


ORIGINAL ARTICLE

The effects of the ratio of pellets of wheat and barley grains to ground corn grain in the diet on sorting and chewing activities of heat stressed dairy cows

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Abstract

Background: Normal feeding behaviours is one of the criteria of the health condition of dairy cows particularly in the condition of heat stress.

Objective: The study evaluated the effects of the ratio of pellets of wheat and barley grains to ground corn grain on sorting activity and chewing behaviour of lactating dairy cows managed under ambient conditions including natural heat stress events.

Materials and Methods: Nine multiparous cows (650 ± 56 kg Body Weight; mean \pm SD) averaging 102 ± 13 days in milk and producing 54 ± 6 kg/d were randomly assigned to a triplicate 3×3 Latin square. During each 21-d period, cows received one of three total mixed rations as dietary treatments. The dietary treatments were three ratio of pellets (containing 50% ground wheat and 50% ground barley): ground corn on a dry matter (DM) basis: 1) 33.3:66.6 (low); 2) 66.6:33.3 (medium); and 3) 100:0 (high). During the experiment, the ambient temperature-humidity index was equal or more than 72, indicating that the cows were predisposed to heat stress condition.

Results: Increasing the proportion of wheat-barley pellets in the diet had a minimal effect on sorting index of different particles during the first 6 h of the day. However, later in the day (6-18h), sorting against long particles (particles >19 mm) and in favour of fine particles (particles <1.18 mm) linearly increased with increasing the proportion of pellet; as a result overall daily sorting against long particles was increased with increasing the proportion of pellets. Although the average of daily eating and rumination was not affected by the treatments in the day times with high ambient THI, time spent for eating and rumination was low and eating time had more fluctuation for diet contained a high level of pellets.

Conclusion: Increasing the proportion of pellets of barley and wheat grains in the diet under conditions of heat stress caused more fluctuation in daily eating behaviour and cows were predisposed to sort against long particles.

KEYWORDS

dairy cow, feeding behavior, grain fermentability, heat stress

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1 | INTRODUCTION

Feeding behaviour of dairy cows has an important role on their health and productivity. Sorting against long particles can cause up to 0.4 unit decreasing in rumen pH and 0.6 decreasing in milk fat percentage (DeVries, 2013). The effect of feeding behaviour may be more important during heat stress as previously described in different studies (Eslamizad et al., 2015; Karimi et al., 2015; Schneider et al., 1988). Dairy cows change their time of eating from a hot to a cooler time of the day (Schneider et al., 1988). Moreover, eating (Eslamizad et al., 2015) and ruminating (Karimi et al., 2015) activities decrease during ambient heat stress conditions. This may exacerbate the condition of heat stressed dairy cows that are struggling with both low rumen pH and productivity problems (West, 2003).

Increasing diet fermentability is suggested as a favourable way for improving productivity of heat stressed dairy cows but too much diet fermentation can cause rumen acidosis (Nasrollahi et al., 2019; Kadzere et al., 2002; West, 2003). The effect of diet fermentability on the increasing risk of acidosis might be dependent on feeding behaviour. Three scenarios describing this link were found in literature. The first one indicates that cows with low rumen pH tend to select long particles that alleviates the risk of rumen acidosis (Keunen et al., 2002; Kargar et al., 2013; Maulfair & Heinrichs, 2013). However, the second one indicates that the development of rumen acidosis is due to the selection of fine particles because they are more appetizing and fermentable (DeVries et al., 2008; Leonardi & Armentano, 2003). In addition, there is a third group of studies reporting no effect of increasing diet fermentability on cow behaviour (Kargar et al., 2015; Ahmadi et al., 2020). Therefore, this discrepancy needs more investigations.

Increasing diet fermentability is usually achieved through modification of grain source with expected different rumen fermentability, and it might be possible that the discrepancy among the results can be related to the severity of the modification. Using the pellets of barley and wheat grain is known as a successive way of increasing diet fermentability up to a severe condition (Li et al., 2012; Nasrollahi et al., 2019), but the effects on feeding behaviour were not evaluated. Therefore, the objective of the present study was to investigate the effect of the ratio of pellets of wheat and barley grains to ground corn grain on sorting index and chewing behaviour in heat stressed dairy cows. We hypothesized that increasing the proportion of pellets would motivate dairy cows to select long forage particles and to increase their chewing activity.

2 | MATERIALS AND METHODS

2.1 | Animals, housing and diets

The research was part of a larger project, and full details of experimental set-up, cow management and housing condition were reported previously (Nasrollahi et al., 2019). Briefly, nine multiparous lactating Holstein dairy cows (650 ± 56 kg BW; mean \pm SD)

averaging 102 ± 13 days in milk (DIM) and producing 54 ± 6 kg of milk daily were used in a replicated 3×3 Latin square design with 21 day periods. The dietary treatments were three ratio of pellets (containing 50% ground wheat and 50% ground barley):ground corn on a dry matter (DM) basis: (1) 33.3:66.6 (low); (2) 66.6:33.3 (medium); and (3) 100:0 (high). The percentage of corn and pellets in the diet was fixed at 35% of diet DM and the three diets were formulated to have similar protein (CP = 14.5% of DM) and energy (NEL = 6.46 MJ/kg of DM) contents with a similar forage to concentrate ratio (40:60). Information on diet ingredients and chemical compositions were reported in companion paper (Nasrollahi et al., 2019). The study was conducted from May 2015 to August 2015. Cows were housed in individual stalls (3×3 m) within an aluminum roofing facility with open sides and clean wood shavings and sand were used for bedding and refreshed daily. There were no cooling system. Feed was supplied twice daily at 1,000 and 1,800 hr. Cows were milked three times daily at 0100, 0900 and 1,700 hr.

2.2 | Measurements

Ambient temperature (T_{db} , °C) and relative humidity (RH, %) were measured in the stalls using a temperature and humidity data-logger (model ST-172; Fotronic Co., Melrose, MA) at 30-min intervals over 24 hr for 2 consecutive (days 19 to 20) days of each sampling period.

On days 18 and 19 of each period, chewing activity was monitored visually for each cow over a 24-hr period using three trained observers. The observation was based on instantaneous recording with each scanning of all nine cows took around 1 min. Eating and ruminating activities were noted at 5-min intervals, and each activity was assumed to persist for the entire 5-min interval (Colenbrander et al., 1991). A period of rumination was defined as at least one observation of rumination occurring after at least 5 min without rumination. A meal was defined as at least one observation of eating activity occurring after at least 30 min without eating activity. The time spent eating, ruminating, and chewing (eating + ruminating) per kilogram of DMI, as well as the number of meals per day, number of ruminations per day, and eating rate (total daily DMI/total eating time; g DM/min) were averaged by cow within the period. To evaluate diurnal changes in eating, rumination and total chewing behaviors, each daily activity was divided into 1-hr intervals.

To measure fermentability, an in situ experiment was conducted on pellet and corn grain samples of present experiment and relative data were reported in previous companion paper (Nasrollahi et al., 2019). Concisely, the in situ experiment was conducted on two nonlactating cannulated Holstein cows. The average of fermentability for pellet at incubation times of 2, 4 and 6 hr was 48.5, 54.4, and 55.6%, respectively, while relative values at those times for corn were 44.1%, 48.9%, and 51.3%, respectively (Nasrollahi et al., 2019).

On days 15–20 of the study in each period, samples of TMR of each treatment diet and refusals of each cows were collected daily

for the determination of particle size. Then, TMR samples were pooled by diet within period and refusal samples were pooled by cows within period. Particle size was measured on each pooled sample in triplicate using the Penn State Particle Separator (PSPS) equipped with 3 sieves and a bottom pan (Kononoff, 2002). After sieving, samples were placed in a forced air oven at 70°C to determine DM of each sieved fraction. Physically effective factor (pef) values were determined as the total proportion of DM retained on the 19- and 8-mm sieves (pef8, Lammers et al., 1996) or on the 19, 8 and 1.18 sieves (pef1.18; Kononoff, 2002) (Table 1). The peNDF was calculated by multiplying pef8 and pef1.18 by the NDF content of the diet (DM basis) to obtain peNDF8 and peNDF1.18, respectively. The geometric mean particle length (GMPL) was calculated according to the ASAE (1995; method S424.1) procedure. On days 16 and 17 of each period, feed bunk contents for each cow were weighed and sampled at 6, and 24 hr after the morning feeding to determine particle size after feeding. The two time points were measured at a same day and repeated for 2 days.

2.3 | Calculations

2.3.1 | Sorting index

Sorting of particles was determined from the actual intake of each fraction compared to the predicted intake of the same fraction had the diet been consumed as formulated (Leonardi & Armentano, 2003). A sorting index score was calculated for each fraction as the percentage of actual intake compared with the predicted intake. When the values were equal to 100% it indicated no sorting, whereas those values <100% indicated sorting against, and >100% indicated sorting for.

2.3.2 | THI calculation

To calculate the temperature-humidity index (THI): $THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)]$ (NRC, 1971). During the experiment, THI along with rectal temperature of cows (39.2°C) indicated that the cows were in heat stress almost the entire duration of the study (average daily THI > 72). The value of THI was consistent among the three experimental periods, with average daily THI > 72 in all periods, maximum THI of 82, 83 and 83 for periods 1, 2 and 3, respectively, and minimum THI of 63, 70 and 70 for the same three periods (Nasrollahi et al., 2019).

2.4 | Statistical analysis

Statistical analysis was conducted using the mixed model procedure of SAS (Proc Mixed; SAS Institute, 2002) to account for the considered fixed and random effects. The treatment, square and period were considered as fixed effects while cow within square was considered a random effect. The data concerning the particle size of the initial diet were analysed using a completely randomized block design with model effects of treatment and period (block). Estimation method was REML and the degrees of freedom method was Kenward-Rogers. The linear and quadratic effects of increasing diet fermentability were tested using polynomial orthogonal contrasts. Statistical significance was declared at $p \leq 0.05$, and tendencies were considered at $0.05 \leq p \leq 0.10$.

3 | RESULTS

Data regarding treatment effect on feed intake, rumen pH and VFA, milk production and composition of the present experiment were

TABLE 1 Physical characteristics of the experimental diets with different proportion of pellets

	Diets				Effects	
Item	low	Medium	high	SEM	Linear	Quadratic
% DM retained on sieve						
>19 mm	8.72	9.22	9.96	0.70	NS	NS
19–8 mm	30.8	40.4	45.4	1.40	***	NS
1.18–8 mm	36.9	33.7	33.7	0.92	*	NS
<1.18 mm	23.6	16.7	11.0	0.59	***	NS
pef8 ^a	0.40	0.50	0.55	0.014	***	NS
pef1.18 ^a	0.76	0.83	0.89	0.006	***	NS
peNDF8 ^a , % DM	14.6	18.6	20.8	0.54	***	NS
peNDF1.18 ^a , % DM	28.1	31.3	33.5	0.22	***	NS
GMPL ^b mm	4.52	5.69	6.66	0.15	***	NS

^apef8 and pef1.18 = physical effectiveness factor determined as the DM proportion of particles retained on 2 sieves (19 and 8 mm) or 3 sieves (19, 8, 1.18 mm), respectively, of the PSPS (Kononoff, 2002; Lammers et al., 1996). peNDF8, peNDF1.18 = physically effective NDF calculated as dietary NDF content (% DM) multiplied by pef8 and pef1.18, respectively.

^bGMPL = Geometric mean particle size as calculated by ASAE (1995; method S424.1).

* $p < 0.05$.

*** $p < 0.001$.

reported in previous companion paper (Nasrollahi et al., 2019). In brief, increasing the proportion of pellets linearly decreased dry matter intake (22.8, 22.5 and 21.8 kg/day for low, medium and high diets, respectively) and rumen pH (6.08, 5.96 and 5.84), but milk production was quadratically affected (40.0, 42.2 and 39.1 kg/day). Milk fat (2.87 ± 0.08) and protein percentages ($2.61 \pm 0.03\%$) were not affected by the treatments (Nasrollahi et al., 2019).

3.1 | Diet particle size and sorting

The change in physical characteristics of the diets is presented in Table 1. Increasing proportion of pellets linearly increased the amount of particles 8–19 mm in length and decreased particles 1.18–8 mm and <1.18 mm in length of the initial diets. As a result, GMPL, pef8, pef1.18, peNDF8 and peNDF1.18 increased linearly with increasing the proportion of pellets. However, the amount of long particles (>19 mm) was not affected by dietary treatments.

Sorting index during the first 6 hr after morning feeding for different particles and pefs were not affected by the proportion of

pellets, except for long particles (>19 mm) that were quadratically affected (Table 2). During the final 18 hr of the day, increasing the proportion of pellets led to a linear increase in sorting against long particles (<19 mm). Furthermore, daily sorting for long particles (i.e., >19 mm) decreased with increasing the proportion of pellets, which led to a linear decrease in sorting for pef8 and pef1.18. This indicated that cows fed with a diet containing greater proportion of pellets tended to reject long particles.

3.2 | Chewing

Data on chewing activity are reported in Table 3. Globally, chewing activity was not affected by the treatment. On average, cows spent 238 min/day and 10.8 min/kg of DMI for eating activity. Cows spent 464 min/day and 21.3 min/kg of DMI for rumination activity.

Daily pattern of chewing activity with the change of ambient THI and for different dietary treatments are reported in Figure 1. In overall, both eating and rumination decreased during times when THI increased (from 1,100 to 1,600). Eating activity during night times was

	Diets				p value	
Item	low	Medium	high	SEM	Linear	Quadratic
0–6 hr after morning feeding, %						
>19 mm	94.5	53.1	85.1	12.2	NS	*
19–8 mm	118.3	125.1	109.4	6.6	NS	NS
1.18–8 mm	93.1	87.1	89.2	3.34	NS	NS
<1.18 mm	62.4	79.8	85.3	16.3	NS	NS
pef8 ^b	114.4	114.8	105.2	5.40	NS	NS
Pef18 ^b	105.1	104.1	99.8	2.50	NS	NS
6–18 hr after morning feeding, %						
>19 mm	93.6	86.7	81.9	3.26	*	NS
19–8 mm	98.7	101.8	100.5	0.88	NS	NS
1.18–8 mm	101.9	101.3	103.2	0.81	NS	NS
<1.18 mm	100.6	101.4	104.3	0.95	**	NS
pef8 ^b	97.6	98.0	96.0	1.00	NS	NS
Pef18 ^b	99.8	99.6	99.2	0.21	NS	NS
Total (0–24 hr), %						
>19 mm	94.0	81.9	82.5	3.26	*	NS
19–8 mm	102.6	104.8	102.1	1.07	NS	*
1.18–8 mm	100.2	99.3	100.7	0.60	NS	*
<1.18 mm	98.1	98.2	100.9	1.61	NS	NS
pef8 ^b	101.0	100.9	98.2	1.12	*	NS
Pef18 ^b	100.7	100.3	99.5	0.42	*	NS

TABLE 2 Sorting index^a for lactating dairy cows fed diets differing in the proportion of pellets under heat stress condition ($n = 9$ cows)

^aSorting index was calculated as the percentage of actual intake relative to the predicted intake of each particle fraction.

^bpef8 and pef1.18 = physical effectiveness factor determined as the DM proportion of particles retained on 2 sieves (19 and 8 mm) or 3 sieves (19, 8, 1.18 mm), respectively, of the PSPS (Kononoff, 2002; Lammers et al., 1996).

* $p < 0.05$.

** $p < 0.01$.

TABLE 3 Chewing activity for dairy cows fed diets differing in the proportion of pellets under heat stress condition ($n = 9$ cows)

	Diets				Effects	
Item	low	Medium	high	SEM	Linear	Quadratic
Eating						
Min/day	229	241	240	12.6	NS	NS
Min/kg of DM	10.2	10.8	11.0	0.71	NS	NS
Meals/day	8.96	8.80	8.66	0.32	NS	NS
Length, min/meals	25.7	27.7	27.9	1.73	NS	NS
Rate, kg of DM/min	0.102	0.097	0.098	0.006	NS	NS
Ruminating						
Min/day	443	446	433	23.6	NS	NS
Min/kg of DM	20.1	20.3	19.9	1.54	NS	NS
Bouts/day	17.7	16.9	17.9	0.88	NS	NS
Length, min/bout	25.6	27.0	25.2	2.01	NS	NS
Total chewing						
Min/day	671	688	673	29.3	NS	NS
Min/kg of DM	30.3	31.2	30.9	2.02	NS	NS

low for all cows, especially from 0,300 to 0,600, but cows fed high proportion of pellets came back to feed later than other treatments and had more fluctuation during early morning. Rumination activity during night times was higher than day times but relatively similar for the three dietary treatments.

4 | DISCUSSION

The average daily THI of equal or more than 72 that was observed in the present study predisposed cows to a period of mild to moderate heat stress (Renaudeau et al., 2012). Due to a greater in situ degradability of the pellets of barley and wheat grain versus corn grain in this present study, we expected that the substitution of ground corn with pellets of ground wheat and barley raised diet fermentability, which is in agreement with previous studies (Ferraretto et al., 2013; Mills et al., 1999). The information of the change in feeding behaviour of heat stressed dairy cows due to the modification of grains source and relative change in diet fermentability is important for defining new strategies for health and production improvement. The results may be important for dairy husbandry in countries like Iran, which face heat stress and climate warming (Pal & Eltahir, 2015).

In spite of a similar amount of forage in the three treatment diets, the physical characteristics were not equivalent. Increasing proportion of pellets increased particle 8–19 mm length and decreased shorter particles. Even peNDF8 and peNDF1.18 were increased with increasing proportion of pellets. This observation is important because despite a similar forage level and particle size, physical characteristics of the diets changed due to nonforage dietary sources. In previous studies, using grain with different processing (Safaei et al., 2017) or different sources (Nasrollahi et al., 2012; Kargar et al., 2013; Kargar et al., 2015) changed effective fiber criteria like pef8 and pef1.18 and even peNDF8 and peNDF1.18. The intensity of the animal biological

response (i.e., sorting or chewing) following a change of fibre effectiveness (i.e., pef or peNDF) needs to be further discussed.

The present study showed that the increase in sorting against long particles was positively correlated with the increasing proportion of pellets in the diet and relative increasing in diet fermentability. This observation is in contrast with our expectation, which was in accordance to previous trials reporting that an improvement of diet fermentability increases selection for long particles (Keunen et al., 2002; Maulfair & Heinrichs, 2013) or does not affect sorting (Kargar et al., 2013). Recently, (Safaei et al., 2017) demonstrated that with improving diet fermentability by decreasing density of flaked barley grain, sorting index of long particles was reduced. The sorting against long particles in the present study might be due to the physical characteristics of pellets used and the condition of ambient heat stress. Indeed, dairy cows are intrinsically prone to select in favour of high energy concentrate and against fibre parts (DeVries et al., 2008; Leonardi & Armentano, 2003). The pellets can be selected more efficiently than mesh form. Condition of heat stress could add an intensified effect. Cows faced heat stress had a more sorting against fibre parts with greater heat production (Renaudeau et al., 2012; Kadzere et al., 2002) and feeding energy-dense pellets with greater discrimination has a potential to induce more sorting activity. This reasoning may be not valid in all conditions while another study reported that dairy cows facing heat stress sorted for long fibre particles (Miller-Cushon et al., 2019). More studies need to address the role of grain sources and diet fermentability on feed sorting of dairy cows under heat stress condition and address its interfering factor.

The present study has also shown that the sorting index of cows was affected by the treatment later in the day. Our previous observations demonstrated that the feeding behaviour of cows during early times of the day is stronger than later times (Nasrollahi et al., 2014). It seems that the occurrence of heat stress discriminates the current study from the previous one. Our present finding on diurnal change

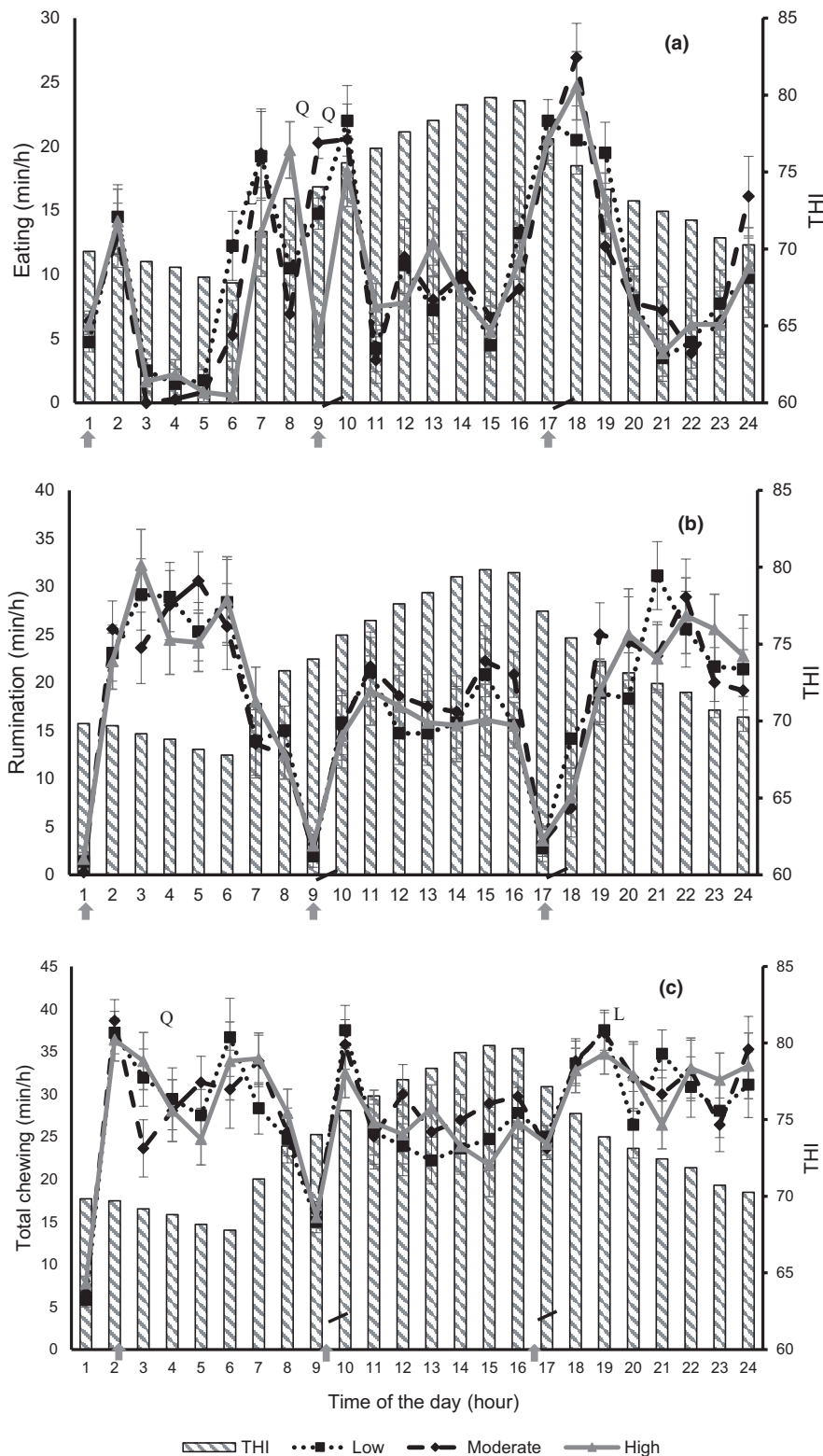


FIGURE 1 Diurnal changes in eating (a), rumination (b) and total chewing (c) contrasted with the change in ambient THI for lactating dairy cows fed diets differing in the proportion of pellets under conditions of heat stress. L and Q indicating on significant linear and quadratic treatment effects, respectively. ↑ indicates on milking times and ↓ indicates on feeding times. Time scales are the real hours of the day

in chewing demonstrated that eating activity decreased during heat stress (i.e., high THI) times of the day (1100–1600) which was coinciding with early time after morning feeding. Similarly, Schneider et al., (1988) reported such a feeding pattern in a chamber experiments in which heat-stressed cows ate more when temperatures were cooler. Moreover, the effect of heat stress on the reduction of feeding behaviour was documented in other studies in dairy cows

(Eslamizad et al., 2015; Karimi et al., 2015). From these observations, it could be hypothesized that dairy cows facing heat stress change their feeding behaviour to eat at cooler times of the day. Although eating at cooler times may help cows to get rid of heat increment due to eating at the warmer times, it may cause the ingestion of a high proportion of daily diet in a short time (i.e., cooler times) that may cause a more acid accumulation and rumen pH dropping.

Despite the significant treatment effect on diet physical form, chewing activity was unexpectedly not affected in our study. This might be partly explained by our findings on sorting indices. Indeed, although the diet with greater fermentability had a coarser physical form, the long particles were rejected by the cows. This reduced the size of particles that was actually ingested. On the other hand, the greater particle size that was achieved by pelleted grain certainly would not be as effective as forage or other NDF sources with a same particle size. Some pellets particles might be broken down during feed processing and delivering, and others might be disrupted in rumen fluid before motivating rumination. In agreement with this point, Yang et al., (2001) observed that although grain with different processing could result in diets with different initial particle sizes, the rumen digesta particle size were similar and did not affect chewing activity per kg of dry mater intake. In the same way, Nasrollahi et al., (2012) demonstrated that the source of the grain could result in increased particle size and even increased peNDF of the diet, without significantly affecting chewing. Interestingly, two experiments from a same team (Kargar et al., 2013, 2015) compared corn versus barley grain and reported a greater peNDF1.18 for barley compared to corn-based diets. Nevertheless, for one study, rumination increased in barley and not in corn-based diets (Kargar et al., 2013), and for the other one, chewing was not affected (Kargar et al., 2015). It may indicate on some interfering factor that affect the relationship of grain sources and fermentability, with chewing activity. Further researches are needed to better understand these interferences. In overall, it is suggested that more criteria of physically effectiveness of diet should be undertaken to proficiently predict animal response; and a simple outcome of PSPS sieves cannot necessarily guarantee animal response regarding chewing activity.

In overall, replacing ground corn with pellets of ground barley and wheat increased particle size and the peNDF of the diet. Moreover, the increased proportion of pellets and the relative increasing of diet fermentability under heat stress conditions resulted in cows sorting against long particles, mainly during later times of the day. Despite the presence of coarser particles in the diet with high proportion of pellets, chewing activity was not affected. Increasing diet fermentability by adding pellets in the ration may affect particle size of initial diet and sorting index but not chewing activity. In the condition of the present study, a low diet fermentability (i.e., pellets:corn ratio of 66:33) is recommended for heat stressed dairy cows to minimize sorting against long particles (particles >19 mm). As the cow behaviors in heat stress condition may also be dependent on day to day variations, the variation is recommended to be evaluated in further research.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

Animals were cared for according to the guidelines of the Iranian Council of Animal Care (1995) and the experiment was approved by the Institutional Animal Care Committee for Animals Used in Research.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.487>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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