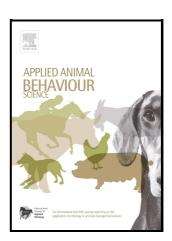
Journal Pre-proof

Forage particle size and forage preservation method modulate lying behaviour in dairy cows.

Andreas Haselmann, Matthias Wenter, Wilhelm F. Knaus, Birgit Fuerst-Waltl, Qendrim Zebeli, Christoph Winckler



PII: S0168-1591(22)00169-1

DOI: https://doi.org/10.1016/j.applanim.2022.105711

Reference: APPLAN105711

To appear in: Applied Animal Behaviour Science

Received date: 28 May 2022 Revised date: 23 July 2022 Accepted date: 27 July 2022

Please cite this article as: Andreas Haselmann, Matthias Wenter, Wilhelm F. Knaus, Birgit Fuerst-Waltl, Qendrim Zebeli and Christoph Winckler, Forage particle size and forage preservation method modulate lying behaviour in dairy c o w s . , *Applied Animal Behaviour Science*, (2022) doi:https://doi.org/10.1016/j.applanim.2022.105711

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 Published by Elsevier.

Journal Pre-proof

Forage particle size and forage preservation method modulate lying behaviour in dairy cows.

Keywords: laterality of lying, eating behaviour, ruminating behaviour, silage, barn-dried hay

Andreas Haselmann^{a, 1}, Matthias Wenter^{a, 2}, Wilhelm F. Knaus^a, Birgit Fuerst-Waltl^a, Qendrim Zebeli^b, Christoph Winckler^a

^aInstitute of Livestock Sciences, Department of Sustainable Agricultural Systems, BOKU—University of Natural Resources and Life Sciences, Vienna, 1180 Vienna, Austria.

^bInstitute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, 1210 Vienna, Austria.

Current address:

¹Department of Livestock Sciences, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland.

²Südtiroler Rinderzuchtverband, 39100 Bozen, Italy.

Corresponding address: andreas.haselmann@fibl.org

Highlights

*We investigated the effects of forage characteristics on cow lying behaviour.

*Reducing the particle size of forages increased cows' time budget for lying.

*Preservation method of forages modulated laterality of lying.

ABSTRACT

It has been suggested that reducing forage particle size as well as feeding hay compared to silage may improve forage intake. Little is known; however, how such ration characteristics affect other aspects of cattle behaviour. Therefore, this study investigated the lying behaviour of cows in response to particle size reduction (LONG *versus* SHORT; Trial 1) and preservation method (SILAGE *versus* HAY; Trial 2) of forages which constituted ≥ 80% of the rations' dry matter. Two feeding groups with 10 (Trial 1) and 9 Holstein cows each (Trial 2) received the experimental rations for approximately 5 weeks. Cows were housed in a free-stall barn with straw-bedded cubicles and rubberized floor in the alleys. Lying behaviour was recorded using HOBO Pendant® acceleration data loggers during the last 3 weeks of the experimental period as well as for 3 consecutive days during the week preceding the experiment, the latter serving as a baseline covariate to consider individual cow lying behaviour.

SHORT-fed cows (Trial 1) had a longer daily lying time (+ 1.1 h/d; P = 0.003) and tended to have more lying bouts (+ 3.1; P = 0.090) than cows fed the LONG ration. In both groups, a preference towards the left lying side (57%) was observed. A similar pattern was observed for both groups with regard to rumination time while lying. Cows fed SILAGE or HAY (Trial 2) did not differ with regard to lying times (12.8 h/d). However, cows on HAY tended to reduce the lying time on the left side (- 1.1 h/d; P = 0.098) and number of lying bouts per

day (-2.5 bouts/d; P = 0.039). Consequently, these cows showed nearly an equal preference for the left and right lying side with 49.1% and 51.6%, respectively; whereas cows on SILAGE tended to prefer the left lying side (55.6%), also when ruminating. In contrast, cows on HAY tended to reduce rumination time on the left lying side (-0.61 h/d; P = 0.079).

Effects observed may be attributed to changes in eating behaviour as well as the amount of feed ingested in a given time period. Under the conditions investigated, time budgets for lying may be limited and changes of the forage characteristics (e.g. particle size reduction) could mitigate these constraints.

Keywords:

laterality of lying; eating behaviour; ruminating behaviour; silage; barn-dried hay

Introduction

Lying behaviour of dairy cattle has been comprehensively studied in recent decades (Tucker et al., 2021) and can be described in terms of total lying time, the number and duration of lying bouts, and the laterality while lying (i.e., left and right lying side) (Ledgerwood et al., 2010). Lying is of high importance as shown by studies of the motivation of cattle to lie down for 12 to 13 h when housed indoors (Jensen et al., 2005) as well as the importance given to lying when under time constraints (Munksgaard et al., 2005).

Lying behaviour of cows is affected by different factors such as housing conditions (e.g. design of cubicles and stocking density at the cubicles), the health status of the animals, as well as their production level and body condition.

Analysing the lying behaviour of cows on nearly 80 farms in the US, Ito et al. (2014) showed that roughly one third of the variation in lying time was due to differences in bedding surface. Cows lie longer on dry *versus* wet bedding surfaces (Fregonesi et al., 2007b) and on deep bedded (sawdust) *versus* inadequately bedded surfaces (Tucker et al., 2003). Lying time depends also on the stocking density (cows/per cubicle) as well as the available space per cow (m² activity area/cow) (Fregonesi et al., 2007a).

Cows with impaired hoof health might also exhibit a modulated lying behaviour with longer lying times per bout but fewer bouts per day. This is probably due to the animals avoiding the transition from lying to standing (Chapinal et al., 2009; Ito et al., 2010). Clinical mastitis might reduce lying times because of cows' discomfort (Tucker et al., 2021). Westin et al. (2016) observed lower lying times in cows with lower BCS scores, probably due to greater time periods spent for feed intake (Munksgaard et al., 2005).

In contrast, the effect of feed characteristics on the lying behaviour of cows has so far scarcely been investigated. Cows on pasture generally exhibit shorter lying times than cows housed indoors (Ito et al., 2014; Tucker et al., 2007), especially when pasture allowance is limited (O'Driscoll et al., 2019). Little is known about whether lying behaviour may also be modulated by feed characteristics when cows are housed indoors.

In organic dairying, it is a common practice to feed high-forage diets to dairy cows during the winter feeding period. High-forage diets require longer eating times as compared to concentrate-rich rations, typically fed in high-input/high-

output dairy production systems (Susenbeth et al., 2004, 1998). As a consequence, cows' lying time budget might be more limited than when cows are fed concentrate-rich rations. In addition, high levels of forages being ingested in a given time period may increase distention of the rumen wall (Allen, 1996) and induce discomfort. Tucker et al. (2009) hypothesized, that cows prefer the left lying side as a relief to balance a full rumen after eating. However, most of the studies addressing laterality provide no information on feed intake. Pucora et al. (2019) noted that the gravity vector of the rumen fill as well as the digestive anatomy of ruminants affect their lying side. Non-feed-associated reasons for laterality of lying, such as the stage of pregnancy of dairy cows or the slope of the lying surface, have been studied by Arave and Walters (1980) and Forsberg et al. (2008).

Our working group has recently shown that both particle size reduction (Haselmann et al., 2019) and preservation method of forages (Haselmann et al., 2020) affect the eating behaviour and feed intake of cows fed a high-forage diet (≥ 80% of dry matter). The aim of this study was to investigate whether these effects correspond with changes in lying behaviour, with respect to total lying time, number of lying bouts, laterality of lying, and rumination time with respect to the cows' body position.

Animals, Materials and Methods

This publication presents the results gathered in the course of 2 feeding trials.

Trial 1 was conducted from January to March 2018, using 20 lactating Holstein cows (Haselmann et al., 2019) and Trial 2 from January to March 2019, using 18

lactating Holstein cows (Haselmann et al., 2020). Both trials were approved by the Institutional Ethics and Animal Welfare Committee of the University of Veterinary Medicine Vienna in accordance with good scientific practice guidelines and national legislation (protocol no. ETK-07/10/2017 and ETK-19/11/2018, respectively). The companion papers give information about chemical composition of feeds, feed intake behaviour, feed intake, and performance of dairy cows.

The trials were conducted at the organic farm of the Secondary School for Agriculture, HBLA Ursprung, Elixhausen, Austria. In Trial 1, cows were kept in a free-stall housing system with a total of 27 cubicles, resulting in a cubicle:cow ratio of 1.35 and 6.65 m² of available space per cow. The cubicles were distributed in 3 rows. Those facing each other had a length of 2.30 m and those facing the wall had a length of 2.5 m. The lying area in the cubicles had a length of 1.85 m and a width of 1.2 m. The cubicle base was a straw mattress (20-30 cm deep) which also contained some ground limestone. Cubicles were cleaned and maintained, e.g. by adding fresh straw, on a daily basis. The width of the alley along the feed bunk was 3.20 m and 2.40 m in the alley in the resting area. The alleys were covered with textured rubber and cleaned with automatic scrapers several times a day. The feeding area was equipped with CALAN gates in order to guarantee individual feeding of cows.

In Trial 2, cows were kept in the same barn, but the two feeding groups were separated from each other using gates, to avoid animals reaching the feed of the respective other treatment. This separation, resulting in two barn areas with 14 and 13 cubicles, was introduced at the onset of the trial, after cows had been assigned to the respective feeding

groups. The cubicle:cow ratio amounted to 1.44 and 1.55, and space allowance in the activity area was 7.3 and 7.5 m²/cow for the SILAGE and HAY groups, respectively.

Experimental Design and Treatment

Both trials were performed under *ceteris paribus* conditions with respect to ration composition, feeding management, and housing conditions. Trials started with a covariate feeding period (roughly five weeks), followed by the experimental feeding period (five weeks), including a two-weeks adaptation period. In week 3 of the covariate feeding period, cows were assigned to one of the two feeding groups according to their previous milk yield, body weight, days in milk, and parity. Cows were equipped with a transponder which enabled them to open an individually assigned CALAN gate (American CALAN Inc., Northwood, NH, USA). Cows were allowed to adapt to the altered conditions for two weeks. During the covariate feeding periods, all cows received a TMR containing hay (grass-based or artificially-dried alfalfa), silage (grass or clover-based), corn silage, and a commercially available concentrate, in approximately equal shares (DM basis), respectively. For the experimental feeding periods cows were then switched to the respective experimental dietary treatments:

In Trial 1, feeding groups either received forages with LONG or SHORT particles included in a TMR at a level of 80% (DM basis). The particle size of the LONG ration was set by mixing all components (43% grass hay, 37% clover-grass silage and 20% concentrate; DM basis) for exactly 20 min in a vertical feed mixer. The SHORT ration was identical but hay and silage were chopped to a theoretical cut

length of 0.5 cm and hay was additionally hammer-milled using a 2-cm sieve, before being introduced into the feed mixer. As a result, the geometric mean length of the forage particles was 52 mm (LONG) and 7 mm (SHORT) (Haselmann et al., 2019).

In Trial 2, feeding groups received either SILAGE or HAY derived from the same parent plant material. A pelleted concentrate was offered to each cow manually three times a day (in total 3.6 kg/d; DM basis). Parent grass forages for both preservation methods were mowed at the same time, treated equally on the field, and windrows were harvested alternately with a loading wagon for either ensiling or barndrying (Haselmann et al., 2020).

In both trials, cows were allowed for at least 11% feed refusals (DM basis), and fresh feed was offered several times per day.

Data collection and calculations

Data collection took place during the last week of the covariate feeding period as well as weeks 3 to 5 of the experimental feeding period. Lying behaviour of cows was recorded using HOBO® Pendant G Acceleration data loggers (Onset Computer Corporation, Pocasset, MA, USA; dimensions: 33 mm high \times 60 mm wide \times 25 mm deep; weight: approximately 17 g) which were validated by Ledgerwood et al. (2010).

Loggers were programmed to record data in a 30-s interval, wrapped in cling film, and fitted in rubber wrappers. Loggers were attached on cows' left or right metatarsi (middle) using Vet Wrap (Wahl GmbH, Dietmannsried, Germany).

Recording of lying data started approximately 18 h after attaching the loggers to

the cows' legs. In the covariate feeding period, it lasted for three consecutive days in both trials to ensure reliable lying behaviour data (Ito et al., 2009). In the experimental feeding period of Trial 1, data were recorded two times for five consecutive days, resulting in 10 observation days per cow. In Trial 2, data were recorded once for five consecutive days per cow. HOBO logger data were exported via an USB adapter and the Onset HOBOware® Lite Software Version 3.0.0 (Onset Computer Corporation, Pocasset, MA, USA) on the computer, and summarized using the algorithms of UBC AWP (2013) in SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA) and Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA).

Parallel to the lying behaviour, chewing behaviour (eating and rumination time) was recorded with RumiWatch halters (ITIN Hoch, Liestal, Switzerland). The operation mode of the HOBO loggers and a validation of this system were published by Kröger et al. (2016) and Rombach et al. (2018). Recorded data of the RumiWatch halters were also transferred to the computer via USB and summarized using the RumiWatch converter (version no. V0.7.4.13) and Microsoft Excel. The data on chewing behaviour have already been reported in Haselmann et al. (2020; 2019). Here, we present the rumination time depending on body position (i.e., standing or lying side). Therefore, we merged the RumiWatch data with those of the HOBO loggers on a 10-min basis, respectively. Rumination was assigned to the respective body position if the recorded position was performed for longer than five min per interval.

Statistical Analyses

Statistical analyses were performed with SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) using linear mixed models. Data were checked for normal distribution and the presence of outliers. The statistical model included the effects of the experimental ration (LONG versus SHORT; Trial 1) or (SILAGE versus HAY; Trial 2), the placement of the logger (left or right leg), and, as a covariate, the corresponding value of the response variable from the covariate feeding period. In Trial 1, cow ID within group was additionally included to account for repeated measurements during the experimental period. In Trial 2, repeated measurements were considered by introducing an effect of the cow ID within group and wearing period of the loggers and the group was additionally included as a crossed random effect, to consider that the SILAGE and HAY treatment was randomly assigned to the two separated groups. A heterogenous first-order autoregressive covariance structure (total lying bouts, left and right lying bouts) or a first-order autoregressive covariance structure (all other parameters) was chosen based on model fit according to the Akaike information criterion. To investigate whether the proportion of lying time on the right or left side differed significantly from the expected 50% (i.e., null hypothesis), a one-sample t-test was performed for each treatment group separately. The level of significance was set at $P \le 0.05$ and $0.05 < P \le 0.10$ was interpreted as indicating a tendency.

Results

Trial 1

Cows consuming the SHORT ration spent more time lying and less time standing, with 1.1 h/d difference (P = 0.003) (Table 1). Cows on the SHORT ration also lay

more on the right side than cows fed the LONG ration (+ 0.67 h/d; P = 0.048). The time spent lying on the left side increased only numerically (+ 0.2 h/d; P = 0.561). Furthermore, SHORT-fed cows showed also a trend towards more daily lying bouts (+ 3 bouts/d; P = 0.090), primarily on the right (+ 1.9; P = 0.024) as compared to the left side (+ 1.29; P = 0.198).

However, the percentage distribution of lying time on the left *versus* right side was not affected (P = 0.692). With an average of 57.4%, more time was spent on the left side than expected by chance (t-test: P < 0.001).

Cows in both groups ruminated on average longer while lying (72%) than while standing (28%) (Table 1). The percentage distribution of ruminating time on the left (57%) *versus* right lying side (43%) followed the same pattern as was laterality of lying in both groups.

Trial 2

Standing and lying time did not differ between both groups (Table 2). Number of daily lying bouts was lower in cows consuming HAY as compared to SILAGE (-2.52 bouts/day; P = 0.039). Lying time on the left side tended to be shorter in cows consuming HAY (-1.1 h/d; P = 0.098). In turn, for the right side we observed only a numerical increase (+0.71 h/d; P = 0.231). As a result, the percentage distribution of lying times tended to be different between groups for the left (P = 0.062) and right side (P = 0.053), respectively. Cows on SILAGE tended to prefer the left side (55.6%; t-test: P = 0.069) while cows fed with HAY had similar proportions of time spent on the left versus the right side (t-test: P = 0.750).

Again, also in Trial 2, rumination occurred mainly while lying (on average 79%; Table 2). Cows fed with SILAGE ruminated 55% of the time on the left and 45% of the time on the right side. Nearly the opposite pattern, namely 46% and 54%, respectively, were observed for cows on HAY. Rumination time on the left side tended to be lower in cows on HAY (- 0.61 h/d; P = 0.079). The opposite, though not significant pattern, was observed for the right side (+ 0.58 h/d; P = 0.130).

Discussion

This is the first study addressing the effects of physical characteristics of forages (particle size or preservation method) on the lying behaviour of organic dairy cows. Cows on the SHORT ration spent more time lying than cows provided a LONG ration (Trial 1). The preservation method of forages (Trial 2) had no effect on total lying time. However, cows on HAY had a lower number of daily lying bouts and spent similar proportions of the lying time on the left and right side. In contrast, cows on SILAGE preferred the left side when lying.

Lying time and lying bouts

In both trials, lying time reached a high level compared with the range presented in the comprehensive review by Tucker et al. (2021). The greater lying time in cows on the SHORT ration was mainly due to additional lying bouts (P = 0.090), while in Trial 2 cows consuming SILAGE had significantly more lying bouts per day compared to those on HAY. For the statistical model of the lying bouts, a heterogenous first-order autoregressive covariance structure was chosen, whereas for lying time, a first-order autoregressive covariance structure was used. The heterogenous covariance structure assumes that between-subject variance changes

over the recording time (Littell et al., 2006). Such variation over days could stem from changes in the eating pattern. Rivera-Chacon et al. (personal communication) showed that reduced feed bunk visits go along with a higher number of daily lying bouts when cows were switched from an all-forage to a high-concentrate (65% concentrate; DM basis). In order to elucidate the associations between the daily pattern of the lying and eating behaviour, the number of meals, visits to the feed bunk, and meal bout duration should be also considered.

Interestingly, comparing the differences in lying time in Trial 1 with those for eating time, cows on the SHORT ration reduced eating time (- 1.15 h/d; Haselmann et al., 2019) and, in turn, extended lying time (+ 1.1 h/d). It appeared that cows spent the time saved on eating, i.e. freed-up time, on additional lying time. A compensatory relationship between eating times and lying times have also been reported by Dohme-Meier et al. (2014) and Munksgaard et al. (2005). In Trial 2, cows consuming SILAGE or HAY showed similar eating times, and, accordingly, lying times also hardly differed between groups (Haselmann et al., 2020).

On the other hand, changes in the cows' physiological state could be also responsible for the longer lying times in cows on the SHORT ration. Westin et al. (2016) reported that high body condition scores are associated with increased lying times. Indeed, we reported a more positive energy balance and a significant higher body weight gain in cows on the SHORT ration (Haselmann et al., 2019).

In conclusion, it could be suggested that feeding a ration with a reduced forage particle size frees up the cows' time budget. Under the current conditions, it could be assumed that cows' time budget for lying was rather constrained. In our companion paper, we reported that chewing times were near the physiological maximum in both trials (Haselmann et al., 2020, 2019). Due to the feeding of high-forage diets, cows may have expended high mastication efforts, which was reflected in elevated eating times (Susenbeth et al., 2004). Overall, these cows spent about 17-18 h per day on lying and eating, supporting the assumption that time was limited.

Laterality of lying behaviour

Similar to cows in Trial 1 (both groups), cows on SILAGE showed also a pronounced preference for the left lying side. An effect of a late stage of pregnancy (9th month; Arave and Walters, 1979; Forsberg et al., 2008) can be excluded, since cows were, depending on the experimental group, on average between 45 and 78 days pregnant at the beginning of the experiments. A further reason for the preference of a specific lying side may be the housing conditions (Phillips et al., 2003). Tucker et al. (2009) suggested that the preference for a specific lying side is generally more pronounced in free-stall housing systems than in deep-bedded pack systems, because choice of lying behaviour is restricted. In the current study, individual characteristics of cows' lying behaviour were considered via the inclusion of data of the covariate feeding period. The stage of pregnancy as well as the housing conditions were thus controlled for, suggesting that the observed effects were induced by the diet. Tucker et al., (2009) speculated that cows prefer the left lying side in order to balance a full rumen, probably as a

reaction to relieve discomfort. The high average daily dry matter intake of roughly 22 ± 0.69 kg (average of both trials) in both groups supports this assumption.

In contrast to the cows' overall preference for the left lying side, cows on HAY showed no side preference. Although the two trials are not directly comparable, it is noteworthy that cows on HAY also had the lowest level in number of daily lying bouts compared to the other treatment groups (i.e., SILAGE, LONG, SHORT). Thus, it appears that cows on HAY avoided the left lying side. Although not precisely determined, we anecdotally observed that the left paralumbar fossa was less deep (or even flat) in cows on HAY as compared to cows on SILAGE. This suggests a greater distension of the rumen wall in HAYfed cows. The early studies by Waldo et al. (1965) suggested that intake of silage is not restricted by the rumen capacity. In our study we observed a greater dry matter intake in HAY-fed cows (+ 0.5 kg/d; Haselmann et al., 2020). Due to the 54% higher content of water-soluble carbohydrates in HAY as compared to SILAGE (Haselmann et al., 2020) the gaseous phase in the rumen may have differed and hence also distension of the rumen wall. *In-vitro* studies by Hindrichsen and Kreuzer (2009) have shown that water-soluble carbohydrates (e.g. sugar) elevate the gas production by rumen microbes. Furthermore, the specific characteristics of HAY, such as the greater water-holding capacity and/or the smaller initial particle size in the ingested feed boli, could have contributed to changes in the formation and thickness of the rumen mat (Schadt et al., 2012; Wattiaux et al., 1992; Zebeli et al., 2012). Hypothetically, these effects could have increased the distension of the rumen wall and caused greater discomfort in cows when lying on the left side. Similar observations were reported by Grant et al.

(1990) for ruminally-cannulated animals, which avoided lying on the left side (i.e., where the cannula was located).

Body position during rumination

Body position of cows while ruminating was investigated by merging the data of lying behaviour and ruminating time on a 10-min basis. Cows spent roughly 72% and 79% of their lying time ruminating in Trials 1 and 2, respectively. While lying, cows generally preferred the left lying side for rumination and time proportions followed nearly the same pattern as observed for laterality of lying. For example, cows on the LONG ration lay 57.9% of the time on the left side and 56.7% of the total rumination time was also performed in this position. Similar values were observed for cows on the SHORT ration and cows on SILAGE. A preference towards the left lying side for ruminating is in accordance to the study by Grant et al. (1990). In contrast, cows on HAY showed a preference towards the right lying side when ruminating (54%) but not with respect to the overall lying time.

General considerations and outlook

With regard to animal welfare, longer lying times together with a greater number of lying bouts (Trial 1) appear to contribute positively to the animals' wellbeing. However, baseline lying times were fairly high (Tucker et al., 2021) and therefore the improvement achieved through a further increase in lying time may be limited. Under standard commercial conditions, cows' lying times are often lower due to a narrower cubicle:cow ratio, and, especially in larger herds, cows expending also more time for access to milking, which further reduces the time available for lying

(Tucker et al., 2021). In this case, the observed increase in lying time associated with shorter particle size may have a more positive effect on animal welfare than under the conditions studied.

However, feeding relatively short feed particles in a homogeneous mixture (i.e., TMR) restricts the animals' need for feed selection (Leiber et al., 2020) and may therefore impair welfare. Satisfying this need may at least partly be achieved by (additionally) providing long, un-chopped forage such as silage or hay or access to pasture.

Whether the choice of a specific lying side affects the cows' welfare remains unclear. In a meta-analysis of six studies, Tucker et al. (2009) found no evidence for a preferred lying side, but individual animals may show pronounced preferences. Philipps et al (2003) observed normally-distributed laterality in an extensively managed dairy herd and non-normally-distributed laterality in cows housed indoors. Although it has been argued that extreme dominance in laterality may indicate discomfort in cows (Forsberg et al., 2008; Grant et al., 1990; Tucker et al., 2009), it remains unclear whether this is associated with physiological stress. If so, diet-induced changes on laterality might be considered in the data interpretation of commercially available lying behaviour sensors in the future (Stygar et al., 2021).

In addition, it should be noted that the effects were investigated with Holstein cows which tend to be subject to pressure to increase feed intake because of their high potential for milk synthesis (Chase, 1993). On average, cows yielded about 29 ± 1.15 kg/d, which is comparatively high for cows on high-forage diets.

Further studies should investigate these effects in other breeds such as dualpurpose dairy cattle (Simmental), whose dry matter intake is less driven by the
milk yield potential. It would also be interesting to assess, whether effects on
laterality of lying behaviour could be observed on herd-level using farms feeding
hay as the sole forage compared to farms feeding silage in the winter feeding
period.

Conclusion

The present study demonstrated that the characteristics of high-forage diets impact cows' available time budget for lying behaviour. Under the conditions studied, a reduced particle size of forages resulted in a longer total lying time. The preservation method for forages did not affect lying times. However, feeding SILAGE resulted in a greater proportion of time lying on the left side whereas cows on HAY did not show a preference for a specific lying side. The effects might stem from dietary-induced changes on eating behaviour as well as the amount of feed being ingested in a given time period. Further studies are necessary to elucidate the relation between the daily patterns of eating and lying behaviour and its consequences on (forage) dry matter intake.

Acknowledgements

The authors are grateful to the Austrian Federal Ministry for Sustainability and Tourism, the Provincial Government of Salzburg (Austria), Raiffeisenverband Salzburg, and the "Ja! Natürlich!" brand of the REWE group (Wiener Neudorf, Austria) for funding this experiment. In addition, we are thankful to the Secondary School for Agriculture, HBLA Ursprung, Elixhausen, Austria, for housing the

experiment, to the farm staff, especially Franz Griessner and Florian Gollhofer. Birgit Fuerst-Waltl and Christoph Winckler cooperated within the framework of the ERA-NET Core Organic Cofund project ProYoungStock. The corresponding author, Andreas Haselmann, is especially grateful to the Research Institute of Organic Agriculture (FiBL Switzerland) for the working time provided to write this publication. We would also like to thank Kathleen Knaus for editing assistance.

References

- Arave, C.W., Walters, J.L., 1980. Factors affecting lying behavior and stall utilization of dairy cattle. Applied Animal Ethology 6, 369–376. https://doi.org/10.1016/0304-3762(80)90137-6.
- Chapinal, N., de Passillé, A.M., Weary, D.M., von Keyserlingk, M.A.G., Rushen, J., 2009. Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. Journal of Dairy Science 92, 4365–4374. https://doi.org/10.3168/jds.2009-2115.
- Chase, L.E., 1993. Developing nutrition programs for high producing dairy herds. Journal of Dairy Science 76, 3287–3293. https://doi.org/10.3168/jds.S0022-0302(93)77665-1.
- Dohme-Meier, F., Kaufmann, L.D., Görs, S., Junghans, P., Metges, C.C., van Dorland, H.A., Bruckmaier, R.M., Münger, A., 2014. Comparison of energy expenditure, eating pattern and physical activity of grazing and zero-grazing

- dairy cows at different time points during lactation. Livestock Science 162, 86–96. https://doi.org/10.1016/J.LIVSCI.2014.01.006.
- Forsberg, A.M., Pettersson, G., Ljungberg, T., Svennersten-Sjaunja, K., 2008. A brief note about cow lying behaviour-Do cows choose left and right lying side equally? Applied Animal Behaviour Science 114, 32–36.

 https://doi.org/10.1016/j.applanim.2008.02.008.
- Fregonesi, J.A., Tucker, C.B., Weary, D.M., 2007a. Overstocking reduces lying time in dairy cows. Journal of Dairy Science 90, 3349–3354. https://doi.org/10.3168/jds.2006-794.
- Fregonesi, J.A., Veira, D.M., von Keyserlingk, M.A.G., Weary, D.M., 2007b.

 Effects of bedding quality on lying behavior of dairy cows. Journal of Dairy

 Science 90, 5468–5472. https://doi.org/10.3168/jds.2007-0494.
- Grant, R.J., Colenbrander, V.F., Albright, J.L., 1990. Effect of particle size of forage and rumen cannulation upon chewing activity and laterality in dairy cows. Journal of Dairy Science 73, 3158–3164. https://doi.org/10.3168/jds.s0022-0302(90)79005-4.
- Haselmann, A., Wenter, M., Fuerst-Waltl, B., Zollitsch, W., Zebeli, Q., Knaus, W., 2020. Comparing the effects of silage and hay from similar parent grass forages on organic dairy cows' feeding behavior, feed intake and performance. Animal Feed Science and Technology 267, 114560 available online 04 June 2020. https://doi.org/10.1016/j.anifeedsci.2020.114560.

- Haselmann, A., Zehetgruber, K., Fuerst-Waltl, B., Zollitsch, W., Knaus, W., Zebeli, Q., 2019. Feeding forages with reduced particle size in a total mixed ration improves feed intake, total-tract digestibility, and performance of organic dairy cows. Journal of Dairy Science 102, 8839–8849. https://doi.org/10.3168/JDS.2018-16191.
- Hindrichsen, I.K., Kreuzer, M., 2009. High methanogenic potential of sucrose compared with starch at high ruminal pH. Journal of Animal Physiology and Animal Nutrition (Berl) 93, 61–65. https://doi.org/10.1111/j.1439-0396.2007.00779.x.
- Ito, K., Chapinal, N., Weary, D.M., von Keyserlingk, M.A.G., 2014. Associations between herd-level factors and lying behavior of freestall-housed dairy cows. Journal of Dairy Science 97, 2081–2089. https://doi.org/10.3168/jds.2013-6861.
- Ito, K., von Keyserlingk, M.A.G., LeBlanc, S.J., Weary, D.M., 2010. Lying behavior as an indicator of lameness in dairy cows. Journal of Dairy Science 93, 3553–3560. https://doi.org/10.3168/jds.2009-2951.
- Ito, K., Weary, D.M., von Keyserlingk, M.A.G., 2009. Lying behavior: Assessing within- and between- herd variation in free-stall-housed dairy cows. Journal of Dairy Science 92, 4412–4420. https://doi.org/10.3168/jds.2009-2235.
- Jensen, M.B., Pedersen, L.J., Munksgaard, L., 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as

- measured by demand functions. Applied Animal Behaviour Science 90, 207–217. https://doi.org/10.1016/j.applanim.2004.08.006.
- Kröger, I., Humer, E., Neubauer, V., Kraft, N., Ertl, P., Zebeli, Q., 2016.
 Validation of a noseband sensor system for monitoring ruminating activity in cows under different feeding regimens. Livestock Science 193, 118–122.
 https://doi.org/10.1016/j.livsci.2016.10.007.
- Ledgerwood, D. N., Winckler, C., Tucker, C.B., 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. Journal of Dairy Science 93, 5129–5139. https://doi.org/10.3168/jds.2009-2945.
- Leiber, F., Walkenhorst, M., Holinger, M., 2020. The relevance of feed diversity and choice in nutrition of ruminant livestock. Journal of Sustainable and Organic Agricultural Systems 70, 35–38. https://doi.org/10.3220/LBF1592393539000.
- Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2006. SAS® for Mixed Models, Second. ed. SAS Institute Inc., Cary, NC.
- Munksgaard, L., Jensen, M.B., Pedersen, L.J., Hansen, S.W., Matthews, L., 2005.

 Quantifying behavioural priorities Effects of time constraints on behaviour of dairy cows, *Bos taurus*. Applied Animal Behaviour Science 92, 3–14. https://doi.org/10.1016/j.applanim.2004.11.005.

- O'Driscoll, K., Lewis, E., Kennedy, E., 2019. Effect of feed allowance at pasture on the lying behaviour of dairy cows. Applied Animal Behaviour Science 213, 40–46. https://doi.org/10.1016/j.applanim.2019.02.002.
- Phillips, C.J.C., Llewellyn, S., Claudia, A., 2003. Laterality in bovine behavior in an extensive partially suckled herd and an intensive dairy herd. Journal of Dairy Science 86, 3167–3173. https://doi.org/10.3168/JDS.S0022-0302(03)73919-8.
- Pucora, E., Schiffmann, C., Clauss, M., 2019. Resting postures in terrestrial mammalian herbivores. Journal of Mammalogy 100, 552–563. https://doi.org/10.1093/jmammal/gyz044.
- Rombach, M., Münger, A., Niederhauser, J., Südekum, K.-H., Schori, F., 2018. Evaluation and validation of an automatic jaw movement recorder (RumiWatch) for ingestive and rumination behaviors of dairy cows during grazing and supplementation. Journal of Dairy Science 101, 2463–2475. https://doi.org/10.3168/JDS.2016-12305.
- Schadt, I., Ferguson, J.D., Azzaro, G., Petriglieri, R., Caccamo, M., van Soest, P., Licitra, G., 2012. How do dairy cows chew? Particle size analysis of selected feeds with different particle length distributions and of respective ingested bolus particles. Journal of Dairy Science 95, 4707–4720. https://doi.org/10.3168/jds.2011-5118.
- Stygar, A.H., Gómez, Y., Berteselli, G. V., Dalla Costa, E., Canali, E., Niemi, J.K., Llonch, P., Pastell, M., 2021. A systematic review on commercially

available and validated sensor technologies for welfare assessment of dairy cattle. Frontiers in Veterinary Science 8. https://doi.org/10.3389/fvets.2021.634338.

- Susenbeth, A., Dickel, T., Südekum, K.-H., Drochner, W., Steingaß, H., 2004.

 Energy requirements of cattle for standing and for ingestion, estimated by a ruminal emptying technique. Journal of Animal Science 82, 129–136.

 https://doi.org/10.2527/2004.821129x.
- Susenbeth, A., Mayer, R., Koehler, B., Neumann, O., 1998. Energy requirement for eating in cattle. Journal of Animal Science 76, 2701–2705. https://doi.org/10.2527/1998.76102701x.
- Tucker, C.B., Cox, N.R., Weary, D.M., Špinka, M., 2009. Laterality of lying behaviour in dairy cattle. Applied Animal Behaviour Science 120, 125–131. https://doi.org/10.1016/j.applanim.2009.05.010.
- Tucker, C.B., Jensen, M.B., de Passillé, A.M., Hänninen, L., Rushen, J., 2021.

 Invited review: Lying time and the welfare of dairy cows. J Dairy Sci 104,

 20–46. https://doi.org/10.3168/jds.2019-18074.
- Tucker, C.B., Rogers, A.R., Verkerk, G.A., Kendall, P.E., Webster, J.R., Matthews, L.R., 2007. Effects of shelter and body condition on the behaviour and physiology of dairy cattle in winter. Applied Animal Behaviour Science 105, 1–13. https://doi.org/10.1016/J.APPLANIM.2006.06.009.

- Tucker, C.B., Weary, D.M., Fraser, D., 2003. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. Journal of Dairy Science 86, 521–529. https://doi.org/10.3168/jds.S0022-0302(03)73630-3.
- UBC AWP, 2013. UBC Animal Welfare Program: SOP HOBO Data Loggers.

 pp. 1 23. University of British Columbia, Vancouver, Canada.
- Waldo, D.R., Miller, R.W., Okamoto, M., Moore, L.A., 1965. Ruminant utilization of silage in relation to hay, pellets, and hay plus grain. II. Rumen content, dry matter passage, and water intake. Journal of Dairy Science 48, 1473–1480. https://doi.org/10.3168/jds.S0022-0302(65)88501-0.
- Wattiaux, M.A., Mertens, D.R., Satter, L.D., 1992. Kinetics of hydration and effect of liquid uptake on specific gravity of small hay and silage particles.

 Journal of Animal Science 70, 3597–3606.

 https://doi.org/10.2527/1992.70113597x.
- Westin, R., Vaughan, A., de Passillé, A.M., DeVries, T.J., Pajor, E.A., Pellerin,
 D., Siegford, J.M., Vasseur, E., Rushen, J., 2016. Lying times of lactating
 cows on dairy farms with automatic milking systems and the relation to
 lameness, leg lesions, and body condition score. Journal of Dairy Science 99,
 551–561. https://doi.org/10.3168/jds.2015-9737.
- Zebeli, Q., Aschenbach, J.R., Tafaj, M., Boguhn, J., Ametaj, B.N., Drochner, W., 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. Journal of Dairy Science 95, 1041–1056. https://doi.org/10.3168/JDS.2011-4421.

Table 1. Trial 1: Least squares means (\pm SE) for the effects of a reduced forage particle size on the lying and rumination behaviour of dairy cows.

Item	LONG	SHORT	<i>P</i> -value
Standing time (h/d)	12.4 ± 0.24	11.3 ± 0.23	0.003
Lying time (h/d)	11.6 ± 0.24	12.7 ± 0.23	0.003
Left side (h/d)	6.98 ± 0.240	7.18 ± 0.233	0.561
Right side (h/d)	4.91 ± 0.233	5.58 ± 0.222	0.048
Left side (%)	57.9 ± 1.69	56.9 ± 1.60	0.692
Right side (%)	42.1 ± 1.69	43.1 ± 1.60	0.692
Lying bouts (n/d)	11.7 ± 1.24	14.8 ± 1.19	0.090
Left	6.78 ± 0.674	8.07 ± 0.643	0.198
Right	$\begin{array}{c} 4.89 \pm \\ 0.568 \end{array}$	6.79 ± 0.553	0.024

Lying time per bout (h/d)

Left	1.41 ± 0.093	1.21 ± 0.090	0.134
Right	1.25 ± 0.119	1.06 ± 0.115	0.269
Rumination behaviour (h/d)			c.
Standing	2.22 ± 0.228	2.68 ± 0.263	0.186
Left lying side	3.52 ± 0.170	3.70 ± 0.212	0.540
Right lying side	2.68 ± 0.277	2.80 ± 0.368	0.833

Table 2. Trial 2: Least squares means $(\pm$ SE) for the effects of the forage preservation method on the lying and rumination behaviour of dairy cows.

Item	SILAGE	НАҮ	<i>P</i> -value
Standing time (h/d)	11.1 ± 0.39	11.4 ± 0.40	0.607
Lying time (h/d)	12.9 ± 0.39	12.6 ± 0.40	0.608
Left side (h/d)	7.31 ± 0.463	6.21 ± 0.456	0.098

Right side (h/d)	5.83 ± 0.386	6.54 ± 0.396	0.213
Left side (%)	55.6 ± 2.35	49.1 ± 2.32	0.062
Right side (%)	45.0 ± 2.221	51.6 ± 2.28	0.053
Lying bouts (n/d)	10.2 ± 0.78	7.68 ± 0.774	0.039
Left	5.62 ± 0.973	3.56 ± 0.963	0.135
Right	4.88 ± 0.551	4.24 ± 0.570	0.439
Lying time per bout (h/d)			
Left	1.40 ± 0.136	1.48 ± 0.132	0.683
Right	1.26 ± 0.239	1.58 ± 0.241	0.355
Rumination behaviour (h/d)			
Standing	1.98 ± 0.173	1.70 ± 0.177	0.270
Left lying side	3.78 ± 0.230	3.17 ± 0.237	0.079

Right lying side

 3.15 ± 0.255

 3.73 ± 0.260

0.130