Livestock Science 251 (2021) 104662

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Evaluation of production parameters, feed sorting behaviour and social interactions in dairy cows: Comparison of two total mixed rations with different particle size and water content

Cecilia Kronqvist, Frida Petters, Ulrica Robertsson, Mikaela Lindberg *

Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, P.O. Box 7024, SE-750 07 Uppsala, Sweden

HIGHLIGHTS

- Compact TMR reduced feed intake and eating time compared with conventional TMR.
- Milk yield was not affected by dietary treatment.
- Less sorting behaviour was observed in cows fed a compact TMR.
- Antagonistic behaviours were less frequent amongst cows fed a compact TMR.
- Cows fed a compact TMR spent more time resting than cows fed a conventional TMR.

ARTICLE INFO

Keywords:
TMR
Compact TMR
Behaviour
Dairy cattle
Dry matter intake
Time budget

ABSTRACT

Dairy cow performance is affected by both the nutritional composition and the physical structure of the diet. The structure of total mixed rations (TMR) largely depends on the ingredient composition and structure of forage, dry matter (DM) content, mixing time and type of mixer used. This study investigated feed intake, eating time, milk production, DM digestibility, ruminal pH, time budget, and sorting and antagonistic behaviours in lactating dairy cows fed grass/clover silage and concentrate, either in a TMR or in a compact total mixed ration (CTMR) with decreased particle size and DM content due to addition of water. Two cross-over experiments (3-week periods), each involving 40 lactating dairy cows of the Swedish Red and Holstein breeds, were run simultaneously. Experiment 1 was designed to measure production parameters, feed digestibility and ruminal pH, while Experiment 2 was designed to measure time budget, feed sorting behaviours and antagonistic behaviours at the feed bunk. Diets were fed ad libitum, with the same ingredient composition and a forage:concentrate ratio of 60:40 (DM basis). Decreased particle size in the CTMR diet was achieved by mixing the grass-clover silage in a feed mixer with a vertical auger with knives, before mixing with concentrate in a feed mixer without knives. Decreased DM content in CTMR was achieved by adding water to the mix. Mean DM content of the diet was 51% DM for TMR and 37% DM for CTMR. The results showed lower daily DM intake (26.7 kg DM) and shorter eating time (242 min) in cows fed CTMR than in cows fed TMR (28.5 kg DM and 278 min). Feed digestibility, ruminal pH, daily milk yield (35.1 kg and 35.8 kg for CTMR and TMR, respectively) and milk composition did not differ between the diets. Cows fed CTMR showed less sorting and fewer antagonistic behaviours at feeding than cows fed TMR. The daily time budget of the cows also differed, with cows fed CTMR spending more time resting (+45 min) than cows fed TMR.

1. Introduction

High-producing dairy cows need to be fed energy-dense diets to supply sufficient energy to meet their requirements for milk production. However, raising energy density by increasing starch content in the diet can potentially cause rapid fermentation and low ruminal pH, which has a negative effect on fibre-digesting microorganisms (Russell and Wilson, 1996). Long periods of low ruminal pH can cause health problems such

* Corresponding author.
E-mail address: Mikaela.Lindberg@slu.se (M. Lindberg).

https://doi.org/10.1016/j.livsci.2021.104662
Received 8 July 2020; Received in revised form 7 July 2021; Accepted 2 August 2021
Available online 3 August 2021

as sub-acute ruminal acidosis (SARA), reduced intestinal motility, ruminal ulceration, laminitis and liver abscesses in the cow (Slyter, 1976; Gozho et al., 2005). One way of enabling higher levels of starch in the diet is to use a total mixed ration (TMR), where starch-rich concentrate is mixed with forage before feeding to achieve uniform composition of the material consumed by the cows (Schingoethe, 2017). In comparison with separate feeding of forage and concentrates, this results in a more stable rumen environment, with less fluctuation in pH (Maekawa et al., 2002). Use of TMR feeding is now well established, partly because the increased herd size seen in many countries world-wide facilitates this feeding system by providing more scope to form groups of animals with similar requirements, which can be met by a specific TMR mix (Schingoethe, 2017).

The nutritional composition and the physical structure of the diet both have an impact on cow performance. Nasrollahi et al. (2015) found in a meta-analysis that decreasing forage particle size increased dry matter (DM) intake, neutral detergent fibre (NDF) intake and DM digestibility, but decreased NDF digestibility. Milk yield and milk protein yield increased, while milk fat concentration decreased, as forage particle size decreased. Similar results were found in a recent study by Haselmann et al. (2019), who showed that dairy cows fed high-forage diets with reduced particle size had higher DM intake and milk yield than cows fed the conventional particle size. Other effects of reduced particle size may be shortened time needed for eating and ruminating (Storm and Kristensen, 2010; Nasrollahi et al., 2014), which would give the cow more time to rest. As time is a limited resource for high-yielding dairy cows (Løvendahl and Munksgaard, 2016), this could result in improved animal welfare. In addition, decreased DM content of TMR diets may decrease DM intake (Felton and DeVries, 2010).

As the TMR concept is based upon achieving consistent composition of the diet, the degree of mixing should result in a final structure of the feed that is sufficiently homogeneous to prevent the cows from sorting the different components of the diet from each other. However, sorting of TMR diets still occurs to varying extents in commercial herds applying TMR feeding systems (Sova et al., 2013). Sorting of the feed affects the individual nutrient intake. Cows typically sort against longer feed particles, so they consume a feed ration that contains more easily soluble carbohydrates and less fibre than intended (Leonardi and Armentano, 2003; Leonardi et al., 2005; Miller-Cushon and DeVries, 2009). There are indications that this sorting of the diet is associated with increased risk of metabolic diseases (DeVries et al., 2008; Jurkovich et al., 2019). Several studies have shown that particle size and DM content in TMR can affect the possibility for cows to sort the diet (Leonardi and Amentano, 2003; Leonardi et al., 2005; Felton and DeVries, 2010). Findings by DeVries et al. (2008) also indicate that subclinical rumen acidosis can influence the sorting behaviour and extent of sorting by individual cows. Sorting can thus affect the diet of individual cows within a group.

Compact TMR (CTMR), a recently developed feed preparation concept, is claimed to reduce variations in ruminal pH, by decreased sorting of feed, and to decrease the time spent at the feed table, thereby allowing the cows to rest more. This concept involves prolonged mixing of the diet, together with soaking the concentrate fraction in added water to improve adhesion of concentrate particles to the larger forage particles. Compact TMR diets have been used in Denmark for some years, and there are indications that the method may result in improvements in milk yield on commercial farms (Kristensen, 2015).

The aim of this study was to determine the effect of feeding lactating dairy cows a diet processed to be similar to CTMR, by prolonged mixing of grass/clover silage and addition of water, in comparison with a conventional TMR with the same ingredients. The measured outcomes were total feed DM intake, eating time, milk production, DM digestibility, ruminal pH, time budget, and sorting and antagonistic behaviours at feeding. The hypothesis was that the diet with decreased DM content and particle size would reduce sorting behaviours and eating time, decrease antagonistic interactions, increase resting time, and improve milk production and digestibility, without altering feed intake or ruminal pH.

2. Material and methods

2.1. Animals and experimental design

The study was conducted during six weeks in October-November 2017 in the research herd at Lövsta National Livestock Research Centre, Uppsala, Sweden. All animal experiments were carried out in accordance with Swedish regulations and complied with EU Directive 2010/63/EU for animal experiments. The local ethics committee in Uppsala approved the animal trial procedures used (ref. 5.8.18-08023/2017). A total of 80 lactating dairy cows of the Swedish Red and Swedish Holstein breeds in their first to seventh lactation were used. Before the trial, the cows were kept in the same barn and fed concentrate according to production in automatic dispensers and grass/clover silage mixed with straw ad libitum in feed troughs or on feed tables.

Two separate experiments were conducted simultaneously, with the same dietary treatments offered to different groups of cows using different feeding equipment. Experiment 1 evaluated the effect of diet on DM intake, eating time, milk production and composition, DM digestibility and ruminal pH. Experiment 2 evaluated sorting behaviour, time budget and antagonistic social interactions at the feed table in cows fed the two experimental diets. Both experiments had a cross-over design, where 40 cows in each experiment were divided into two equal-sized groups that were randomly assigned to each treatment (TMR, CTMR) during two experimental periods. Experimental periods were 21 days in duration (14 days of treatment adaptation and 7 days of data collection) and after the first period, the cows changed diet for the second period.

Experiment 1 involved 14 primiparous cows and 26 multiparous cows, with 18 of the cows of Holstein breed and 22 of the Swedish Red breed. Cows were on average 48 (range 17–86) days in milk (DIM) at the start of the experiment. Similarly, Experiment 2 involved 13 primiparous and 27 multiparous cows, of which 12 were Holstein and 28 Swedish Red. At the start of the experiment the cows in Experiment 2 were at 106 (range: 73–160) DIM. In Experiment 1, all cows were fitted with transponders to record milk yield and feed intake and four of the cows had ruminal cannulas (Bar Diamond Inc., Parma, ID, USA). The cannulated cows were all second lactation or older, all had their surgery at least 4 weeks prior to the experiment, and were fully recovered and considered healthy at the start of the experiment. Cows were blocked according to parity (first or older) and days in milk (DIM), with four to seven cows per block. The cannulated animals formed a separate block, resulting in seven blocks in total. Within each block, the treatment order was randomly assigned to the animals, so that in total 20 animals received each diet during each experimental period. In addition to the 40 experimental cows, 18 non-experimental cows used the same barn area and feeding facilities during the study period.

In Experiment 2, 40 mid-lactation cows were fed in two groups, one on each side of a feed table, i.e. housed in two compartments sharing one feed bunk. The cows were blocked according to parity (first or older) and DIM, resulting in six blocks with six or seven cows in each block. Within each block, treatment order was randomly assigned to the animals so that in total 20 animals received each treatment during each experimental period. In addition to the 20 cows assigned to each treatment, 41 and 39 other cows in each group were not included in the experiment, but used the same housing and feeding facilities during the study period, so that each compartment housed around 60 cows. Cows from both experiments were milked twice daily (starting at 06:00 h and 16:00 h) in an automatic rotary system (DeLaval AB, Tumba, Sweden).

2.2. Experimental feeds and feeding

The ingredient composition of the diets offered in both experiments was the same. Both diets consisted of 60% grass/clover silage and 40%

concentrate (DM basis). The grass/clover silage was a second-cut ley consisting of timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and approximately 100 g/kg DM of red clover (*Trifolium pratense* L.), harvested during summer 2017 with a theoretical chopping length of 20 mm and stored in a bunker silo. The concentrate was in the form of crushed pellets composed of (g/ kg feed) wheat (370), oats (280), soy expeller (140), wheat bran (104), toasted soybeans (70), limestone (13), molasses (10), and minerals and vitamins (13). All feed components were chosen to be allowed in organic and conventional production. The TMR treatment diet consisted of silage and concentrate, mixed together in a stationary mixer without knives (DeLaval Vertical Mixer 10 m^3, DeLaval AB, Tumba, Sweden) for 5 min at 50 rpm and then transported on a conveyor belt to the feed waggon (Experiment 1) and the feeding table (Experiment 2) in the different compartments of the barn. The reason for not having knives in the mixer was to minimise residual feed when emptying. The CTMR treatment diet was prepared using the same ingredients, but the silage was first processed for 60 min in a feed mixer with a vertical auger with knives at 60 rpm (Taarup Silo King Premium 14 m^3, Mayer Maschinenbau GmbH, Tittmoning, Germany) before transfer to the stationary mixer, where it was mixed at 50 rpm for 10 min with concentrate pellets and water to allow some swelling of concentrate pellets in the water. Water was added to adjust the DM content of the total mix to 37%. Using a silage with 40% DM required addition of 28% water of the total DM weight of the mix. This procedure is not considered "standard Compact TMR mixing", but the deviations were required for practical reasons at the research facility.

In Experiment 1, the feed was distributed in 20 troughs placed on scales (CRFI, BioControl A/S, Rakkestad, Norway) with transponder-controlled head gates for the cows (BioControl A/S, Rakkestad, Norway). Each diet was given in the same adjacent troughs during the entire experiment. Six of the troughs contained CTMR and 14 contained TMR, since the non-experimental cows were allowed to eat from the TMR troughs. The cows had access to all troughs within the allotted diet. The feed was distributed from the conveyor belt into an automated feed waggon (FS 1600, DeLaval AB, Tumba, Sweden) and into the troughs 2–3 times daily, aiming at ad libitum access to the feed, and the troughs were emptied and cleaned daily, in the morning. Uneaten feed was not sampled within this experiment. Due to the repeated distribution of feed in troughs over the day and the bucket-like design of the troughs, which held 640 litres, it was assumed that the cows would be prevented from sorting both diets to a measurable extent. The scales under the troughs were calibrated twice weekly.

In Experiment 2, the feed was distributed on a feed table, i.e. an alley separating the barn in two areas where the cows were able to eat from each side. A barrier approximately 60 cm tall was erected along the centre of the feed table to separate the feed for the two treatment groups, which accessed their diets at opposite sides of the feed table through a head locking feed barrier. However, the head locking was not in use during the experiment. The feed was mixed and distributed from the conveyor belt twice daily, aiming at ad libitum access to the feed and maintaining at least 2–3% orts per day. The feed table was cleaned and a sample of the orts was collected daily in the morning (05.30 h), immediately prior to first feeding.

2.3. Data recorded and samplings

Milk yield was automatically recorded at every milking. In each sampling period, milk samples were collected at all four milkings during two consecutive days, preserved with bronopol, kept in a refrigerator at +4 °C and sent to the laboratory for analysis of fat, protein and lactose within five days.

Samples of the silage were collected from the silo twice weekly for DM determination and correction of the water volume added to the CTMR. Samples were also taken five days per week, frozen individually and later pooled for further analysis. Silage was analysed for DM, crude protein (CP), NDF, ash, organic matter digestibility (OMD), metabolisable energy (ME) and acid-insoluble ash (AIA). Samples of the concentrate batches from the feed factory were collected twice during the sampling week, pooled into one sample per sampling week and used for DM, CP, NDF, ash and AIA determination.

In Experiment 1, feed intake was recorded as weight difference of the trough and assigned to the cow whose identification tag was logged in the feed gate. The time between entering and exiting the feed gate was considered "eating time". For digestibility estimation, spot samples of faeces were collected as three grab samples per cow per sampling week (Mehtiö et al., 2016), and frozen individually in plastic bags. Ruminal pH was measured in the cannulated cows throughout the sampling week on a total of 24 hourly occasions per cow and period spread around the clock, so that there was one sample from every hour, although from different days. These measurements were made using a manual pH metre that was calibrated daily during the experiment with pH buffers 7 and 4 (Mettler Toledo, Schwerzenbach, Switzerland). The cannula was opened and a 50 mL Falcon tube was filled with rumen fluid from central rumen, approximately 30 cm below the surface of feed material and liquid, and immediately used for pH measurement.

In Experiment 2, effects of feed sorting, estimated as changes in diet particle size over time, were measured as follows: At feeding, a sample was collected from the conveyor belt immediately before the feed table. When the cows had been given access to the feed for 3 h, all feed on an area of approximately 50–100 cm of the feed table was collected. To decrease the sample size without manipulating the composition of the sample, it was thoroughly mixed and split into quarters, of which two diagonally situated quarters were retained. Mixing and splitting was repeated several times until there were around 5–7 L of feed left, and the sample was then kept for particle size analysis. This sampling procedure was repeated 6 h after feeding in another area of the feed table. To estimate particle size distribution, a Penn State particle separator was used according to Lammers et al. (1996). For the determination, 250 g of feed was used for separation each time, and three repeated separations were made per sample. The three fractions from each separation were weighed, pooled to one sample per feed sample and fraction and placed at 60 °C overnight for DM determination.

Time budget of the 40 focal animals was examined by direct observations in daytime and from video recordings at night. Large numerals were painted on the cows for easy identification. Every 10 min, the observer noted the position and activity of the focal animals. Observation times were spread over the days in the sampling week, but in total a full 24-h day was covered. Sorting behaviour and antagonistic behaviours (see Table 1 for a list) were continually recorded during two 60-min periods on two occasions during each experimental period, commencing at time of feeding and 2 h post feeding.

2.4. Analyses

Samples of silage and concentrate were dried at 60 °C during 10–18 h. Faeces samples were thawed and pooled to one sample per cow during each sampling week. These samples were stored in Petri dishes at −20 °C, then moved to −80 °C prior to freeze-drying. Feed and faeces samples were milled to pass a 1 mm sieve (Kamas, Malmö, Sweden). Dry matter was determined by drying the milled samples at 103 °C for 16 h, followed by ash determination through combustion at 550 °C for 3 h. Feeds were analysed for CP using an automated Kjeldahl procedure (Foss, Hillerød, Denmark) and for NDF according to Chai and Udén (1998). Metabolisable energy content and organic matter digestibility (OMD) in the silage was estimated by the 96-h in vitro digestible OM (VOS) method, as described by Åkerlind et al. (2011) and calculated according to Lindgren (1983). Mineral analyses of forage samples were performed at a commercial laboratory (AgriLab AB, Uppsala Sweden) using inductively coupled plasma emission spectroscopy. Acid-insoluble ash content of the samples were determined using the method of Van Keulen and Young (1977). Milk samples were heated to 37 °C and analysed for fat, protein and lactose using MIRS (CombiScope FTIR 300

Table 1
Behaviours evaluated in Experiment 2.

Behaviour	Definition
Time budget behaviour[1]	
Eating	The cow's head is inside the feed gate
Resting	The cow is lying in a cubicle
Standing in feed area	The cow is standing in the alley in the feeding area
Standing in alley	The cow is standing in an alley (not in the feeding area)
Standing in cubicle	Standing with at least two claws in a cubicle
Drinking	Nose in the water cup
Milking	The cow is not in the house
Ruminating	Chewing without eating
Antagonistic interactions[2]	
Lowered head	The cow lowers the head and/or gores at another cow
Goring	The cow gores another cow's head or body
Pushing	The cow uses a part of the body (not the head) to push another cow
Squeezing	The cow squeezes in between two cows that are eating
Bulldozing	The cow violently approaches a feed space by making all cows in the way move
Blocking	The cow uses her body to keep other cows away from the feed table
Sorting behaviour[2]	
Digging	The cow use her muzzle to move feed aside until a pit is formed
Eating from below	The cow eats from the bottom layers of the feed pile
Eating from the side	The cow stretches her neck to reach more distant feed
Throwing feed	The cow grabs feed and throws up her head, so the feed is thrown through the air

[1]Behaviours used for the time budget examination.
[2]Behaviours used for continuous recordings in the feeding area.

HP, Delta Instruments B.V., Drachten, the Netherlands).

2.5. Calculations and statistical analysis

Data on milk production and feed intake from Experiment 1 were collected automatically at every milking and feeding bout during the sampling weeks, and daily mean values per cow and experimental period were calculated. The proportional daily average concentrations of fat, protein and lactose in milk were calculated based on milk yield at sampling. Feed efficiency was calculated as energy-corrected milk yield (ECM)/DMI. Total eating time in Experiment 1 was calculated as the sum of time for all eating bouts during one day. Apparent total tract digestibility was calculated based on the assumption that total daily intake of AIA in feed and total daily output of AIA in faeces were constant (Huhtanen et al., 1994). Milk yield was recalculated to ECM using the equation developed by Sjaunja et al. (1990). Ruminal pH was plotted as a function of time and total time below pH 5.8 was calculated from the curves. Time budget data were summarised per cow and period and expressed as total time for which the actions were performed during a 24-hour period. Resting was calculated both as the time when the cow was lying down without ruminating and as total time resting, including the time when the cow ruminated in a lying position. Total time ruminating was calculated as the sum of ruminating in standing or lying position. In Experiment 1, one cow was ill for the first period and therefore data for that cow in the first experimental period were excluded from the analyses. Proportion of particles in the different fractions of the diet was calculated based on the sum of DM in all fractions.

Statistical analyses were performed using procedures in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Feed intake, eating time, feed efficiency and milk data were modelled using a mixed model. Block ($n = 7$), breed ($n = 2$), period ($n = 2$), treatment ($n = 2$) and the interaction between period and treatment were used as fixed variables and cow was included as a random factor. Effect of diet on ruminal pH was evaluated using a mixed model where treatment was included as fixed variable, cow as random variable and time since the end of the last meal as covariate. Repeated measures within cow were modelled with an autoregressive covariance structure. Time below ruminal pH of 5.8 was analysed with a model including cow and treatment. Digestibility was analysed in a model accounting for block, breed, period and treatment. Particle distribution of the diets at feeding and 3 h and 6 h after feeding, measured as% of total DM, was modelled including treatment, time of sampling (feeding, 3 h after feeding, 6 h after feeding) and fraction (upper sieve, middle sieve or pan).

The count data from observations on eating behaviour and interactions were modelled in a negative binomial model. Period ($n = 2$), treatment ($n = 2$) and time since feeding ($n = 2$) were used as class variables. Time budget data were modelled with a mixed model, with period ($n = 2$), treatment ($n = 2$) and block ($n = 6$) as fixed factors and cow as a random variable.

3. Results

3.1. Feed and diet

The nutrient composition of the feeds and the calculated composition of the mix of forage:concentrate offered are shown in Table 2. There were differences in particle size distribution between the diets, with TMR having a higher proportion of long (>19 mm) and short (< 8 mm) particles than CTMR (Table 3). However, there were no consistent changes in particle size distribution when comparing the feed at feeding and after 3 h and after 6 h on the feed table (Experiment 2).

In Experiment 1, milk yield, ECM yield and milk composition did not differ between the diets, but total DM intake was lower for cows on the CTMR treatment compared with cows fed TMR (Table 4). Total time spent eating was also lower for cows fed CTMR. Feed efficiency, measured as ECM/DMI, was not different between the diets (Table 4). There were no differences between cows fed the two diets in apparent DM digestibility (Table 4). Average ruminal pH or time below pH 5.8 did

Table 2
Nutrient composition of components in the feedstuffs (g/kg dry matter ± standard deviation unless otherwise stated), dry matter content of the total mixed ration (TMR) and compact TMR (CTMR) and calculated values on a dry matter basis for a mix with forage:concentrate ratio of 60:40 as in the experimental diets.

	Silage	Concentrate*	Mixed ration
Dry matter (g/kg)	398 ± 15	881	513 ± 26 (TMR) 370 ± 20 (CTMR)
Crude protein	161 ± 2.3	193	174
Starch	n.d.	373	149
Crude fat	n.d.	62	25
Neutral detergent fibre (NDF)	454 ± 9.2	211	357
P	2.5 ± 0.07	5.1	3.5
Ca	9.4 ± 0.49	6.8	8.4
K	30.0 ± 1.63	10.4	22.2
Mg	2.2 ± 0.07	3.4	2.7
Na	1.0 ± 0.00	3.1	1.8
S	2.2 ± 0.07	0	1.3
OMD,%	79 ± 5.0[1]	n.d.	–
MJ ME/kg DM	11.4 ± 0.8[1]	13.4	12.2

*Values obtained from feed manufacturer.
[1]Calculated from analysis of VOS (Lindgren, 1983).
n.d., not determined.

Table 3
Particle size distribution in percentage of dry matter for the total mixed ration (TMR) and the compact TMR (CTMR) at feeding, 3 h after feeding and 6 h after feeding. Least squares means ± SEM. Data from 4 days in each sampling period ($n = 8$). Means within rows with different superscripts differ significantly ($P < 0.05$).

	TMR			CTMR		
	Feeding	3h	6h	Feeding	3h	6h
>19 mm	$27^a \pm 1$	$27^a \pm 1$	$25^a \pm 1$	$6^b \pm 1$	$7^b \pm 1$	$5^b \pm 1$
8–19 mm	$30^b \pm 1$	$30^b \pm 1$	$33^b \pm 1$	$60^a \pm 1$	$59^a \pm 1$	$59^a \pm 1$
<8 mm	$43^a \pm 1$	$42^a \pm 1$	$42^a \pm 1$	$33^b \pm 1$	$35^b \pm 1$	$36^b \pm 1$

Table 4
Feed dry matter (DM) intake, milk production, milk composition, DM digestibility, ruminal pH, time below ruminal pH 5.8, time spent eating and feed efficiency (ECM/DMI) in cows fed a total mixed ration (TMR) or compact TMR (CTMR). Data from Experiment 1 ($n = 40$ cows). Data shown as least squares means.

Item	TMR	CTMR	SEM	P-value
Dry matter intake (kg/day)	28.5	26.7	0.6	0.038
ECM* yield (kg/day)	34.2	33.0	0.7	0.259
Milk yield (kg/day)	35.8	35.1	0.8	0.549
Milk fat%	3.82	3.72	0.08	0.373
Milk protein%	3.24	3.24	0.03	0.978
Milk lactose%	4.77	4.77	0.02	0.934
DM digestibility,%	56	57	0.7	0.144
Ruminal pH	5.76	5.75	0.04	0.756
Ruminal pH<5.8 (h)	14.6	14.9	1.6	0.902
Time spent eating (min/day)	278	242	11	0.001
ECM/DMI	1.24	1.23	0.03	0.836

*Energy-corrected milk.

not differ between the diets (Table 4).

3.2. Behavioural data

3.2.1. Feed sorting

Cows displayed significantly more sorting behaviours (Experiment 2) when fed TMR, 42.6 recorded sorting events per hour, compared with CTMR, 16.9 sorting events per hour (LSmeans, SEM = 4.91, $P = 0.004$). The specific sorting behaviours that differed between the treatments were “digging”, 23.4 and 6.8 events per hour for TMR and CTMR cows, respectively (SEM = 3.25, $P = 0.004$), and “eating from below”, 10.1 and 1.0 events per hour, respectively (SEM = 1.11, $P < 0.0001$). The other two sorting behaviours studied, “eating from the side” and “throwing feed”, did not differ in frequency between the diets (Fig. 1).

3.2.2. Antagonistic behaviours

There were more antagonistic interactions when the cows were fed TMR compared with CTMR, 14.8 and 8.5 recorded events per hour, respectively (SEM = 1.30, $P = 0.006$). The number of antagonistic interactions was higher in the TMR group during the first hour after feeding, 17.8 observations per hour, compared with the CTMR group, 11.5 observations per hour. This was also shown in the third hour after feeding, 11.8 and 5.5 observations per hour in the TMR and CTMR group, respectively (SEM = 1.84, $P = 0.04$). The specific behaviours that differed between treatments were “lowering head”, 3.9 and 2.0 events per hour for TMR and CTMR cows, respectively (SEM = 0.58, $P = 0.042$), and “goring”, 5.6 and 2.5 events per hour for TMR and CTMR cows, respectively (SEM = 0.61, $P = 0.004$). These behaviours were also observed at higher frequencies in the third hour after feeding in the TMR group compared with the CTMR group. The frequency of the other behaviours assessed did not differ between treatments or over time (Fig. 2).

3.2.3. Time budget

The data on time budgets of the cows (Table 5) showed that cows fed TMR spent a shorter time resting without ruminating, 6.0 h per 24-hour period, than cows fed CTMR, 6.8 h per 24-hour period. However, total time resting, e.g. lying down in the cubicle whether the cow ruminated or not, did not differ between treatment groups and was 11.0 h and 11.6 h for TMR and CTMR, respectively. Cows fed TMR spent more time standing in the aisle in the feeding area than cows fed CTMR, 62 and 52 min, respectively.

4. Discussion

The present study is to our knowledge the first exploring effects on behaviour and production in dairy cows fed a mix similar to CTMR, which is a feeding strategy used by quite a few commercial dairy farmers, particularly in Denmark. Our hypothesis was partly confirmed by the results, as the cows’ time for rest increased, feed sorting behaviours were reduced and the numbers of antagonistic interactions between cows were lower when feeding CTMR compared with feeding conventional TMR.

4.1. Feed intake and milk production

The results showed differences between the two treatments in feed intake and feeding behaviour. The feeds used in the study are typical in Swedish dairy farming, combining grass/clover silage with purchased concentrate. The concept of CTMR includes three specific targets for the feed mix: uniform particle length < 25 mm, the mix should be free of grass lumps, and there should be no particles separated from the mix (Kristensen, 2015). These criteria were set for the CTMR diet in this study, although for practical reasons the CTMR was prepared somewhat differently than recommended. The extensive mixing of the silage in the CTMR diet resulted, as planned, in fewer long particles (> 19 mm), but also showed lower proportion of the smallest particles (< 8 mm). One possible reason for a decrease in the smallest particle size in CTMR compared to TMR could be the addition of water, reducing DM with 30%, possibly causing small particles to adhere to larger particles. The combination of changed particle size distribution and decrease in DM resulted in decreased feed intake in CTMR cows with 1.8 kg DM, corresponding to 6%. A decrease in long particles could be expected to increase feed intake, as reported by Kononoff and Heinrichs (2003) and Maulfair et al. (2010). However, both these studies had a consistent increase in the smallest particle size fraction corresponding to the decrease in the largest particle size fraction. Miller-Cushon and DeVries (2009) and Felton and DeVries (2010) added water to decrease the DM concentration in TMR diets, and found a positive correlation between DM concentration and DM intake in diets with similar DM concentrations as in the present study. Miller-Cushon and DeVries (2009) concluded that the water-filling effect of TMR diets with addition of water caused differences in DMI. This may be the reason for the lower DMI for the CTMR diet in the present study. Because of the design of the present study it was not possible to distinguish between the effect of particle size and the effect of DM concentration in the diets.

In spite of decreased DMI in cows on the CTMR treatment in the present study, DMI was high in both treatments. Feed intake affects rate of passage of the digesta, i.e. higher intake increases passage rate and thus reduces the digestibility (Colucci et al., 1982). In the present study, we found no differences in DM digestibility between treatments, however the estimated digestibilities were low. The high DMI may have affected the digestibility negatively, and resulted in feed conversion rates (kg ECM/ kg DMI) that were lower than expected for these diets, and not different between treatments. A low feed conversion rate may also indicate a gain in body reserves, but the short experimental periods did not allow a conclusive estimation of this.

We found no differences in milk yield or milk composition between the treatments, in spite of the observed differences in DMI. These results are in line with those from Miller-Cushon and DeVries (2009), who also found no effect on milk yield even if there was differences in DM intake

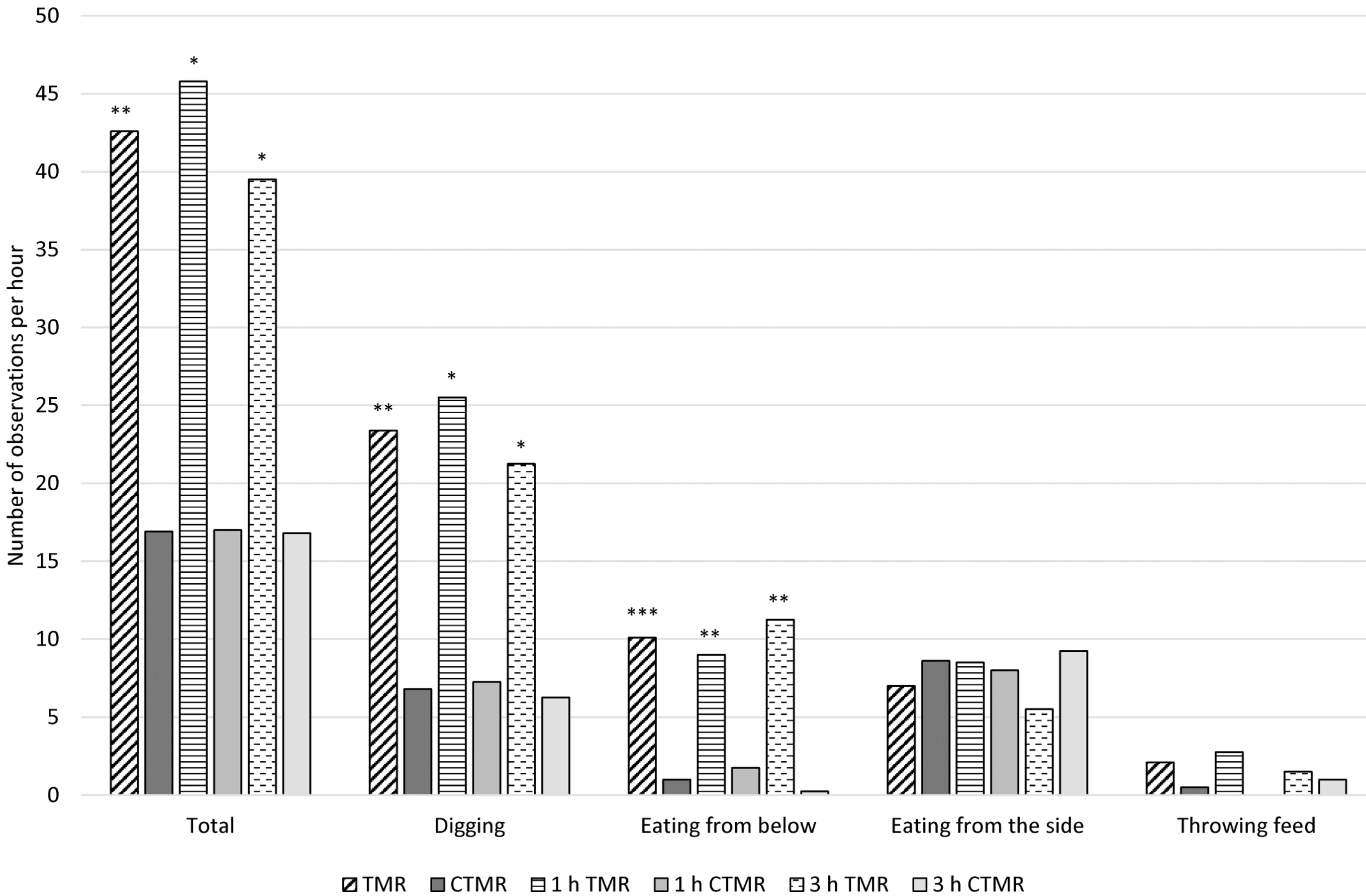

Fig. 1. Least square means of different feed sorting behaviours (observations per hour) of cows fed a total mixed ration (TMR) or compact TMR (CTMR). The bars show the sum of observations per treatment and for 0–1 hour and 2–3 h after feeding. Statistically significant differences between treatments are indicated by asterisks (*P<0.05, **P<0.01, ***P<0.001).

when altering TMR particle structure. In the present study, the nutrient concentration of the rations in combination with the high DMI well covered the nutrient requirements of the cows in both treatments, which can explain why milk yield did not differ. This could have been affected by the production level of the cows, and higher production potential in the cows may have given a different result.

4.2. Eating behaviour

The cows in the present study spent an average of 4 to 5 h per day eating, with similar results in both experiments, which is in line with the 3 to 5 h reported by Grant and Albright (2001). In Experiment 1, the cows fed CTMR had on average 36 min shorter eating time than the cows fed TMR, which can be partly explained by the lower DMI, but may also indicate that the sorting behaviour observed for cows fed TMR in Experiment 2 somewhat extended the eating time. A positive correlation between sorting and eating time has been reported previously by Greter and DeVries (2011). The reduction in eating time for CTMR compared with TMR was 13%, twice the reduction in DM intake. Thus, cows fed CTMR were able to consume their DM at a higher rate than cows fed TMR. There were clear visible differences in eating behaviour as the cows consumed the different feeds in Experiment 2, however the reduction in eating time was not significant and smaller than in Experiment 1, which possibly was influenced by the methodology in the behavioural study. When eating, the cows on TMR moved their muzzle back and forth in the feed pile to form a pit and then licked small particles (concentrate) from the bottom of that pit. These behaviours were recorded as “digging” and “eating from below” and can be categorised as typical sorting behaviours (Leonardi and Armentano, 2003). The cows fed CTMR did not create pits in the feed, which lay in an even layer along the entire feed table during the day. When eating, the cows fed CTMR took mouthfuls from the top, indicating that they did not select any individual feed particles.

4.3. Particle sorting

The TMR had 32% long particles (> 19 mm), which is too high according to the recommendation by Heinrichs and Kononoff (2002) for a maximum level of 8% long particles to avoid sorting. The proportion of long particles in CTMR was 6%. However, the increased frequency of sorting behaviours in cows fed TMR did not result in any significant differences in particle size distribution over time measured using the method described in Section 2.3 of this paper. It could be that the methods used were unable to show differences in particle size distribution over time given the experimental design with sampling of feed on a large area of the feed table. It is sometimes claimed that it is more difficult for cows to sort their feed if it is made more homogeneous by adding water (Leonardi et al., 2005; Endres and Espejo, 2010; Fish and DeVries, 2012). However, the DM content of the diet in those studies was not as low as in the present study and the proportion of long particles in the feed did not differ as much between treatments in those studies. Felton and DeVries (2010) found that sorting of long particles increased when the DM content decreased from 56% to 44%, although differences in particle size were not reported. This suggests that it may not be sufficient to add water to the feed mix to reduce sorting, and the feed mix may also need to have a low proportion of long particles.

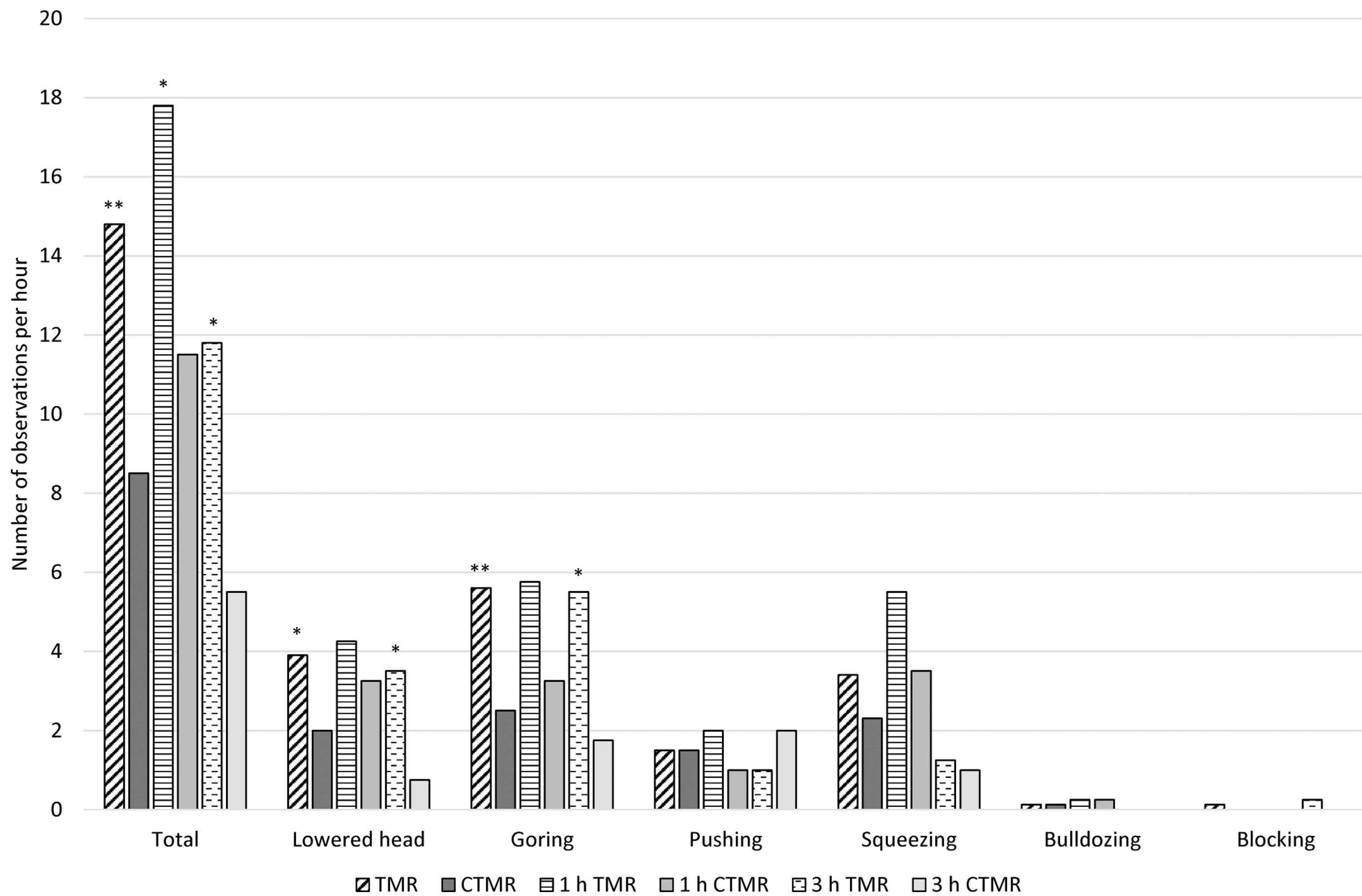

Fig. 2. Least square means of different antagonistic behaviours (observations per hour) of cows fed a total mixed ration (TMR) or compact TMR (CTMR). The bars show the sum of observations per treatment and for 0–1 hour and 2–3 h after feeding. Statistically significant differences between treatments are indicated by asterisks (*P<0.05, **P<0.01).

Table 5
Time spent performing different behaviours, in minutes during 24 h, by cows in Experiment 2 fed a total mixed ration (TMR) or compact TMR (CTMR). Data shown as least squares means.

	TMR	CTMR	SEM	P-value
Eating	282	261	11.3	0.141
Resting	361	406	14.0	0.007
Standing in feed area	62	52	5.7	0.049
Standing in alley	49	61	5.1	0.057
Standing in cubicle	77	78	6.3	0.868
Drinking	37	37	4.8	0.942
Milking	146	136	5.1	0.027
Ruminating	426	408	11.6	0.108
Resting (including rumination)	658	695	18,8	0.109

4.4. Antagonistic behaviours

A lower number of antagonistic behaviours was observed when the cows were fed CTMR compared with TMR. This may be associated with less competition for the feed (Olofsson, 1999; Huzzey et al., 2006). When the CTMR concept is used on commercial farms, feed is usually distributed once daily to decrease the workload for the farmer and to minimise synchronised rising behaviour of cattle with delivery of fresh feed. The decreased sorting, resulting in a more even feed composition over time, also makes the fresh feed more similar to the residual feed on the feed table. It has thus been suggested that feeding CTMR provides a quieter existence for cows in loose housing systems, since they do not rush to the feeding table when new feed is distributed (Kristensen, 2015). Due to a management routine whereby the feed was laid out when the cows were at milking, the behaviour of the cows when new feed was given could not be investigated in the present study. However, the hypothesis of a reduced number of antagonistic interactions and reduced competition between cows at the feeding table was confirmed, since it was found that cows showed a lower frequency of such behaviours when fed CTMR than when fed TMR. The cows fed CTMR also spent less time inactive in the feeding area than the cows fed TMR. This difference may indicate that competition for feed was greater in the TMR group. Similarly, Huzzey et al. (2006) found that cows had longer idle standing time at the feed table when competition for feed increased. The groups in the present experiment had the same number of eating spaces in total but differed slightly in total number of cows, which could have affected competition in the groups. However, as the experiment had a cross-over design, this variation was taken into account.

4.5. Resting time

The resting time of the cows in the time budget study, which included both ruminating and non-ruminating time, was on average 11.3 h per day for all cows on both diets, but the time the cows spent resting without ruminating differed between treatments. Resting is a high priority for cows. Munksgaard et al. (2005) showed that resting has higher priority than eating time and social contact in dairy cows. Similar results as in the present study on lying time for cows kept in loose housing systems have been reported previously (Ito et al., 2009; Gomez and Cook, 2010). The cows fed CTMR rested on average 45 min more per day than the cows fed TMR, indicating that they had more time left in their time budget for rest than cows fed TMR. Thus, the hypothesis of longer resting time, one of the basic arguments for feeding CTMR, was supported. However, there were no differences in ruminating time between the groups. The cows on the CTMR and TMR diet spent on average 6.8 and 7.1 h per day ruminating, respectively, which is well in line with e.g. values reported in a review by Beauchemin (2018).

4.6. Ruminal pH

Particle size in the diet may affect rumination and thereby buffering in the rumen. It is thus possible that the CTMR diet could result in lower average ruminal pH or longer time with low pH values in the rumen. However, in the present study, ruminal pH and the time for which ruminal pH was below pH 5.8 were not affected by diet. The latter can be used as a marker for SARA when sampling from a cannula (Duffield et al., 2004). Beauchemin et al. (2003) found a decrease in average pH in the ventral sac and an increase in hours with pH below 5.8 with decreasing forage particle size in alfalfa-based diets. However, Storm and Kristensen (2010) found no effect on pH in the ventral or medial rumen of altering forage particle size. Addition of water to TMR has been shown not to affect ruminal pH measured at different locations (Leonardi et al., 2005). In the present study, cows on both diets spent much more time below ruminal pH 5.8 than recommended by Zebeli et al. (2008) in order to avoid SARA, even though the diets fed in the experiment were not unusually high in starch or low in NDF. Calculated starch concentration was 15% of DM and calculated NDF concentration was 36% of DM. The risk of SARA could be affected by the time needed to adapt to rapidly fermentable carbohydrates in the diet (Humer et al., 2018), but in the present experiment the cows had at least 2 weeks of adaptation to the diet. In addition, prior to the experiment all cows were fed similar or higher levels of starch.

5. Conclusions

Compact TMR, characterised by smaller particles and lower DM content achieved by addition of water and prolonged mixing of the feed, resulted in decreased feed intake, decreased eating time, fewer sorting behaviours and fewer antagonistic interactions compared with conventional TMR. No effect of CTMR on milk yield, ruminal pH or digestibility was found. Thus CTMR has the potential to improve cow welfare by improved time budget, reduced sorting and reduced aggression.

CRediT author statement

Cecilia Kronqvist Conceptualization, methodology, investigation, data curation, validation, formal analysis, writing - review & editing

Frida Petters Investigation, original draft preparation

Ulrica Robertsson Investigation, original draft preparation

Mikaela Lindberg Conceptualization, methodology, investigation, data curation, validation, formal analysis, writing - review & editing, funding acquisition

Declaration of Competing Interests

None.

Acknowledgements

This work was funded by SLU EkoForsk - field research on organic production. The authors wish to thank the staff at the dairy facilities for managing the animals and the laboratory staff at the Department of Animal Nutrition and Management, SLU for conducting the laboratory analyses.

References

Pages 41–54 inVol. 130 Åkerlind, M., Weisbjerg, M., Eriksson, T., Udén, P., Ólafsson, B. L., Harstad, O., Volden, H, 2011. Feed analyses and digestion methods, Pages 41–54 in. In: Volden, H. (Ed.), NorFor-The Nordic Feed Evaluation System. Wageningen Academic Publishers, Wageningen, the Netherlands. Vol. 130https://doi .org/ 10 .3920/ 978 -90-8686 -718 -9.

Beauchemin, K.A., 2018. Invited review: current perspectives on eating and rumination activity in dairy cows. J. Dairy Sci. 101, 4762–4784. https://doi.org/10.3168/jds.2017-13706.

Beauchemin, K.A., Yang, W.Z., Rode, L.M., 2003. Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. J. Dairy Sci. 86, 630–643. https://doi.org/10.3168/jds.S0022-0302(03)73641-8.

Chai, W., Udén, P., 1998. An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fibre. Anim. Feed Sci. Technol. 74, 281–288. https://doi.org/10.1016/S0377-8401(98)00187-4.

Colucci, P.E., Chase, L.E., Van Soest, P.J., 1982. Feed Intake, apparent diet digestibility, and rate of particulate passage in dairy cattle. J. Dairy Sci. 65, 1445–1456.

DeVries, T.J., Dohme, F., Beauchemin, K.A., 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: feed sorting. J. Dairy Sci. 91, 3958–3967. https://doi.org/10.3168/jds.2008-1347.

Duffield, T., Plaizier, J.C., Fairfield, A., Bagg, R., Vessie, G., Dick, P., Wilson, J., Aramini, J., McBride, B., 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J. Dairy Sci. 87, 59–66. https://doi.org/10.3168/jds.S0022-0302(04)73142-2.

Endres, M.I., Espejo, L.A., 2010. Feeding management and characteristics of rations for high-producing dairy cows in freestall herds. J. Dairy Sci. 93, 822–829. https://doi.org/10.3168/jds.2008-2007.

Felton, C.A., DeVries, T.J., 2010. Effect of water addition to a total mixed ration on feed temperature, feed intake, sorting behavior, and milk production of dairy cows. J. Dairy Sci. 93, 2651–2660. https://doi.org/10.3168/jds.2009-3009.

Fish, J.A., DeVries, T.J., 2012. Short communication: varying dietary dry matter concentration through water addition: effect on nutrient intake and sorting of dairy cows in late lactation. J. Dairy Sci. 95, 850–855. https://doi.org/10.3168/jds.2011-4509.

Gomez, A., Cook, N.B., 2010. Time budgets of lactating dairy cattle in commercial freestall herds. J. Dairy Sci. 93, 5772–5781. https://doi.org/10.3168/jds.2010-3436.

Gozho, G.N., Plaizier, J.C., Krause, D.O., Kennedy, A.D., Wittenberg, K.M., 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J. Dairy Sci. 88, 1399–1403. https://doi.org/10.3168/jds.S0022-0302(05)72807-1.

Grant, R.J., Albright, J.L., 2001. Effect of animal grouping on feeding behavior and intake of dairy cattle. J. Dairy Sci. 84, E156–E163. https://doi.org/10.3168/jds.s0022-0302(01)70210-x.

Greter, A.M., DeVries, T.J., 2011. Effect of feeding amount on the feeding and sorting behaviour of lactating dairy cattle. Can. J. Anim. Sci. 91, 47–54. https://doi.org/10.4141/CJAS10067.

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. J. Dairy Sci. 102, 8839–8849. https://doi.org/10.3168/jds.2018-16191.

Heinrichs, A., Kononoff, P., 2002. Evaluating Particle Size of Forages and TMRs using the New Penn State Forage Particle Separator, 42. Pennsylvania State University, College of Agricultural Sciences, Cooperative Extension DAS, pp. 1–15.

Huhtanen, P., Kaustell, K., Jaakkola, S., 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. Technol. 48, 211–227.

Humer, E., Petri, R.M., Aschenbach, J.R., Bradford, B.J., Penner, G.B., Tafaj, M., Südekum, K.-.H., Zebeli, Q., 2018. Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. J. Dairy Sci. 101, 872–888.

Huzzey, J.M., DeVries, T.J., Valois, P., von Keyserlingk, M.A.G., 2006. Stocking density and feed barrier design affect the feeding and social behavior of dairy cattle. J. Dairy Sci. 89, 126–133. https://doi.org/10.3168/jds.S0022-0302(06)72075-6.

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. J. Dairy Sci. 92, 4412–4420. https://doi.org/10.3168/jds.2009-2235.

Jurkovich, V., Könyves, L., Bakony, M., 2019. Association between feed sorting and the prevalence of metabolic disorders in Hungarian large-scale dairy herds. J. Dairy Res. 86, 162–164. https://doi.org/10.1017/S002202991900027X.

Kristensen, N.B., 2015. Implement Compact TMR to increase productivity, feed efficiency and health in dairy herds. In: Proceedings from Djurhälso- och utfodringskonferensen 2015, pp. 68–79 (in Swedish). https://www.vxa.se/globalassets/dokument/fordjupningar/dou/2015/implement-compact-tmr-to-increase-productivity-feed-efficiency-and-health-in-dairy-herds—article.pdf.

Lammers, B.P., Buckmaster, D.R., Heinrichs, A.J., 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79, 922–928. https://doi.org/10.3168/jds.S0022-0302(96)76442-1.

Leonardi, C., Armentano, L.E., 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86, 557–564. https://doi.org/10.3168/jds.S0022-0302(03)73634-0.

Leonardi, C., Giannico, F., Armentano, L.E., 2005. Effect of water addition on selective consumption (sorting) of dry diets by dairy cattle. J. Dairy Sci. 88, 1043–1049.

Lindgren, E., 1983. Nykalibrering Av VOS-metoden För Bestämning Av Energivärde Hos Vallfoder. Department of Animal Nutrition and Management, Uppsala, Sweden. Working paperIn Swedish.
Løvendahl, P., Munksgaard, L., 2016. An investigation into genetic and phenotypic variation in time budgets and yield of dairy cows. J. Dairy Sci. 99, 408–417. https://doi.org/10.3168/jds.2015-9838.
Maekawa, M., Beauchemin, K.A., Christensen, D.A., 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows1. J. Dairy Sci. 85, 1165–1175. https://doi.org/10.3168/jds.S0022-0302(02)74179-9.
Mehtiö, T., Rinne, M., Nyholm, L., Mäntysaari, P., Sairanen, A., Mäntysaari, E.A., Pitkänen, T., Lidauer, M.H., 2016. Cow-specific diet digestibility predictions based on near-infrared reflectance spectroscopy scans of faecal samples. J. Anim. Breed. Genet. 133, 115–125. https://doi.org/10.1111/jbg.12183.
Miller-Cushon, E.K., DeVries, T.J., 2009. Effect of dietary dry matter concentration on the sorting behavior of lactating dairy cows fed a total mixed ration. J. Dairy Sci. 92, 3292–3298. https://doi.org/10.3168/jds.2008-1772.
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. Appl. Anim. Behav. Sci. 92, 3–14.
Nasrollahi, S.M., Ghorbani, G.R., Khorvash, M., Yang, W.Z., 2014. Effects of grain source and marginal change in lucerne hay particle size on feed sorting, eating behaviour, chewing activity, and milk production in mid-lactation Holstein dairy cows. J. Anim. Physiol. Anim. Nutr. (Berl). 98, 1110–1116. https://doi.org/10.1111/jpn.12185.
Nasrollahi, S.M., Imani, M., Zebeli, Q., 2015. A meta-analysis and meta-regression of the effect of forage particle size, level, source, and preservation method on feed intake, nutrient digestibility, and performance in dairy cows. J. Dairy Sci. 98, 8926–8939. https://doi.org/10.3168/jds.2015-9681.
Olofsson, J., 1999. Competition for total mixed diets fed for ad libitum intake using one or four cows per feeding station. J. Dairy Sci. 82, 69–79. https://doi.org/10.3168/jds.S0022-0302(99)75210-0.
Russell, J.B., Wilson, D.B., 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci. 79, 1503–1509. https://doi.org/10.3168/jds.S0022-0302(96)76510-4.
Schingoethe, D.J., 2017. A 100-year review: total mixed ration feeding of dairy cows. J. Dairy Sci. 100, 10143–10150. https://doi.org/10.3168/jds.2017-12967.
Sjaunja, L.O., Baevre, L., Junkkarinen, L., Pedersen, J., Setälä, J., 1990. A Nordic proposal for an energy corrected milk (ECM) formula. In: Performance Recording of Animals: 27th Biennial Session of the International Committee for Animal Recording. Paris, France, pp. 156–192.
Slyter, L.L., 1976. Influence of acidosis on rumen function. J. Anim. Sci. 43, 910–929.
Sova, A.D., LeBlanc, S.J., McBride, B.W., DeVries, T.J., 2013. Associations between herd-level feeding management practices, feed sorting, and milk production in freestall dairy farms. J. Dairy Sci. 96, 4759–4770. https://doi.org/10.3168/jds.2013-6679.
Storm, A.C., Kristensen, N.B., 2010. Effects of particle size and dry matter content of a total mixed ration on intraruminal equilibration and net portal flux of volatile fatty acids in lactating dairy cows. J. Dairy Sci. 93, 4223–4238. https://doi.org/10.3168/jds.2009-3002.
Van Keulen, J., Young, B.A., 1977. Evaluation of acid insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44, 282–287.
Zebeli, Q., Dijkstra, J., Tafaj, M., Steingass, H., Ametaj, B.N., Drochner, W., 2008. Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet. J. Dairy Sci. 91, 2046–2066. https://doi.org/10.3168/jds.2007-0572.