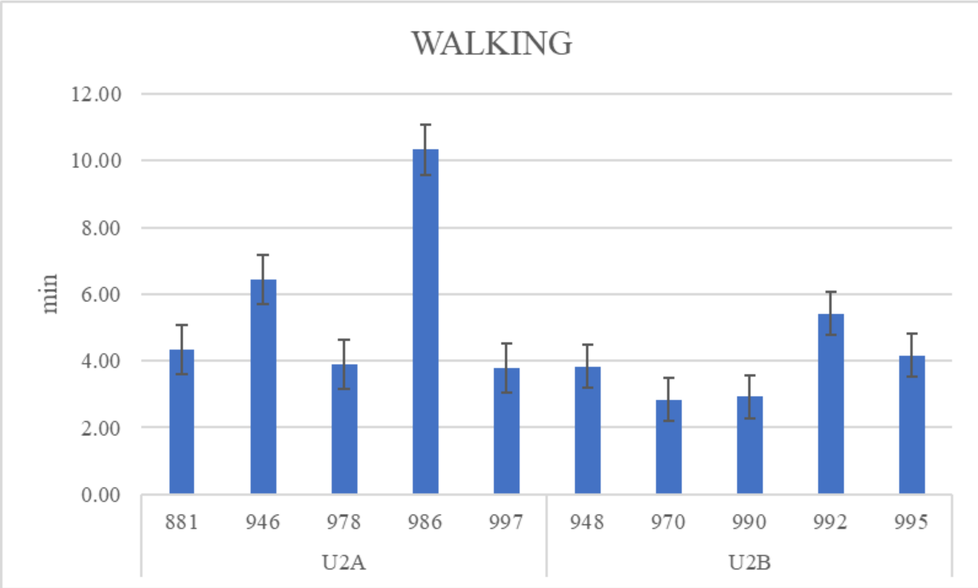

Figure 16. Least Square meansof walking behaviour considering the cow effect ($P<0.001$).

Figure 17 represents how long cows stood during the 2 hours of outings. Cows 881 and 948, as both had lameness issues, stood for about 80 and 100 minutes, respectively. Cow 997 not only walked less than other cows, but also stood for a shorter time (about 45 minutes).

As described above, cows that spent more time walking, on the other hand, spent less time standing.

Figure 18 shows how cows grazed. In particular, cows 881 and 948 spent 15 minutes and 8 minutes grazing, respectively. Cow 986, which spent the highest amount of time waking during the 2 hours of external activity, spent only 11 minutes grazing. On the contrary, cow 997, which walked for about 4 minutes, grazed for 60 minutes. U2B cows grazed significantly more than U2A cows, as the Least Square meansshow (44.88 vs. 30.47 min, respectively). Also, cows 992 and 995 spent more than 60 minutes grazing. In fact, they express a lower time walking (about 5 minutes).

In general, it was possible to say that when cows grazed for several minutes during outings, the time spent walking by cows themselves was as low as the time spent standing, and vice versa.

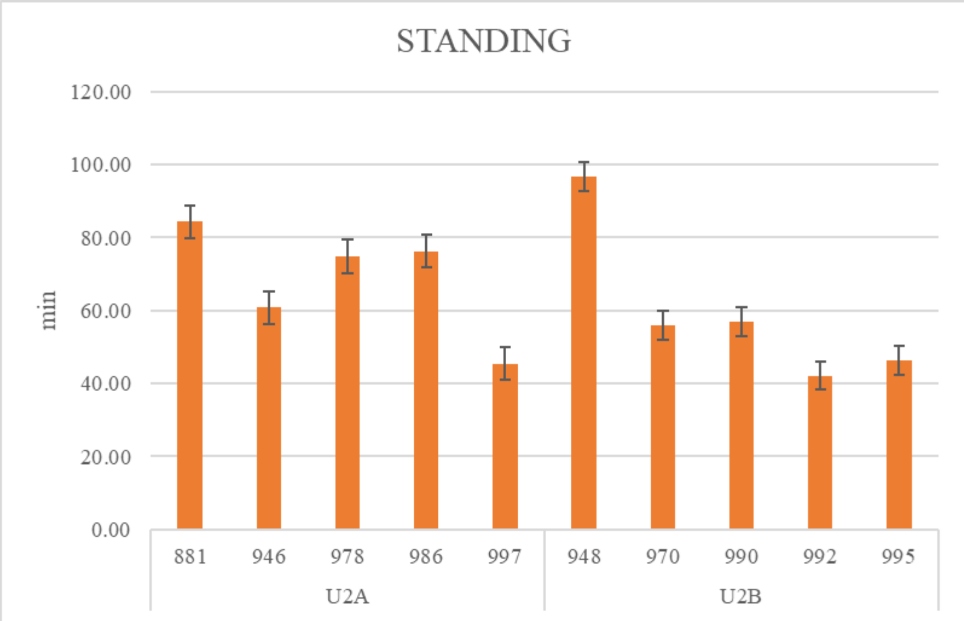

Figure 17. Least Square meansof standing behaviour considering the cow effect ($P<0.001$).

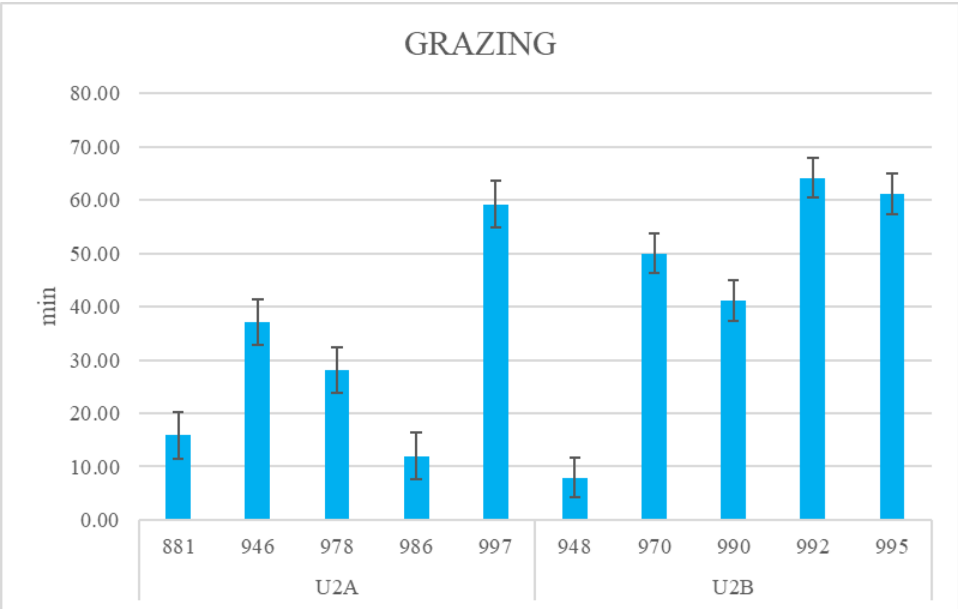

Figure 18. Least Square meansof grazing behaviour considering the cow effect ($P<0.001$).

Regarding selfgrooming behaviour, three cows exhibited it for more than 6 minutes: 986 (U2A), which spent about 8 minutes taking care of itself, 970 (U2B), which spent 6.5 minutes licking itself, and 990 (U2B), which spent 6 minutes on expressing selfgrooming in 2 hours. It could be assumed that cow 986, which spent about 10 minutes grazing, could be stressed about being in a different environment and expressed the highest amount of time licking itself.

Other cows spent about 3- or 4-minutes tacking care of their body (Figure 19).

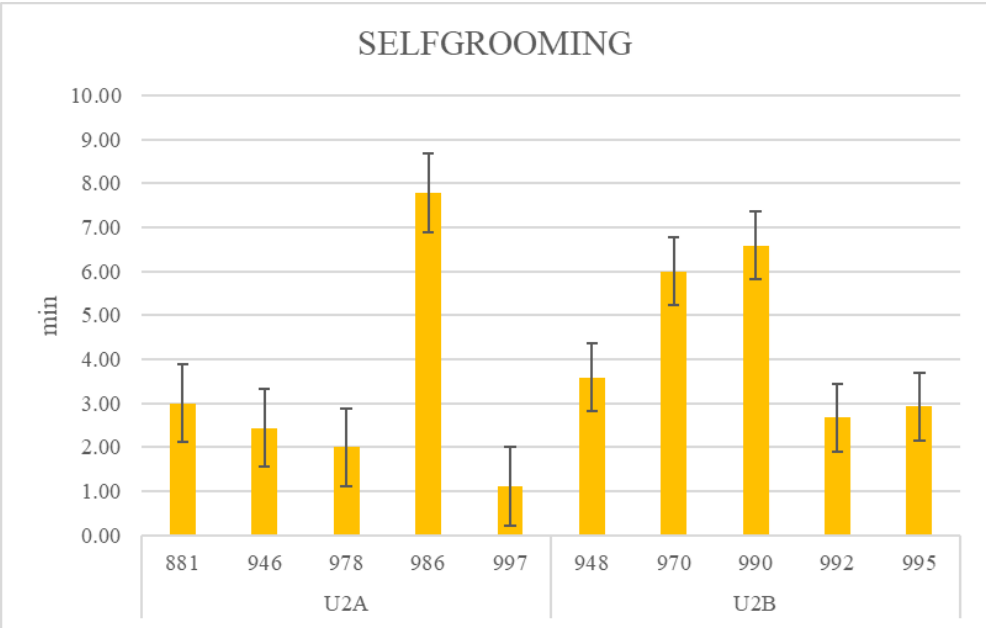

Figure 19. Least Square meansof selfgrooming behaviour considering the cow effect ($P<0.001$).

Considering lying behaviour and playing behaviour (Figure 20 and Figure 21, respectively), it was evident that the cow effect was very consistent in this trial. Only two cows were laid down even if for a short period. Cow 990 was laid for 7 minutes and cow 948 was laid for 3 minutes. Regarding playing behaviour, only one cow (997) attempted to play with the others for a brief moment, less than 1 minute.

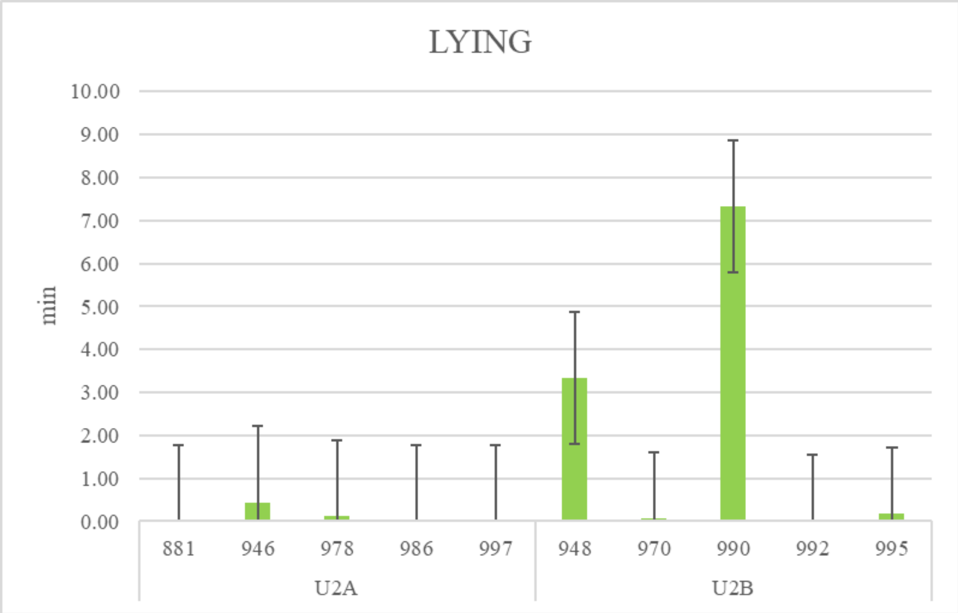

Figure 20. Least Square meansof lying behaviour considering the cow effect (P<0.01).

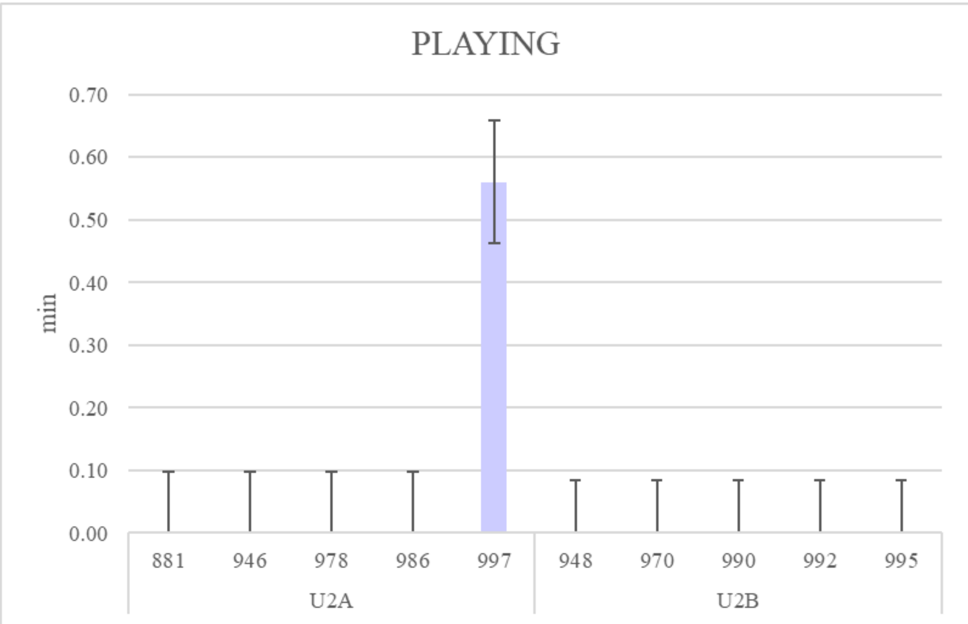

Figure 21. Least Square meansof playing behaviour considering the cow effect (P<0.01).

Also, urination behaviour (Figure 22) and negative interactions (Figure 23) were manifested briefly during the 2-hour outings. Cow 948, which stood for the higher amount of time, exhibited the higher time urinating (0.90 minutes) and expressing negative interactions with the other cows in the outdoor fence (0.80 minutes), probably associated with the presence of lameness causing great stress to the cow.

Negative interactions occurred with different frequencies among cows, with peaks of about 0.8 min for cows 986 (U2A) and 948 (U2B). Only one cow, the 978 (U2A), did not express negative interactions towards the other bovines in the external fence.

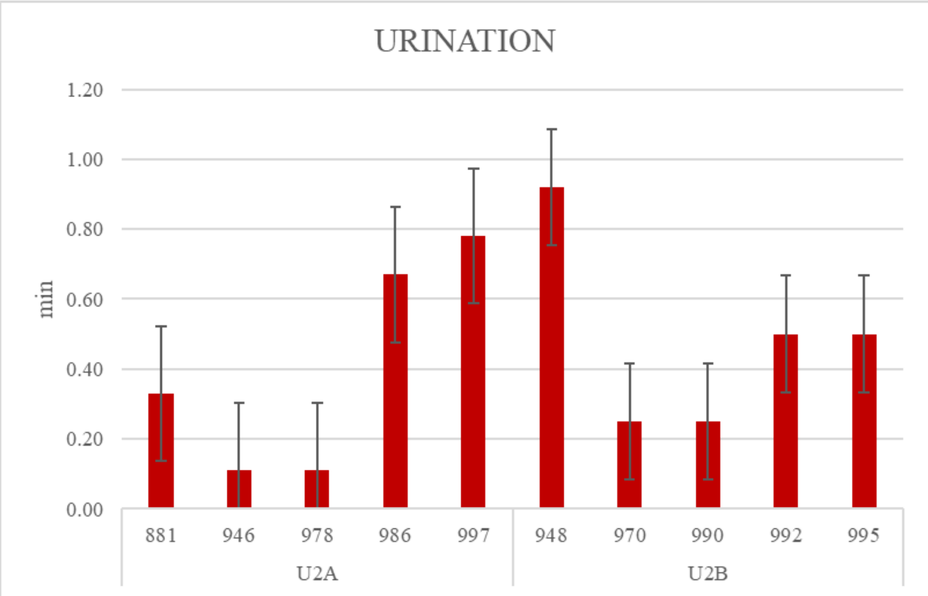

Figure 22. Least Square meansof urination behaviour considering the cow effect (P<0.05).

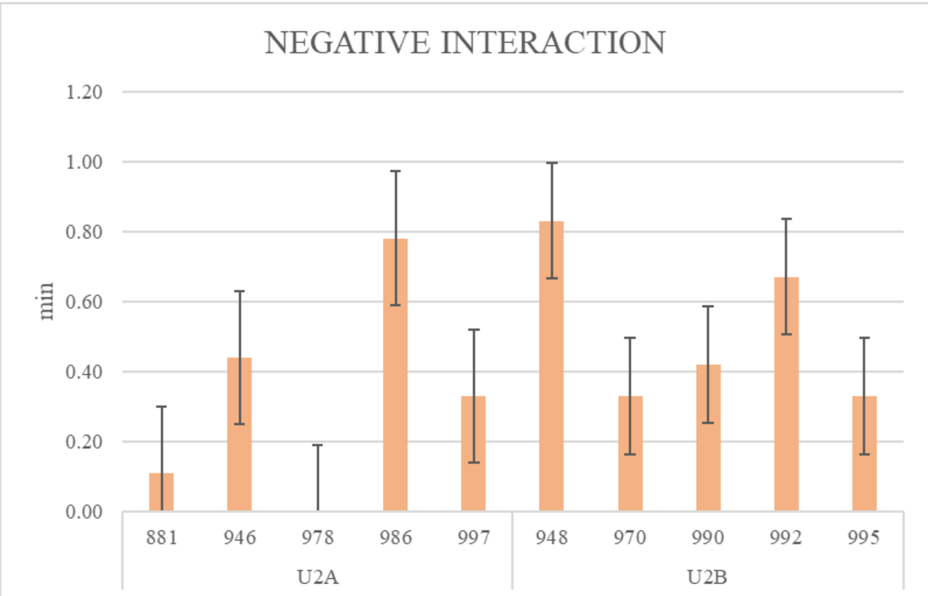

Figure 23. Least Square meansof negative interaction considering the cow effect (P<0.05).

4.2. Rumination behaviour

The data about the time budget of rumination were recorded hourly, making it possible to carry out a specific analysis and to compare the rumination time during the two-hour exit with that manifested for the subsequent two hours upon return to the housing environment.

The ANOVA of the data obtained from the activity monitoring system of the milking robot used in the herd is reported in Table 11, showing the significance of the four effects considered in the analysis. The effects found to be significant for the rumination variable were the thesis within the time slot (P<0.001), bovine interaction by thesis within the time slot, and the day effect (P<0.01).

The cow effect was not significant, assuming that the outing to the external fence influenced the rumination behaviour during the trial in the same way for each cow.

Table 11. ANOVA (F-values and P-values) of rumination data obtained with Lely activity monitoring system (AMS).

EFFECTS	F-value	P-value
Cow	1.53	0.134
Thesis within time slot	12.22	<0.001
Cow * Thesis within time slot	1.84	<0.01
Days	8.64	<0.01

The control group (CTR) was included in this analysis, as the activity monitoring system recorded the data regardless of whether cows went out to the pen. So, it could be possible to compare the rumination time between U2 cows and CTR cows (which stayed in the housing system during the 2-hour outings) and to verify if there were any statistical differences between the two theses. The influence of the two experimental periods was considered in the analysis by including the thesis within the time slot effect.

The Latin Square design 2x2, detailed in paragraph 3.2 and represented in Table 6, was used in this analysis.

Also, it could be possible to compare the rumination time expressed as minutes between both time slots considered in the analysis and see if the 2-hour exit affected rumination upon the return to the barn.

In this part of this study, the "thesis within time slot" is considered to compare U2 vs. CTR within the two time slots (15.00 - 17.00 and 17.00 - 19.00). Table 12 shows the Least Square meansof the minutes of rumination regarding the effect above cited, showing that there was a significant difference between the CTR thesis and U2 theses in the first time slot, from

15.00 to 17.00 (P<0.05). Cows ruminated with a least square mean of 33.03 minutes when they belonged to the CTR thesis, and ruminated with a mean of 22.89 minutes when they belonged to the U2 thesis. In fact, cows did not eat during the activity in the external area, and CTR cows were given an ad libitum ratio instead. Grazing behaviour should not have affected the rumination behaviour in this trial so much. Indeed, the amount of grass ingested was low as the grass was cut before the beginning of the trial and did not stimulate rumination behaviour, even if the time spent grazing during the 2-hour outing was high, as described above in paragraph 4.1.

There was no statistical difference between CTR and U2 theses in the second time slot, from 17.00 to 19.00, even though U2 cows ruminated about 4 minutes longer than CTR cows (37.52 vs 34.11 minutes, respectively).

Table 12. Least Square meansof rumination regarding thesis within time slot effect.

THESIS WITHIN THE TIME SLOT			
15.00-17.00		17.00-19.00	
CTR (min)	U2 (min)	CTR (min)	U2 (min)
33.03^{a}	22.89^{b}	34.11	37.52

^{a,b} Means with different superscript letters are statistically different (P<0.05)

4.3. Eating behaviour

Eating data obtained from the AMS installed in the herd were registered daily, so the comparison between the time slots above considered, as well as the differences among the moments of the day spent inside or outside, was not possible to apply.

The cow effect was the one with the greater significance for both variables (P<0.001), minutes of feeding, and Kg of compound feed ingested, as shown in Table 13.

The thesis effect did not affect the eating behaviour during the day of the trial, but the interaction between the cow effect and thesis effect resulted in a greater significance only for the minutes of feeding (P<0.001), as it resulted from the day effect.

In this analysis, the minutes of feeding were related to the time the cows spent in the feeding lane after ratio administration, whereas kg of compound feeding was related to the quantity of compound feed cows ingested during milking in a day, referring to the standard estimated per cow.

Table 13. ANOVA (F-values) of eating data obtained with Lely activity monitoring system (AMS).

	COW	THESIS	COW *THESIS	DAY
Eating				
Minutes of feeding	24.19***	1.97	9.31***	41.75***
Kg of compound feed	12.43***	0.26	1.13	0.24

*P<0.05; **P<0.01; ***P<0.001

The variables found to be significant are discussed below.

Figure 24 shows the minutes of feeding per cow during the experimental trial (cow*thesis effect). In this analysis, all cows were both in treatment (U2) and control (CTR) groups in the different experimental periods, following the Latin Square design 2x2. So, Figure 24 represents the time cows spent eating in the feeding lane when they were both U2 and CTR thesis. It should, however, be noted that the AMS instrument recorded the eating behaviour just by looking at the head movements; therefore, some false positive or negative results could have happened.

The first four cows (881, 946, 978, and 986), which belonged to the first group of exits (U2A), spent more time in the feeding lane when they were in the CTR group. Cows 881 and 946 spent about 260 minutes in the feeding lane, cow 978 spent about 280 minutes in the feeding lane, and 986 spent less than 250 minutes in the feeding lane (235 minutes). The differences in time spent eating between CTR and U2 groups for 881, 976, and 986 cows were statistically different. In particular, cow 986 spent less than 200 minutes in the feeding lane when it belonged to the U2 group (180 minutes), assuming that the outing negatively affected eating behaviour.

Cow 997, which belonged to the first group of exits (U2A), spent about 20 minutes longer eating in the feeding lane when it was in the U2 group (224 minutes) than when it was in the CTR group (207 minutes). The same trend was observed for cows 970, 990, 992, and 995, belonging to the second exits group (U2B). Differences between the time spent eating from when they were CTR or U2 group were statistically significant for 970, 990, and 992 cows. Cow 970 spent about 300 minutes in the feeding lane when it belonged to U2 (292 minutes) and about 220 minutes when it belonged to CTR (219.5 minutes). Conversely, cow 990 spent about 330 minutes eating when it belonged to the U2 group (327 minutes) and about 290 minutes when it belonged to the CTR group (286.5 minutes). Lastly, cow 992 ate for 248 minutes when belonged to U2 group and 180 minutes when belonged to CTR group. In this case, the outings to the external area positively affected the eating behaviour of these cows.

Cow 248, which had lameness issues, spent more time in the feeding lane when it belonged to the CTR group (279 minutes) than the U2 group (227 minutes).

Studying the quantity of compound feed the cows ingested during the trial, no statistical differences resulted concerning the thesis effect and its interaction with cow effect, as reported in Figure 25. All cows ingested the same amount of feed when they belonged to the control or treatment group.

As the cow effect was found to be the effect with the greatest significance for the ingestion of compound feed, the Least Square meansof it were reported in Figure 26. It was evident that U2A cows, on average, ingested a higher amount of compound feed than U2B cows (4.89 kg vs 4.39 kg, respectively). As reported in paragraph 4.1.1, U2A cows express, on average, more motion behaviour than U2B cows, which spent more time eating during the activity in the external area. This result explained that the higher ingestion of compound feed from U2A cows was to satisfy their energy needs.

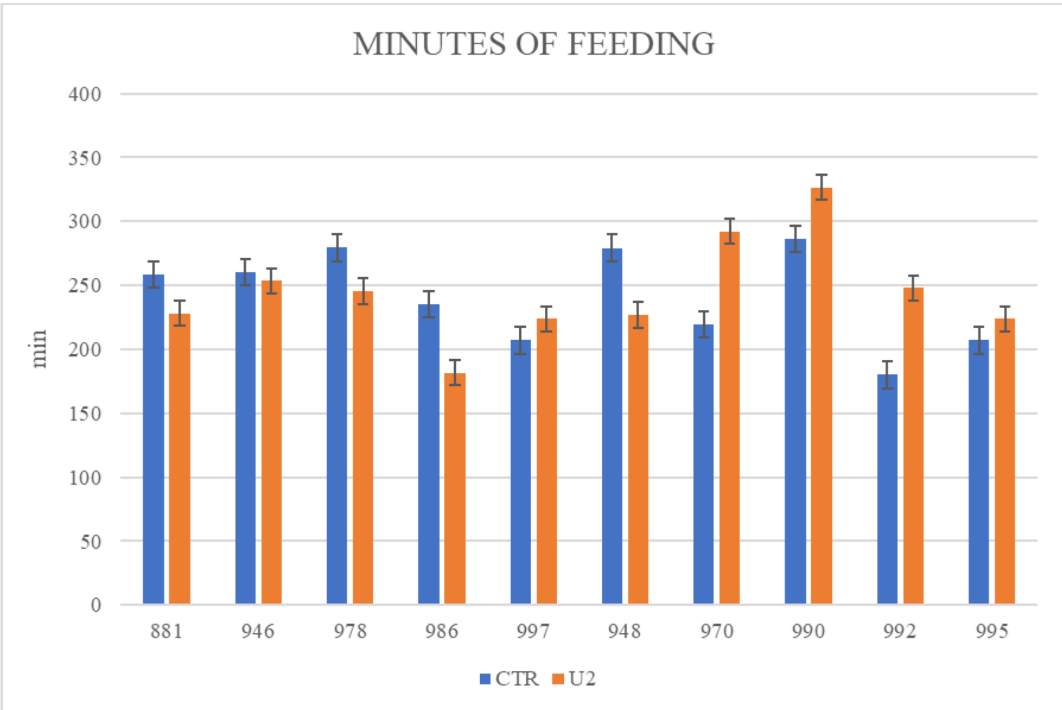

Figure 24. Least Square meansof the time spent eating in the feeding lane (thesis effect) (P<0.001).

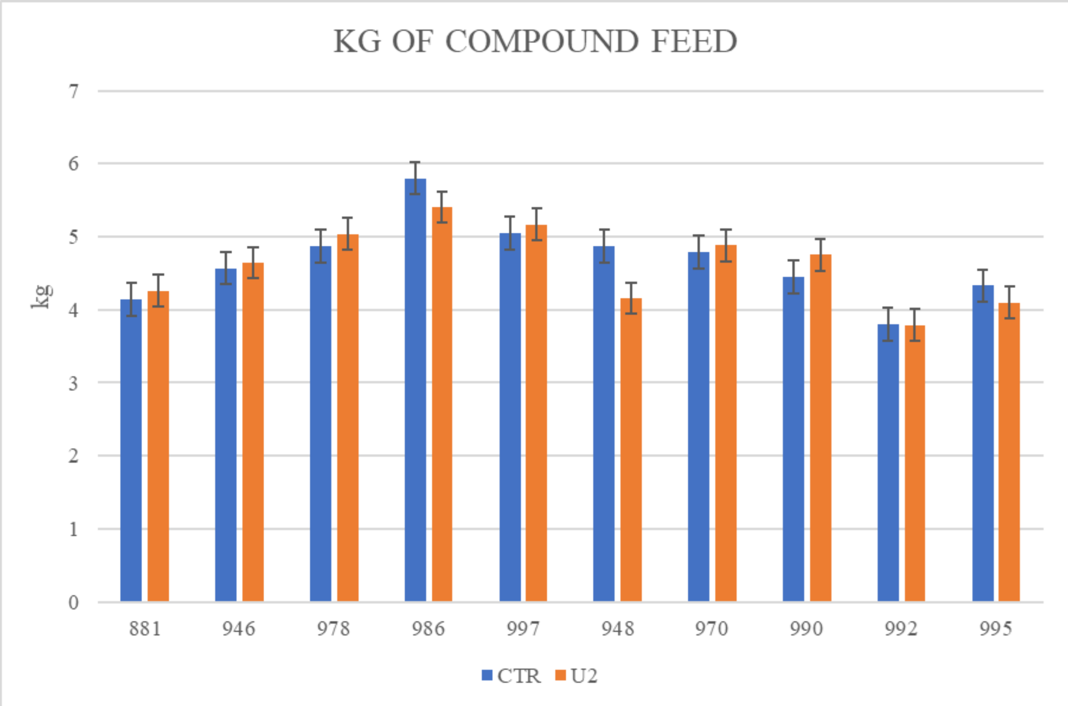

Figure 25. Least Square meansof the ingestion of compound feed (thesis effect).

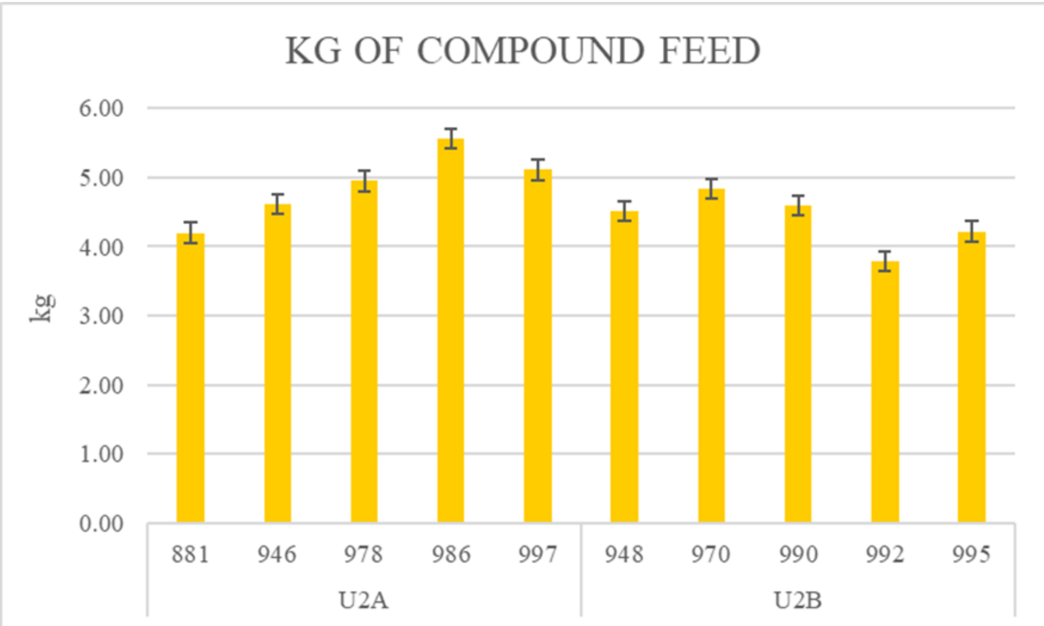

Figure 26. Least Square meansof the ingestion of compound feed (cow effect) (P<0.001).

4.4. Quantity and quality of milk

4.4.1. Daily production and number of milkings

Milk production and the number of milkings data obtained from the AMS installed in the herd were registered daily, as well as the data about eating behaviour represented in paragraph 4.3. The same statistical analysis was used to evaluate the variables.

Milk production refers to the daily amount of milk (kg) produced by cows in the experimental trial. The number of milkings refers to the times each cow went to the AMS per day during the trial.

Table 14 shows the ANOVA analysis of the variables described above. Milk production resulted significant for the cow effect (P<0.001) and the interaction between the two fixed effect cow and thesis (P<0.001). The number of milking was significant for the cow effect (P<0.001), thesis effect, and their interaction (P<0.05) instead.

Table 14. ANOVA (F-values) of milk production and number of milkings data obtained with Lely activity monitoring system (AMS).

	COW	THESIS	COW *THESIS	DAY
Milk production	141.98***	1.17	10.96***	1.97
N° of milkings	14.70***	4.43*	2.26*	0.22

*P<0.05; **P<0.01; ***P<0.001

The Least Square meansof the variables found out to be significant are studied below.

Figure 27 shows the amount of milk produced by each cow during the experimental trial. Milk production was higher for cows which ate a larger amount of compound feed. Comparing Figure 27 with Figure 26 (ingestion of compound feed vs. milk production), the trend per cow was the same. For example, cow 986, the cow with the highest milk production (37.88 kg), was the same cow that ate the highest quantity of compound feed (5.56 kg). On the other side, cow 992, which ate the lowest amount of compound feed (3.79 kg), produced the lowest amount of milk (25.68 kg).

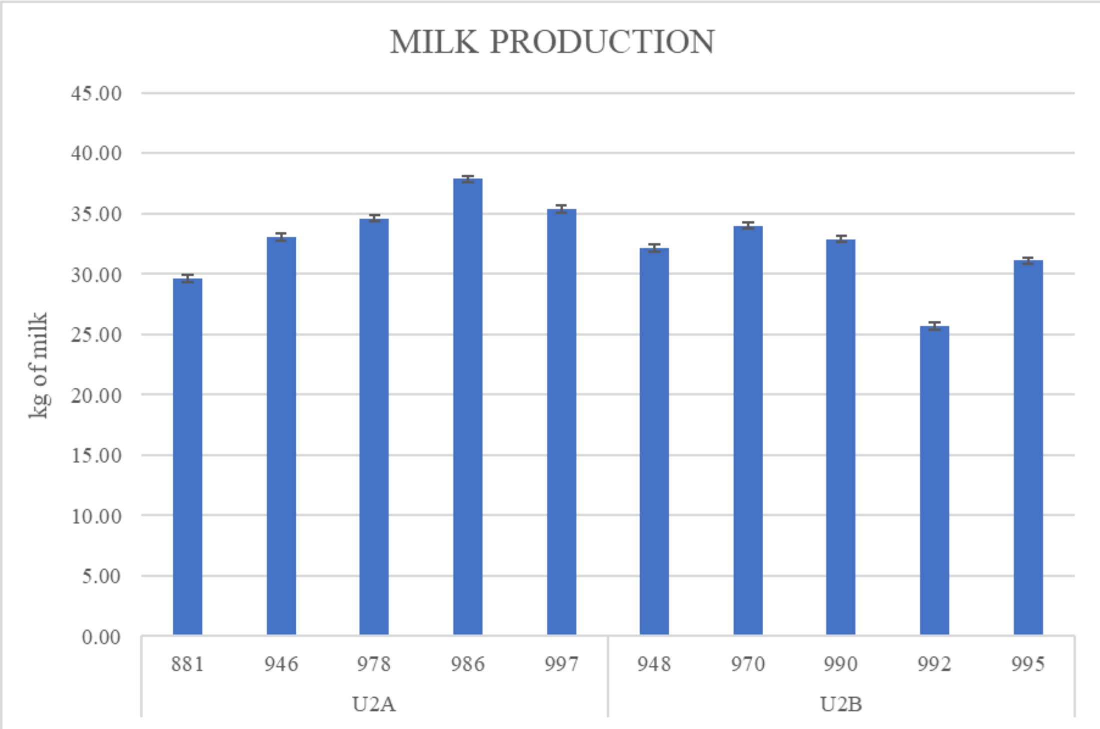

Figure 27. Least Square meansof the daily milk production (cow effect) (P<0.001).

Milk production was also significantly influenced by the interaction between cow and thesis effect, as shown in Figure 28. The figure reports the Least Square meansfor each cow within thesis, allowing the comparison of each cow of the experimental trial when it belonged to the control (CTR) vs. the treatment (U2) group. Looking at the cows that went to the external fence in the first period (U2A), cow 986 produced a lower amount of milk in the days with access to the external fence (39.07 vs. 39.69 kg of milk for U2 vs, CTR respectively).

Studying the milk production of cows of the second period of outings (U2B), cows 970 and 990 significantly increased their milk production when they were in the U2 group, while cow 948 significantly reduced the amount of milk produced.

Cow 970 produced about 35 kg of milk when in the U2 group (in the first experimental period) and 32.7 kg of milk when in the CTR (in the second period), and cow 990 produced 33.75 and 32.07 kg of milk when in the U2 and CTR groups, respectively. Conversely, cow 948 produced 30.21 kg of milk when it belonged to the U2 group (in the second period), and 34.15 kg of milk when it belonged to the CTR group (in the first period).

On average, the amount of milk produced by the G2 cows (that went out in the second period) was almost lower than that of the G1 cows (went out in the first period), following the same trend obtained in Figure 26 regarding the ingestion of compound feed. Anyway, there was no significant difference between the two theses, as Table 14 reported.

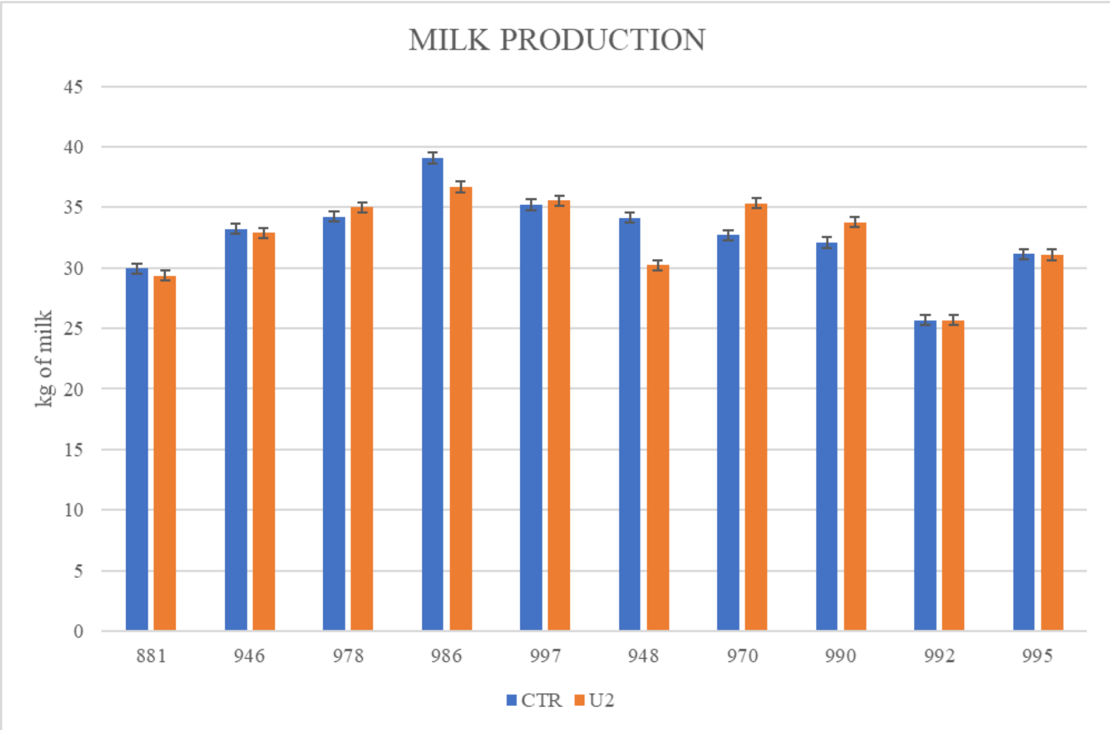

Figure 28. Least Square meansof the daily milk production (cow per thesis effect) (P<0.001).

Considering the effect of the thesis in relation to the number of milkings, it resulted that CTR cows went to the AMS with more frequency than U2 cows. Particularly, CTR cows on average, went to the AMS about 3.25 times per day, and U2 cows went 3.08 times per day (Figure 29). It is important to note that while CTR cows had free access to the milking robot, U2 cows could not access it while they were in the outdoor pen.

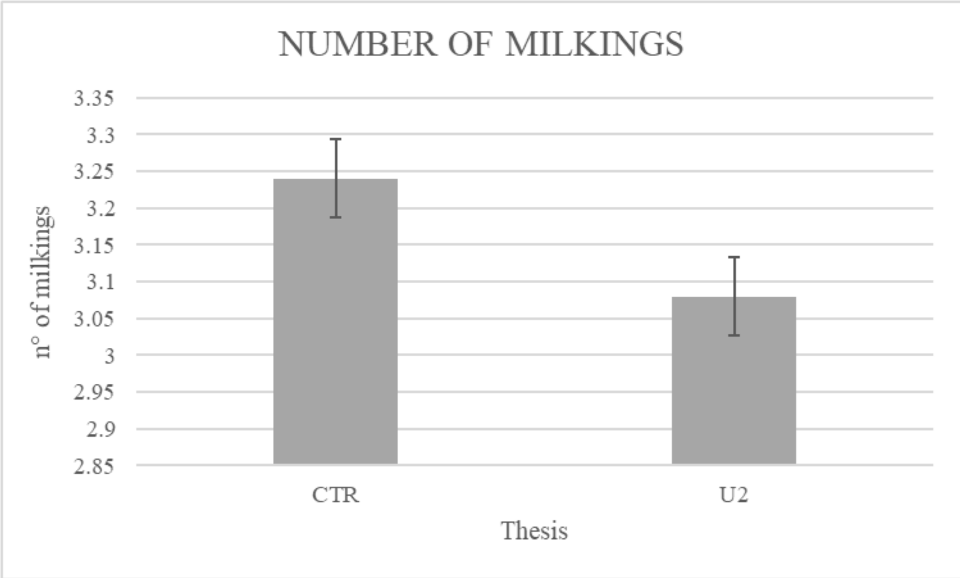

Figure 29. Least Square meansof the number of milking (thesis effect) (P<0.05).

The number of milkings on average was inversely proportional to the amount of milk produced by the cows. Figure 30 shows the times each cow went to the AMS during the experimental trial. Cow 986, which had the highest milk production, went to the milking robot about 3.5 times under the CTR thesis (second period) and about three times under the U2 thesis. Cows 881 and 978, which had a lower milk production than cow 986, had the highest number of milkings: cow 881 went to the AMS 3.70 times per day under both CTR and U2 thesis; cow 978 went to the AMS 4.11 times per day when belonged to CTR thesis and 3.83 times when belonged to U2 thesis.

Cow 992, which had the lowest milk production (25 kg), had the highest number of milkings of all the cows of the second period of outings (U2B), going to the AMS about thrice daily. Cows 970, 990, and 995 produced an amount of milk higher than the one of cow 992 without going to the milking robot such frequently.

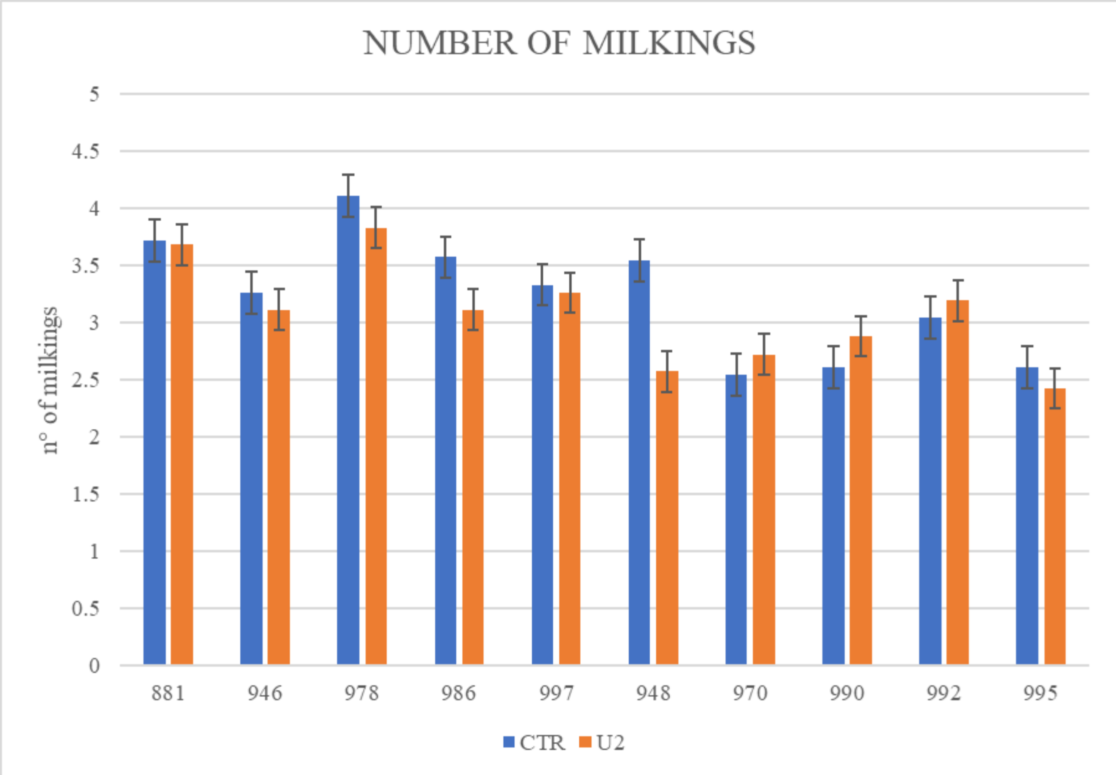

Figure 30. Least Square meansof the number of milkings (cow per thesis effect) (P<0.001).

4.4.2. Milk Quality

Table 15 shows the ANOVA results in relation to the variables of milk quality (protein, fat, lactose, casein, and urea) quantified using the MilkoScan FT2 milk analyser. The table reports the F-values and significance of the thesis, sample, and cow effects.

The content of protein resulted statistically significant concerning the cow effect (P<0.001) and to the sample effect (P<0.05). Lactose percentage resulted significant for the sample effect (P<0.01) and tended to be significant for the cow effect (P<0.10). The casein percentage was significant only for the cow effect (P<0.01). Conversely, the content of urea resulted in a greater significance for the sample effect (P<0.001), but it was also the only variable that was significant in relation to the thesis effect (P<0.05)

Table 15- ANOVA (F-values) of the chemical composition of milk (MilkoScan FT2).

MILK COMPOSITION	THESIS	SAMPLE	COW
PROTEIN	0.00	6.79*	27.73***
FAT	0.11	0.71	1.78
LACTOSE	2.46	16.94**	3.95°
CASEIN	0.18	3.78	12.29**
UREA	6.18*	194.58***	2.40

°P<0.10; *P<0.05; **P<0.01; ***P<0.001

The Least Square meansof the thesis effect were considered, as the thesis was the effect that most explains the aim of the trial. The Least Square meansof the chemical composition of milk samples collected during the trial are shown in Table 16. As reported in Table 15, only one variable resulted in statistical significance for the thesis effect: urea (mg/dl). The content of urea in milk samples collected by CTR cows was lower than the content in milk samples of U2 cows (36.22 vs 38.39 mg/dl, respectively), suggesting that the activity in the outdoor fence increased its content in milk. It could be possible that the grazing behaviour, and thus the ingestion of fresh grass, increased the proportion of protein ingested by cows during the experimental trial, consequently increasing the amount of ruminal ammonia and so urea. Using optimized feed rations results in an average urea concentration in bovine milk of 28-32 mg/dl.

Protein content did not have any significant differences as for fat content, but the percent of protein from milk samples of the cows in this trial was higher than the mean population of Simmental cows (Spigarelli G., 2021) (3.44% vs. 3.64% for CTR group and 3.61% for U2 group of this study), and the percent of fat in the same milk samples was lower than the mean population (3.91 % vs 3.86% for CTR group and 3.77% for U2 group). These results support what has just been described.

Lactose content wasn't affected by the thesis effect, and was in line with the average content.

Casein makes up 80% of the protein in cow's milk. In this trial, the percentage of casein content has been representative of what has just been stated.

Table 16. Least Square meansof the chemical composition of milk (thesis effect).

	THESIS	
MILK COMPOSITION	**CTR**	**U2**
PROTEIN (%)	3.64	3.61
FAT (%)	3.86	3.77
LACTOSE (%)	4.88	4.92
CASEIN (%)	2.88	2.87
UREA (mg/dl)	36.22^{b}	38.39^{a}

^{a,b} Means with different superscript letters are statistically different (P<0.05)

Studying the cow effect for protein and casein content (Figure 31 and 32), cows with the higher production of milk (Figure 27) had the lowest content of protein and so casein and vice versa. Also, cows that ate less compound feed and spent more time grazing had the highest percent of protein and casein content in milk (cows 992 and 995).

Figure 31. Least Square meansof protein content (%) in cow’s milk (cow effect) (P<0.001).

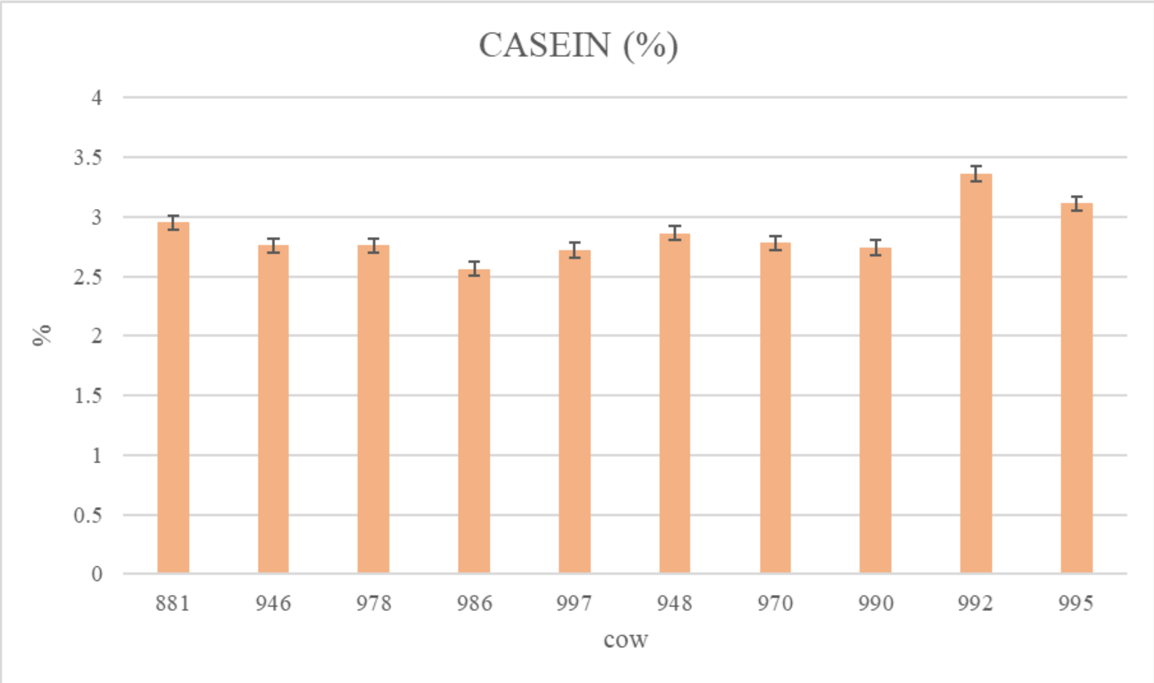

Figure 32. Least Square meansof casein content (%) in cow’s milk (cow effect) (P<0.001).

5. DISCUSSION

In this study the effects of allowing dairy cows programmed access to an outdoor exercise area on behavioural expression and milk production were researched. The observation of animals' behaviour helps to assess the health status of reared cows, but also the observation of any abnormal behaviours they express helps to define the welfare degree provided in the herd. Furthermore, also the amount of time spent for each behaviour helps in assessing the welfare because if behaviours deviate from the normal time budget of a species, welfare problems may occur. Abnormal behaviours and deviations from time budget occur if the management of the animals does not meet the physiological and species-specific needs of the individuals.

Behavioural expression

Pasture-based systems enable cattle to express their natural behaviour and are thus expected to provide better welfare than most confinement systems (Alsaaod et al., 2022), and may reduce lameness. In addition, consumers strongly prefer natural livestock rearing with pasture and grazing (Kismul et al., 2018). Previous studies also reported a significant effect on pasture preference: the longer calves/heifers/ cows were reared without pasture experience, the stronger was their preference for housing (Charlton and Rutter, 2017).

Also, activity monitors are becoming increasingly common due to their ability to monitor behavioural patterns and to alert the farmer when key indicators are too high or too low for each animal raised (Zambelis et al., 2019).

In this thesis, the behavioural patterns were different between the two periods of the experimental trial, resulting in a higher locomotion activity during the first two weeks and higher grazing and positive interactions between conspecifics in the last two weeks of the trial.

During the experimental trial, cows were not supposed to eat. The grass in the outdoor fence was cut before the beginning of the trial itself. The manifestation of this behaviour supports what was described earlier, namely, the availability of space in an outdoor enclosure encourages the expression of the innate and natural behaviours of each species.

As Alsaaod et al. (2022) studied, cows spent less time lying down when in the outdoor area and had higher locomotor activity levels, in particular, walking behaviour was higher than in cubicle cows (75.6±25.9 min/day vs. 38.8±15.8 min/day; $P<0.0001$), standing longer than 12 hours/day standing. These results are in accordance with what was studied in this thesis.

During the trial U2 cows spent more than 60 minutes in 2 hours of outdoor activity standing, about 6 minutes walking, and 0.11 minutes lying.

In the study of Grant (2007), cows spend from 12 to 14 hours lying in free-stall environment, which is at odds with the results of this thesis, concluding that different housing facilities influence the expression of behavioural patterns in cows. Grant (2007) also reported that cows devote from 7 to 10 hours ruminating per day. This result is in accordance with what was carried out in this thesis from both in vivo behavioural observation and the Lely activity monitoring system. When cows have access to an outdoor area, they will spend most of their time grazing and ruminating, as is inherent in their behavioural repertoire.

The results of this thesis are also in accordance with what Shepley et al. (2020) state in their review: cows provided with outdoor access, either through exercise yards or pasture, express more locomotor activity than those that are not. These conditions ensure the correct level of animal welfare, so animals can perform a particular behaviour when they are highly motivated (Dawkins, 2004).

Kismul et al. (2018) led a study similar to this experimental trial. They compared the effect of production pasture (PROD) and exercise pasture (EX) on milk production and cow behaviour in an automatic milking system, obtaining that cows in the PROD group spent more time outdoors than those in group EX in 8.5 hours of daily access. Also, Group PROD cows spent more time grazing and resting. Cows in group PROD spent most of their time outdoor access time outdoors in both mornings and afternoons, but in the afternoon, grazing activity was much more pronounced in this group, in accordance with the results of this thesis. Kismul et al. (2019) also found out that for both treatments (Ex and PROD), the total outdoor time increased by approximately 1 hour during the experiment, even if EX group spent a lower time exhibiting grazing behaviour than the PROD group.

Kismul et al. (2018) stated that the eating activity increased from 5.00 to 9.00, flattened during the warmer hours of the day, and then from 13.00 onwards, the hourly intake increased again, with a distinct dip from 16.00 to 19.00. In this experimental trial, the grazing behaviour was consistent during the 2-hours of outdoor activity from 15.00 to 17.00, even if the grass was cut before the beginning of the test.

Time of allocation to a fresh plot altered the distribution of grazing behaviour variables over the day, even in the study carried out by Abrahamse et al. (2009). Grazing time was longer, and the number of bites was greater following allocation to a new plot when compared to allocation to the same plot. The results of this study are in accordance with what was obtained in this experimental trial, where the time spent grazing (in the same outdoor fence) was higher in the first days of outings, then reached a peak and decreased during the experiment in each period (U2A and U2B).

Results obtained from the study conducted by Loberg et al. (2004) have shown that adult dairy cows used the time to walk, trot, and explore the environment when given access to an outdoor paddock, and that exercise had a positive effect on the claw conformation without affecting the milk production.

As Stygar et al. (2021) reported, the precision and accuracy of feeding and drinking assessment vary depending on measured traits and the used sensor. Lying and standing behaviour, but also rumination, are variables validated with high performances in automatic system tools.

In the present study, no significant correlation was found between the time of rumination observed in vivo and the time of rumination registered by the AMS, as there was an objective difficulty in seeing whether the cows ruminated or not. Often, the field views of the cameras did not allow to see the chewing movement. However, Elischer et al., 2013 found out that activity monitored by the AMS (ACT) and rumination monitored by the AMS (RUM) do reflect cow walking and rumination, respectively, but not with a high degree of accuracy, and lying cannot be distinguished from standing. Significant but moderate correlations were found between ACT and observations of walking (r_p=0.61), standing (r_p= 0.46), lying (r_p = −0.57), and activity (r_p = 0.52), as well as between RUM and observations of rumination (r_p= 0.65).

Zambelis et al., 2019 reported that the sensors (ear-tag accelerometer) can accurately monitor dairy cows' active and non-active behaviors. The results also suggest that although the sensor shows promise for identifying feeding behaviours in general, the independent classification of rumination and eating requires additional sensitivity.

Milk production

With the increasing intensification of the dairy sector in many countries and with the introduction of automatic milking, exercise paddocks combined with full indoor feeding, as an alternative to production pasture, are being used as a compromise between farm economics and cow welfare (Kismul et al., 2019). Cows with free access to pasture and indoor housing also produce more milk than those continuously housed (Charlton and Rutter, 2017)

Kismul et al., 2019 proved that cows on the exercise paddock (EX) had a greater milk yield (kg of milk) over the experimental period than the production pasture (PROD), which had a drop in milk yield during the trial. This conclusion differs from the results of this experimental trial, where cows had a lower milk production when they spent more time grazing in the outdoor fence and eating in the feeding lane inside the housing system. Anyway, they had the higher percent content of protein and so casein in milk. Cows with the lower milk yield in this trial were the ones that ingested the smallest amount of compound feed and went less time to the automatic milking system. On the contrary, Kismul et al., 2019 did not find any effect of the treatment on the parameters estimated from test milking data (kg of milk fat and kg of milk protein), and the number of milking was quite similar for the two groups. A drop in milking frequency was observed for both groups, but this drop was greater and lasted longer for PROD compared with EX cows.

Abrahamse et al., 2009 found out that milk production and milk protein and lactose content did not differ between treatment MA (daily move to a previously un-grazed strip after morning milking) and AA (daily move to a previously un-grazed strip after afternoon milking). Milk fat content was lower in MA than in AA instead.

Changes in milk production do not necessarily indicate a change in animal welfare. Thus, a direct and simple effect of altered lying time on milk yield seems unlikely. Milk production is more likely to change when feed intake is lower (Tucker et al., 2019). Cows in the experimental trial did not lay down during the 2-hour outing, and milk production did not change between control group (CTR) and treatment (U2). On the contrary, cows that spent more time walking and standing grazed and laid down less, reducing the time spent eating in the feeding lane inside the housing facilities but increasing the amount of compound feed ingested and the milk production. The latter contained a lower percentage of protein and casein content.

No significant differences in milk yield and composition were found between walking activity group (WA) and non-walking activity group (NWA) in the study of Dong-Hyun et al., 2018. Regarding milk yield, WA produced about 1.5 kg of milk less than NWA (32.06 vs 33.65 kg/die, respectively). In the experimental trial, not all the cows were affected by the outdoor activity on milk production. In particular, U2B cows increased the milk yield during the 2-hour outings, and only one cow on U2A thesis decreased it. Considering milk fat and milk protein, similar results were obtained in both Dong-Hyun (2018) study. Milk fat was lower in WA cows than in NWA cows as in the present trial, and milk protein was higher in WA cows than in NWA cows. In the present study, the percent protein content was mostly the same.

The determination of urea in milk is of relevant importance, being an indicative parameter for improving farm management and herd welfare. The transformation of ruminal ammonia into urea is a metabolic process of defence of the organism, as ammonia is toxic. The main factor that determines the achievement of a balanced urea content in milk is the diet, the total protein intake of the ration, and the nitrogen balance in the rumen-thus the correct and simultaneous availability of nitrogen and energy for ruminal bacteria and their ability to degrade ammonia. Other factors include seasonal variations, recent ingestion of the feed ration, the residence time of the ration in the rumen, or the number of daily milkings. Urea concentration is thus subject to the metabolic fluctuation that each biological analyte has due to changes in one or more of the factors listed above (Orlandini, 2014).

Milk samples in this thesis had a high urea content (36.22 and 38.39 mg/dl for CTR and U2 groups, respectively), just because cows on the external fence ate a certain intake of fresh grass.

The objective of Leso et al. (2023) study was to investigate the effects of providing high-yielding dairy cows with free-choice pasture access during the dry period on their health, behaviour, and milk production. Results showed that free-choice pasture access affected cows' feeding behaviour. Before calving, the animals in PAST (housing with free-choice pasture access during the dry period) spent more time feeding than in CTRL (housing continuously without any access to the outdoors), persisting for several weeks after calving. Also, cows that spent the dry period in PAST produced more milk than their CTRL counterparts. In the present experimental trial similar results were obtained regarding the feeding time, but they differ in milk production as not all the cows increased the milk yield during the trial.

6. CONCLUSIONS

Under the present conditions, interesting findings have been found, regarding both cows' behavioural expressions and milk production.

The behaviours that mainly concerned the present study, as expressed most of the time during the trial by cows, were standing, grazing, and rumination. Walking activity was significant for all the three effects (thesis, cow within thesis, and day within thesis) considered in the statistical analysis but expressed for a shorter period during the outings.

By comparing the behavioural patterns expressed by cows during the two weeks outings, five cows left in the external fence during the first 2 weeks exhibited more movement behaviour (walking and running) and an increased interest in the external environment. On the contrary, cows that went outside in the second period spent more time grazing, grooming themselves, and interacting positively among conspecifics. Therefore, individual variability always plays an important role in the behaviour of the group when the cows are outside the stable.

The day within thesis was also an important source of variation. The time spent on different activities (i.e., walking, standing, grazing, exploring the environment, interactions) was affected by the day and showed a different pattern within each group of cows. Consequently, also the adaptation to different external conditions is significantly affected by animals and, for this reason, it is difficult to predict.

Rumination is considered, as is known, a parameter linked to animal welfare. In this experiment, it can be concluded that cows in an environment closer to the natural one of the pastures dedicate more time to grazing activities, reducing the time for rumination, which is partially compensated after returning to the stable. Climatic conditions probably influenced these results, and it is conceivable that rumination could also increase outdoors with more favourable external conditions (for example in spring-summer).

In this study, no correlation was found between rumination time monitored by the automatic milking system (Lely) and direct observations of rumination, probably due to the difficulties in detecting this behaviour when the animals were very far from the camera.

As expected, when the cows stay in the stable all day, they spent more time eating in the feeding lane and have a greater intake of compound feed than when they spend 2 hours in the outside fence. The cows in the first group showed this expected behaviour. In contrast, animals in the second group spent more time eating in the feeding lane during the two weeks with 2-hours outing and increased milk production and number of milkings. So, we can conclude that the time spent outside in the paddock can also be compensated by more time spent feeding in the stable without showing negative effects on milk production.

Chemical composition of milk, in particular the protein and casein content, is significantly affected by the milk yield. Cows with a lower milk production and a higher percentage of protein and casein were the ones that spent more time grazing and that ingested the lower amount of compound feed. The urea content in milk was higher in cows during the two weeks outdoors compared to those left in the stable all day, due to the greater amount of fresh grass ingested by the cows during outings.

The preliminary findings presented here offer valuable insights for farmers considering the integration of outdoor spaces to enhance locomotion activity in cows. This approach holds promise for positively impacting various facets of dairy livestock farming, encompassing both animal welfare and milk production. Nevertheless, to refine the practical application of this strategy, additional research is imperative. Specifically, there is a need for comprehensive investigations to pinpoint the optimal management of outdoor time within herds employing automatic milking systems. The experiment should also be conducted in different climatic seasons to better understand the adaptation of animals to favourable situations. Additionally, careful attention should be directed toward ensuring the presence of shaded areas and access to water in the external enclosure, particularly during summer outings.

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