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Dynamics of chewing and eating behavior, lying behavior, and salivary characteristics associated with duration of high grain feeding in cows with or with no phytogenic supplement

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ABSTRACT

This study evaluated the adaptive changes in chewing and eating behavior, lying behavior, and salivary properties due to the switch from forage to high grain and the duration of high grain feeding in cows, with or without a phytogenic feed additive. Nine non-lactating cannulated Holstein cows were used in a cross-over design with two experimental periods. Each period included one week of forage feeding, one week of diet transition, and four weeks of high grain feeding (35:65 forage to concentrate ratio; DM basis). Cows were either not supplemented (CON) or supplemented with a phytogenic additive (PHY) characterised by menthol, thymol and eugenol. Switching to high grain decreased ($P < 0.01$) rumination time compared to forage feeding; however, compared to week 2, rumination time increased ($P < 0.01$) by 73.8 and 53.21 min/d in weeks 3 and 4 on high grain, respectively. In week 4 on high grain, the PHY-supplemented cows tended ($P = 0.08$) to ruminate (263 vs. 204 min/d) and chew (406 vs. 347 min/d) longer compared to CON counterparts. The change to high grain increased ($P < 0.05$) sorting for long and medium size feed particles compared to forage diet, and there was a further increase ($P < 0.05$) in sorting for long and medium size feed particles in week 3 and 4 on high grain compared to the initial stage of the high grain challenge. Interestingly, PHY supplementation contributed to a more uniform intake of the diet by reducing ($P < 0.05$) the sorting of both medium and fine particles. Compared to week 1, lying time increased ($P < 0.05$) by 50 min/d in week 3 on high grain. High grain diet decreased ($P < 0.05$) salivary pH in week 1, while PHY supplementation helped maintaining this variable at physiological level during this initial grain challenge. Salivary bicarbonate was lower ($P < 0.05$) in weeks 3 and 4 compared to week 2 on high grain. Overall, the switch to high grain and the duration of feeding influenced chewing and eating behavior, lying behavior and salivary characteristics. Effects were still found 3 or 4 weeks after the diet switch; whereas PHY supplementation contributed to a more uniform nutrient intake by decreasing sorting behavior, which was reflected in stabilized salivary pH at the beginning of the grain challenge, and a tendency for increased chewing activity. However, more research is warranted to evaluate the positive role of PHY supplementation in decreasing feed sorting on metabolic health status and welfare of high-producing dairy cows, which are commonly affected due to high grain feeding.

1. Introduction

The use of energy-dense diets with high proportions of rapidly fermentable carbohydrates has contributed to enhance production performance of dairy cattle (VandeHaar, St-Pierre, 2006). This achievement has been possible mostly because of the increase in the supply of digestible nutrients and metabolizable energy (Valadares Filho et al., 2000). High grain diets, however, may negatively impact cattle

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behavior. For example, the lack of sufficient physically effective fiber in the rations impairs chewing activity and influences feed sorting behavior (Oh et al., 2016; Kröger et al., 2017, 2019). Being an essential physiological process in cattle, the depression in chewing may negatively impact salivary secretions (Maekawa et al., 2002; Chibisa et al., 2016), and may influence production performance or milk composition (Andreen et al., 2021; Souza et al., 2021). In addition, high grain rations have been associated with several metabolic changes in the rumen that can ultimately affect cow health and production (Plaizier et al., 2009) as well as animal lying behavior (Maynou et al., 2018).

Despite extensive research on the effects of high grain diets, the adaptive changes on chewing and eating behavior, lying behavior or salivary properties after the diet switch remain to be clearly elucidated. Addressing this research gap will contribute to elucidate the time needed for animal adjustment to a diet change, and would be especially important with regard to experimental planning. For example, in change-over experimental designs with dairy cattle, researchers have typically implemented experimental periods allowing animal adaptation for around 2 or 3 weeks before samplings are performed (Chibisa and Mutsvangwa, 2013; Kairenius et al., 2018; Ranathunga et al., 2018; Nasrollahi et al., 2019). However, there is a need of further quantitative evidence to show whether this time is sufficient for adequate adjustment to the dietary switch. Elucidating this aspect is important in order to prevent potential carry-over effects, notably when researchers compare diets with different chemical composition.

Investigation on feeding strategies to stimulate chewing or eating behavior and improve salivary buffers in cattle is needed. Reports from pioneering investigation have demonstrated the crucial role of saliva on rumen health, bolus deglutition and nutrient recycling (Reid and Huffman, 1949; Meyer et al., 1964). In this context, phytogenic compounds have been reported to influence salivary characteristics in non-ruminants (Eccles, 1994; Haahr et al., 2004; Shin et al., 2016) and ruminants (Ricci et al., 2021) due to their organoleptic properties. In addition, our research group recently reported that the duration on high grain may influence the ruminal fermentation, and that the impacts of grain-rich diets on gut fermentation are still observed 3 and 4 weeks after the diet change (Castillo-Lopez et al., 2022). However, there is limited understanding of the effects of the duration of supplementation with phytogenic feed additives on chewing and salivary physico-chemical properties. Preliminary reports evaluating the short-term supplementation with individual phytogenic compounds have shown improvements on chewing activity (Kröger et al., 2017; Castillo-Lopez et al., 2021b). These studies indicate the potential of phytogenic feed additives in cattle fed high grain rations, and suggest the need of further experiments to evaluate the effects of the combination and the extended exposure to the phytogenic compounds.

The objective of this study was to evaluate the adaptive changes in chewing and eating behavior, lying behavior, salivation and salivary properties due to the switch from all forage to high grain diet and the length of time of high grain feeding in cows with or with no phytogenic feed supplement. We hypothesized that not only the diet change, but also the duration of a diet based on a high proportion of grain will affect chewing and eating behavior, lying behavior, and salivary characteristics, and that cows will require at least 4 weeks to adapt to the diet change. We also hypothesized that the phytogenic feed additive would improve chewing behavior and salivary buffer content.

2. Materials and methods

2.1. Animals, experimental design and animal management

The methods and protocols followed in this experiment were approved by the institutional ethics and animal welfare committee and the national authority according to §§ 26ff. of Animal Experiments Act, Tierversuchsgesetz 2012 – TVG 2012 (protocol number: BMBWF-68.205/0003-V/3b/2019).

This study is part of a larger experiment, data for changes in gastrointestinal fermentation, feed digestion and liver enzymes have been reported (Castillo-Lopez et al., 2022). In the companion study, changes in gut fermentation could still be observed after 3 and 4 weeks post diet transition, there was an increase in ruminal acetate due to advanced duration of high grain, a change that was accompanied by variations in butyrate in the opposite direction. In addition, gut fermentation increased but differently according to location, with a stronger build-up of short chain fatty acids in the rumen compared to the hindgut; a longer duration on high concentrate increased gut acidification. Furthermore, the liver enzyme glutamate dehydrogenase further increased in weeks 3 and 4 compared to week 1 on high grain. Nine non-lactating Holstein dairy cows (921 ± 86.3 kg) fitted with ruminal cannulae (Bar Diamond, Parma, ID) were used in a cross-over experimental design. The experiment consisted of two periods of six weeks each. In each experimental period, cows were first fed a forage diet for one week, which contained (g/kg DM) 450 of grass silage, 450 of corn silage and 100 of grass hay. Then, cows were adapted to a high grain diet containing 650 g/kg DM of concentrate over one week by increasing the concentrate 10 % daily. This high grain diet was then fed for additional four weeks and contained (g/kg DM) 262.5 of grass silage, 87.5 of corn silage and 650 of pelleted concentrate (Supplementary Table 1). Between the two experimental periods, there was an interval of 10 weeks, where cows grazed on the same pasture as before starting the trial to fully recover from the high grain diets. After the interval period, groups were switched to the corresponding treatment. As detailed below, variables evaluated in this study were measured in the week of forage feeding and during the four weeks of high grain feeding (excluding the diet transition week).

At the initiation of the study, cows were divided according to body weight in two groups of four and five cows, so that body weight was balanced across the 2 groups. Cows were randomly allocated to either a control TMR with no supplementation (CON; average initial body weight of 916 kg) or a TMR supplemented with 0.4 g/kg DM of a phytogenic feed additive (PHY; average initial body weight of 926 kg). The PHY (Digestarom®) was characterised by a combination of the phytogenic compounds menthol (*Mentha arvensis* L.), eugenol (*Syzygium aromaticum*), and thymol (chemically synthesized). From past studies in ruminants (Ricci et al., 2021) and monogastrics (Dawes, Kubieniec, 2004), we know that phytogenic compounds can exert an effect shortly after consumption. Therefore, we consider that the duration of supplementation of the phytogenic compound in this study was enough to detect effects on the animals.

Throughout the experimental periods, cows were housed in a free-stall barn equipped with 10 deep litter cubicles (2.6 × 1.25 m, straw litter). Additionally, an open space within the stall of 10 × 7 m with straw bedding was available as a resting area, where cows can lie down or move freely. This area was located next to the deep litter cubicles and the feeders. Free-choice mineral blocks were available. Water was available for ad libitum consumption in 4 water troughs, each of them of 50 cm wide and 2 m long. The TMR was mixed once daily at 06:00 using an automated feeding system (Trioliet Triomatic T15, Oldenzaal, Netherlands), and was offered in individual automatic feeders to the cows to target 10 % of feed refusals (as is). Before the start of the study, cows were randomly assigned to the automatic feeders, so that each cow was allowed access to 1 independent automatic feeder throughout the experiment through an ear tag transponder (9 automatic feeders used from a total of 20 feeders in the system; Insentec B. V., Marknesse, Netherlands). The dietary treatments were independently given to each cow. Thus, measurements of feed intake and related data were collected individually as automatic feeders were equipped with electronic weighing scales and computer-regulated access gates (Insentec B.V., Marknesse, Netherlands). With this feeding approach, we could also prevent potential issues related to feed bunk competition due to space limitation or social hierarchy among cows. Due to the low proportion of moisture of feed ingredients used in the high grain rations, water was added to the TMR during mixing to achieve a target of DM content of approximately 470 g DM/kg fresh feed. Feed refusals were weighed, and automatic feeders were cleaned every day before the morning meal.

2.2. Sampling and chemical analyses of feeds

Samples of individual feed ingredients were collected at the start and at the end of each period, and TMR offered to the cows were collected and pooled every week and stored at −20 °C for later analyses. Dry matter concentrations of silages were determined by drying samples at 100 °C for 24 h. Using the resulting DM data, diets were then adjusted as needed to ensure proper inclusion of ingredients in the TMR. At the end of the experiment, samples were analyzed for chemical composition. Briefly, ash was analyzed by combustion in a muffle furnace overnight at 580 °C. Crude protein was analyzed following the Kjeldahl method (VDLUFA, 2012) and ether extracts (EE) using the soxhlet extraction system (Extraction System B-811, Büchi, Flawil, Switzerland). The NDF and ADF contents were determined with sodium sulfite and reported exclusive of residual ash following the official analytical methods of VDLUFA (2012) using the Fiber Therm FT 12 (Gerhardt GmbH & Co. KG, Königswinter, Germany) with heat-stable α-amylase for NDF analysis. Starch content was measured with the K-TSTA kit (Megazyme Ltd., Wircklow Ireland). Non-fiber carbohydrates were calculated as 1000 - (content of crude protein + content of NDF + content of ether extract + content of ash). Residual OM was calculated by partitioning non-fiber carbohydrates into starch and residual OM (Weiss and Tebbe, 2018).

Particle size distribution of TMR was measured in triplicate within each week using the method described by Kononoff et al. (2003) with a Penn State Particle Separator equipped with 3 screens (19.0, 8.0, and 1.18 mm) and a pan. Using these data, physically effective NDF (peNDF) and physically effectiveness factor (pef) were calculated as outlined by Beauchemin and Yang (2005). Briefly, the peNDF content of the diet was determined by multiplying the NDF content of the diet by its pef. The pef > 8 mm (ranging from 0 to 1) was calculated as sum of the proportion (as is basis) of particles retained on the corresponding sieves (19.0- and 8.0-mm sieves).

2.3. Evaluation of chewing behavior and eating behavior

Chewing activity and drinking time were monitored according to Kröger et al. (2017), a method also used by Brandstetter et al. (2019). Briefly, noseband sensor halters were used (RumiWatch System, ITIN +HOCH GmbH, Fütterungstechnik, Liestal, Switzerland) during three consecutive days within each week in all animals simultaneously. Approximately 12 h before the start of data collection, halters were placed on the cows for animal adaptation. At the end of measurements, the recorded raw data were transferred through the interface software RumiWatch Manager (version 2.2.0.0; Itin and Hoch GmbH), and processed using the evaluation software RumiWatch Converter (Version 0.7.3.2). Chewing activity data were summarized and calculations included ruminating time, eating time, total chewing time, rumination expressed as chews/min, rumination expressed as chews/bolus and chewing index (total chewing time/kg DMI) according to Kröger et al. (2017). In addition, diurnal variations of rumination and eating patterns were illustrated for a comprehensive description throughout a 24-h period within each week.

Feed sorting was assessed in triplicate within each evaluated week in all animals using an approach similar to Haselmann et al. (2019). Briefly, particle size distribution was measured in refusals collected from the bunk in the morning before new feed was offered. Feed selection of each cow was expressed through the change in particle size distribution (as-is basis) of the provided TMR in relation to the refusals. According to Leonardi and Armentano (2003), feed selection of a particle size fraction was calculated as the percentage of the actual as-fed intake from the predicted as-fed intake, expressed by the selection index. The predicted intake of a specific particle fraction is calculated as the product of as-fed intake and as-fed proportion of this specific fraction in the TMR offered. This analysis allowed the evaluation of the variations in the amount of diet consumed according to feed particle fraction (long, medium, small and fine, as-is basis).

2.4. Evaluation of lying behavior

Lying behavior was recorded during the same three consecutive days used for the evaluation of chewing activity within each evaluated week in all animals, and this was performed using electronic data loggers (HOBO Pendant G Acceleration Data Logger, Onset Computer Corp., Bourne, MA, USA). To do so, loggers were attached to the lateral side of the left hind leg (distal metatarsus) of each cow using vet wrap. Raw data of lying behavior were downloaded with the HOBOware PRO and then processed using the cut point reported by Ledgerwood et al. (2010) and as described in Zobel et al. (2015). Measures of lying behavior included total lying time per day, total number of lying bouts per day, laterality (right or left), and the number of lying bouts to the right or left. Additionally, combining data of rumination and lying behavior, we evaluated ruminating time while standing, as well as the ruminating time when lying on the right or left side.

2.5. Saliva sampling and evaluation of salivary properties

Saliva samples were collected orally in all cows in every evaluated week, with one sampling day during the forage feeding and four sampling days during the entire high grain feeding period. Approximately 100 mL of saliva were collected at each sampling immediately before the morning feeding, each sample was divided in 10 aliquots, and stored in 15 mL vials. Samplings and measurements of salivary properties are detailed in Castillo-Lopez et al. (2021a).

2.6. Evaluation of saliva production

Saliva production was measured in all cows in the week of forage feeding and in week 4 of grain feeding, following a method similar to Maekawa et al. (2002) and Chibisa et al. (2016). The swallowed feed boli were collected at the cardia while the cows were eating by inserting an arm through the ruminal cannula and using a plastic bag (17 × 30 cm) secured on the wrist. Up to five feed boli were collected from each cow. At the end of the experiment, saliva present in feed boli, feed ensalivation and salivation rate (g/min) were measured.

2.7. Statistical analysis

A priori statistical power analysis was performed according to Stroup (1999) and Kononoff and Hanford (2006) with Proc Power of SAS (version 9.4; SAS Institute, Cary, NC). This analysis was conducted using similar data of chewing (Castillo-Lopez et al., 2021a) as main response variables. The results indicated a statistical power ≥ 85 % for this study with α = 0.05, an acceptable level of statistical power. In addition, we observed that a minimum of n = 4 is required to obtain an average statistical power of 0.82 with α = 0.05. Data were checked for the presence of outliers, resulting in 6 outliers for chewing index, 2 outliers for drinking time, and a total of 14 outliers for all salivary variables, which were removed. Normal distribution was checked with Proc Univariate. If the normality assumption was not met, Proc transreg performing a Box-Cox was used to determine the transformation mode (i.e. log or root square transformation), which was performed before the ANOVA. Data collected were analysed with Proc Mixed of SAS with experimental period, measurement week nested within high grain feeding, and treatment (CON and PHY) as fixed effect. In order to compare the effect of duration of high grain feeding on the variables investigated, the PDIFF option was also tested, which allowed multiple comparisons of means throughout the evaluated weeks. Thus, the multiple comparisons allowed the assessment of the changes occurring not only immediately after the diet switch, but also due to prolonged consumption of high grain. Cow within experimental period was included as random effect. Furthermore, the interaction between supplementation treatment × week was assessed. Data collected in different weeks within each experimental period from the same cow within CON or PHY were considered as repeated measures. To do so, a first order autoregressive covariance matrix (ar (1)) was used taking into consideration that the covariance decays with time. Cow was considered as the experimental unit. The largest standard error of the mean (SEM) is reported. Significance was declared when $P \leq 0.05$ and a tendency is indicated if $0.05 < P \leq 0.10$.

3. Results

3.1. Dietary characteristics

The change from forage to high grain ration decreased the content of NDF from 555 to 316 g/kg DM as well as the content of peNDF with size >8 mm from 472 to an average of 190 g/kg DM in the high grain rations. Concomitantly, there was an increase in the content of starch from 170 to an average of 287 g/kg DM (Supplementary Table 1).

3.2. Dry matter intake, chewing activity and eating pattern

Table 1 lists DMI, rumination, eating, total chewing time, and drinking times. Dry matter intake increased ($P < 0.01$) with the change from forage to the high grain ration, with the maximum DMI observed during week 1, independent of PHY supplementation.

Changing the diet from the week of forage feeding to week 1 on high grain reduced rumination time by 270 min/d (from 506.5 to 236.5 ± 30.8 min/d) ($P < 0.01$), with the lowest rumination time found in week 2 of high grain feeding (160.5 ± 30.8 min/d). However, compared to week 2, rumination time increased ($P < 0.01$) by 53 and 73 min/d in weeks 3 (213.5 ± 30.8 min/d) and 4 (233.5 ± 30.8 min/d) on high grain, respectively. Likewise, total chewing time ($P < 0.01$) followed a similar pattern with lowest values observed in week 2 (288.5 ± 34.6 min/d), but showed a recovery ($P < 0.01$) in weeks 3 (360.5 ± 34.6 min/d) and 4 (376.5 ± 34.6 min/d) on high grain, which represented an increase of 72 and 88 min/d, respectively. In week 4 on high grain, the PHY-supplemented cows tended ($P = 0.08$) to ruminate (263 vs. 204 min/d) and chew (406 vs. 347 min/d) longer compared to CON counterparts.

Eating time was strongly reduced ($P < 0.01$) by diet change, with a difference of 54 min/d between the week of forage feeding and week 1 of the high grain diet (197.5 and 144 ± 17.6 min/day, respectively), and reached the lowest value in week 2 on high grain (128.5 ± 17.6 min/d). However, eating time showed a recovery and was greater in week 3 (145.5 ± 17.6 min/d) compared to the previous week. The chewing index was also negatively affected ($P < 0.05$), and reached its lowest value in week 2 on high grain (27.0 ± 2.94 total chewing time/kg DMI). However, chewing index increased in weeks 3 (32.4 ± 2.94 total chewing time/kg DMI) and 4 (33.2 ± 2.94 total chewing time/kg DMI), values that were greater ($P < 0.05$) compared to week 2 on high grain. Results also showed that the number of chews per bolus was strongly affected in week 2 on high grain (48.8 ± 2.13 chews/bolus), but this variable showed a recovery ($P < 0.05$) in week 3 (53 ± 2.13 chews/bolus) of high grain feeding.

Drinking time was affected by diet change, with an increment ($P < 0.05$) of time spent drinking by 20 % when the ration switched to high grain (from 3.95 to 5.35 ± 2.09 min/d). There was an interaction between week on high grain and supplementation on drinking time ($P < 0.05$). Specifically, PHY cows spent less time drinking ($P < 0.05$) in week 1 (6.7 ± 2.09 min/d) compared to the rest of weeks of high grain feeding.

Supplementary Fig. 1 illustrates the diurnal variation of eating time. Overall, in all evaluated weeks, cows visited the automatic feeders within the same interval of the day, but each visit at the automatic feeder lasted shorter during the weeks of high grain feeding compared to the forage diet, with a difference of 30 % less time spent at the automatic feeders during high grain feeding. Supplementary Fig. 2 illustrates the diurnal variation of rumination time. In the week of forage feeding, the overall level of rumination was evidently greater with a peak from approximately 20:00 pm to midnight for both CON and PHY, with values reaching up to 34 min/h. However, in week 1 of high grain feeding, rumination time did not peak in the same manner and it was distributed throughout the day, with an average of 9 min/h. From week 2–4 of high grain feeding, rumination peaks were delayed compared to the week where cows received the forage diet, with maximum peaks early in the morning before the first meal, reaching up to 24 min/h. The lowest rumination activity in the weeks of high grain feeding was generally found in the afternoon.

3.3. Feed sorting and intake according to feed particle fraction

Feed sorting behavior data are shown in Table 2. During the forage feeding, cows selected against long feed particles. However, high grain feeding changed the sorting behavior by increasing ($P < 0.05$) selection for long and medium size feed particles, with maximum sorting activity

Table 1
Effect of switching from forage and the length of time of high grain feeding on feed intake, chewing activity and drinking time of non-lactating Holstein cows supplemented with or with no phytogenic additive supplement[1].

	Forage diet		High grain diet												
	Week 0		Week 1		Week 2		Week 3		Week 4			*P*-values[3]			
Item	CON	PHY	CON	PHY	CON	PHY	CON	PHY	CON	PHY	SE[2]	Change	Dur	S	I
DMI, kg	9.39	9.40	12.91	12.1	10.9	11.2	11.7	10.5	11.5	11.4	0.58	< 0.01	< 0.01	0.18	0.25
Eating time, min/d	203	192	152	136	128	129	143	148	140	138	17.6	< 0.01	0.07	0.67	0.88
Ruminating time, min/d	525	488	241	232	172	149	238	189	204^{x}	263^{y}	30.8	< 0.01	< 0.01	0.49	0.06
Ruminating, chews/min	64.9	64.8	62.9	62.4	62.3	62.4	61.8	61.3	61.7	61.3	0.89	< 0.01	0.05	0.15	0.93
Ruminating, chews/bolus	52.1	52.5	54.8	50.7	48.8	48.9	54.7	50.6	50.8	50.3	2.13	0.40	< 0.05	0.19	0.07
Total chewing time, min/d	719	688	385	376	293	284	377	344	347^{x}	406^{y}	34.6	< 0.01	< 0.01	0.82	0.24
Chewing index, total chewing time/kg DMI	78.9	73.2	29.7	33.7	26.7	27.4	31.8	33.0	29.4^{x}	37.0^{y}	2.94	< 0.01	< 0.05	0.38	0.15
Drinking time, min/d	4.4	3.5	6.7	4.0	6.7	6.9	6.1	8.7	6.1	7.2	2.09	< 0.01	0.07	0.92	0.02

[1]CON: A control diet with no phytogenic supplementation; PHY: supplementation with 0.4 g/kg DM of a phytogenic feed additive based on a combination of menthol, thymol and eugenol.
[2]The largest standard error of the mean.
[3]P-value for the effect of the change from forage feeding to the first week of high grain feeding (Change); the overall P-value for the duration in weeks within the high grain feeding (Dur); the P-value for the effect of phytogenic supplementation (S); and the P-value for the interaction of phytogenic supplementation × weeks of high grain feeding (I).
[x,y]Within week, means with different superscripts indicate a tendency for significant difference ($0.05 < P \leq 0.10$) between CON and PHY.

Table 2
Effect of switching from forage and the length of time of high grain feeding on feed sorting of feed particle fractions in non-lactating Holstein cows supplemented with or with no phytogenic feed additive[1].

	Forage diet Week 0		High grain diet Week 1		Week 2		Week 3		Week 4			*P*-values[4]			
Feed particle fraction as is[2]	CON	PHY	CON	PHY	CON	PHY	CON	PHY	CON	PHY	SE[3]	Change	Dur	S	I
Sorting, %															
Long	95.6	91.7	108.3	113.0	121.0	115.6	123.4	124.9	119.1	117.9	5.65	< 0.01	< 0.05	0.81	0.80
Medium	105.3	108.2	116.3[a]	105.3[b]	120.8	120.3	116.8	115.8	106.9	102.1	2.93	< 0.01	< 0.05	0.31	< 0.05
Short	104.3	104.5	66.0	67.6	51.0	48.8	40.9	52.4	70.1	64.7	8.26	< 0.01	< 0.05	0.78	0.77
Fine	98.9	105.0	72.9[a]	37.2[b]	53.2	50.3	27.6	47.1	50.9	40.6	12.9	< 0.01	0.20	0.76	0.03
Intake, kg as fed															
Long	18.0	21.6	5.94[b]	11.5[a]	5.4	6.63	7.20	7.27	9.08	11.4	1.28	< 0.01	< 0.01	0.21	0.03
Medium	5.74	7.95	13.3	14.5	12.5	13.4	16.4	15.8	9.92	11.7	1.70	< 0.01	< 0.01	0.60	0.39
Short	3.76	4.87	3.09	2.87	2.35	1.95	2.18	1.21	2.87	2.75	0.49	< 0.01	0.09	0.51	0.62
Fine	0.14	0.20	0.05	0.11	0.12	0.14	0.19	0.09	0.11	0.19	0.09	0.36	0.52	0.49	0.80

[1]CON: A control diet with no phytogenic supplementation; PHY: supplementation with 0.4 g/kg DM of a phytogenic feed additive based on a combination of menthol, thymol and eugenol.
[2]Particle fractions determined by Penn State Particle Separator with a 19-mm screen (long), 8-mm screen (medium), 1.18-mm screen (short), and a pan (fine) according to Kononoff et al. (2003). Values = 100 indicate no sorting, < 100 indicate selective refusals (sorting against), and > 100 indicate preferential sorting (sorting for; Leonardi and Armentano, 2003).
[3]The largest standard error of the mean.
[4]*P*-value for the effect of the change from forage feeding to the first week of high grain feeding (Change); the overall *P*-value for the duration in weeks within the high grain feeding (Dur); the *P*-value for the effect of phytogenic supplementation (S); and the *P*-value for the interaction of phytogenic supplementation × weeks of high grain feeding (I).
[a,b]Within week, means with different superscripts indicate a significant difference ($P < 0.05$) between CON and PHY.

observed during week 2 (118.3 ± 5.65 %) and 3 (124.2 ± 5.65 %) of high grain feeding (up to 27 % units greater selection index of long particles between the average of these weeks and the week of forage feeding, 93.6 ± 5.65 %). There was a differential sorting pattern across treatments ($P < 0.05$), so that selection for medium size feed particles was less pronounced in the PHY supplemented cows compared to CON in week 1 on high grain (116.3 and 105.3 ± 2.93 %, for CON and PHY, respectively). The selection index for fine particles during high grain feeding displayed less variation for PHY cows compared to CON, as also evidenced by the interaction between the supplementation and week of grain feeding ($P < 0.05$).

With regard to the amount of diet consumed by cows according to feed particle size (as fed), as expected, there was a reduction ($P < 0.05$) in the intake of long feed particles (>19-mm) due to diet change (from 19.8 to 8.72 ± 1.28 kg/d). However, after reaching the lowest intake of long particles in weeks 2 (6.0 ± 1.28 kg/d) and 3 (7.2 ± 1.28 kg/d), there was an increase ($P < 0.05$) in the consumption of this particle fraction in week 4 on high grain (10.24 ± 1.28 kg/d). In addition, PHY supplementation resulted in greater ($P < 0.05$) intake of long feed particles in week 1 on high grain (5.94 vs. 11.5 ± 1.28 kg/d for CON and PHY, respectively). The intake of medium size feed particles (between 19 and 8 mm) increased with diet change (6.8 vs. 14.0 ± 1.70 kg/d), reaching the highest ($P < 0.05$) intake in week 3 (16.1 ± 1.70 kg/d), but displayed a decrease ($P < 0.05$) in week 4 on high grain (10.8 ± 1.70 kg/d) compared to the previous week.

3.4. Animal lying behavior

Table 3 lists variables associated with cows' lying behavior. Duration on high grain diet increased lying time ($P < 0.01$). Although there was no significant change in lying time immediately after the diet switch; compared to week 1 (13.8 ± 0.42 h/d), lying time increased ($P < 0.05$) by 58 and 50 min/d in week 2 (14.8 ± 0.42 h/d) and 3 (14.7 ± 0.42 h/d) on high grain, respectively.

Table 3
Effect of switching from forage and the length of time of high grain feeding on lying behavior of non-lactating Holstein cows supplemented with or with no phytogenic feed additive[1].

	Forage diet Week 0		High grain diet Week 1		Week 2		Week 3		Week 4			*P*-values[3]			
Item	CON	PHY	CON	PHY	CON	PHY	CON	PHY	CON	PHY	SE[2]	Change	Dur	S	I
Lying time (right), h/d	7.0	6.9	6.8	7.0	7.5	7.3	7.1	6.9	7.3	7.1	0.51	0.71	0.45	0.64	0.97
Bouts to the right, n/d	9.8	8.6	8.6	10.9	8.3	9.0	7.5	8.8	8.2	9.8	1.40	0.72	0.66	0.26	0.66
Lying time (left), h/d	6.6	6.4	7.3	6.8	7.4	7.5	7.4	7.8	7.2	7.0	0.54	<0.05	0.52	0.78	0.94
Bouts to the left, n/d	8.9	8.0	8.4	11.1	8.4	9.7	8.0	9.4	8.5	10.2	1.32	0.42	0.80	0.10	0.61
Total lying bouts, n/d	18.7	16.7	17.0	22.0	16.7	18.7	15.5	18.3	16.6	19.9	2.63	0.83	0.72	0.15	0.63
Total lying time, h/d	13.5	13.5	13.9	13.8	14.8	14.8	14.5	14.9	14.4	14.2	0.42	<0.01	<0.05	0.92	0.91
Rumination time standing, h/d	2.03	2.05	1.02	1.25	0.75	1.07	1.07	0.90	0.78	1.47	0.25	<0.01	0.55	0.16	0.13
Rumination time lying on the right, h/d	2.75	2.58	1.13	1.03	0.80	0.72	0.90	1.03	0.97	1.23	0.26	<0.01	0.19	0.97	0.71
Rumination time lying on the left, h/d	3.68	3.18	1.90	1.35	1.98	1.10	1.83	1.42	1.50	1.48	0.43	<0.01	0.92	<0.05	0.70

[1]CON: A control diet with no phytogenic supplementation; PHY: supplementation with 0.4 g/kg DM of a phytogenic feed additive based on a combination of menthol, thymol and eugenol.
[2]The largest standard error of the mean.
[3]*P*-value for the effect of the change from forage feeding to the first week of high grain feeding (Change); the overall *P*-value for the duration in weeks within the high grain feeding (Dur); the *P*-value for the effect of phytogenic supplementation (S); and the *P*-value for the interaction of phytogenic supplementation × weeks of high grain feeding (I).

Results also revealed that the change to high grain feeding negatively affected ($P < 0.05$) ruminating time when cows were either standing or lying, with a decrease by 50 % and 57 % when standing (from 2.04 to 1.10 ± 0.25 h/d) or lying on the right side (from 2.6 to 1.10 ± 0.25 h/d), respectively. We also found that PHY supplementation decreased ($P < 0.05$) the time that cows spent ruminating when lying on the left side (2.17 vs 1.70 ± 0.43 h/d for CON and PHY, respectively).

3.5. Salivary properties

Salivary physico-chemical properties are listed in Table 4. Salivary bicarbonate was not affected immediately after the diet change. However, this variable decreased ($P < 0.05$) in weeks 3 (76.7 ± 2.64 mM) and 4 (74.2 ± 2.64 mM), with these values being lower compared to week 2 on high grain (82.2 ± 2.64 mM). Buffer capacity and osmolality increased immediately after the diet change ($P < 0.05$; from 0.11 to 0.13 ± 0.01 decamols HCl/L/ΔpH), and then remained constant. Salivary mucins tended ($P = 0.07$) to be greater for PHY supplemented cows. Lysozyme activity was greater in week 4 on high grain ($P < 0.05$) for CON cows (61.3 and 41.0 ± 6.99 U/mL/min for CON and PHY, respectively). High grain diet decreased ($P < 0.05$) salivary pH in week 1, while PHY supplementation helped maintaining this variable at physiological level during this initial grain challenge (8.55 and 8.87 ± 0.08 for CON and PHY, respectively).

3.6. Feed bolus characteristics and salivation

Table 5 shows data for feed bolus characteristics and salivation. Feed bolus size was greater ($P < 0.05$) when cows consumed the high grain ration (34 and 69 ± 6.71 g DM for forage and high grain, respectively) without any interaction between the diet and supplementation. The feed ensalivation was reduced ($P < 0.01$) by nearly half during high grain feeding due to greater bolus size (5.19 and 2.85 ± 0.48 g saliva/g feed DM for forage and high grain, respectively).

Over the 30-min meal, feed bolus size was similar throughout the meal with the forage diet, with an average of 34 g ± 6.71. However, when cows consumed the high grain diet, the first feed bolus was the largest and averaged 92 ± 6.71 g; then, there was a gradual reduction ($P < 0.01$) in the size of the feed boli (Fig. 1). In addition, over the 30-min meal, there was no effect of feed bolus number ($P = 0.67$) on feed ensalivation. However, feed ensalivation was generally greater throughout the meal when cows consumed the forage ration, (averaging 5.19 ± 0.48 g saliva/g feed DM; Fig. 2).

4. Discussion

The transition from a forage diet to high grain rations has commonly been associated with effects on rumen and systemic health in dairy cattle. These detrimental effects are mainly due to a strong reduction of chewing behavior (Maekawa et al., 2002; Kröger et al., 2017, 2019) and impaired salivation (Chibisa et al., 2016; Castillo-Lopez et al., 2021a). In this context, in switch over experimental designs, it has been commonly suggested in the literature that cattle adapt to a different diet around 2–3 weeks after the diet transition (Kairenius et al., 2018; Ranathunga et al., 2018; Nasrollahi et al., 2019). Nonetheless, there are limited quantitative data showing how cattle adjust and cope to the change due to advanced duration of feeding after the diet switch. Our main hypothesis was that adaptive changes with regard to chewing and eating behavior, lying behavior and salivary characteristics will be observed not only immediately after the diet switch, but also 3 and 4 weeks later. We also hypothesized that supplementing a phytogenic feed additive can mitigate such detrimental effects mainly by modulating chewing behavior and salivary characteristics, stimulated by the organoleptic properties of the phytogenic compound.

Our findings are in agreement with the first hypothesis. The reduction in rumination and total chewing times observed immediately after the switch to high grain diet support previous reports (Beauchemin, 2018; Cao et al., 2020). Additionally, we found that rumination and chewing times are negatively affected particularly 2 weeks after the diet change. This may be explained by the decreased eating time in combination with the lowest amount of long feed particles consumed by cows in week 2 on high grain, factors that decreased the stimulus that triggers rumination, since rumination is stimulated by the activation of tensile receptors in the foregut (Brewer, 1987; Ruckebusch, 1988). Interestingly, we found a recovery in rumination time in week 3 on high grain compared to the initiation of the high grain feeding. This increased in rumination time may reflect the increased sorting in favor of long size and medium size feed particles and the increased amount of long feed particles consumed in weeks 3 and 4 on high grain. However, the increase in rumination time did not improve ruminal pH in week 4 compared to week 2 on high grain (Castillo-Lopez et al., 2022). Nonetheless, despite no improvement in ruminal pH in week 4, results suggest that cows showed a positive adaptation to high grain with greater chewing activity, which is known to have beneficial effects on cattle health (Souza et al., 2021). The reduction in DMI observed in week 3 and 4 compared to week 1 on high grain may reflect adaptation to mitigate gut acidification.

Regarding the adaptation of cattle after a diet change, previous

Table 4
Effect of switching from forage and the length of time of high grain feeding on salivary physico-chemical properties in non-lactating Holstein cows supplemented with or with no phytogenic feed additive[1].

	Forage diet		High grain diet													
	Week 0		Week 1		Week 2		Week 3		Week 4			*P*-values[3]			
Item[4]	CON	PHY	CON	PHY	CON	PHY	CON	PHY	CON	PHY	SE[2]	Change	Dur	S	I
pH	8.91	8.86	8.55[b]	8.87[a]	8.84	8.85	8.80	8.95	8.92	8.90	0.08	0.40	0.07	0.15	0.07
Bicarbonate, mM	80.6	74.1	75.1	78.8	84.7	79.8	77.2	76.2	75.0	73.4	2.64	0.91	<0.05	0.32	0.23
Buffer capacity, decamols HCl/L/ΔpH	0.11	0.12	0.13	0.13	0.12	0.13	0.13	0.13	0.13	0.13	0.01	<0.05	0.69	0.69	0.98
Osmolality, mOsm/Kg	243	238	245	249	255	257	256	248	251	257	4.1	<0.01	0.11	0.88	0.32
Mucin, mg/mL	0.86	1.14	1.01	0.98	0.88	1.23	1.04	1.08	1.03	1.13	0.17	0.58	0.86	0.07	0.20
Lysozyme activity, U/mL/min	41.8	35.7	40.8	46.8	43.4	37.1	34.1	40.3	61.3[a]	41.0[b]	6.99	0.32	0.10	0.33	0.08
Total protein, μg/mL	352	483	416	473	380	462	443	444	396	393	63.4	0.72	0.65	0.16	0.52
Phosphate, mM	10.8	10.6	11.0	12.0	11.7	11.0	12.4	11.0	12.9	12.8	0.85	0.06	0.17	0.59	0.54

[1]CON: A control diet with no phytogenic supplementation; PHY: supplementation with 0.4 g/kg DM of a phytogenic feed additive based on a combination of menthol, thymol and eugenol.
[2]The largest standard error of the mean.
[3]*P*-value for the effect of the change from forage feeding to the first week of high grain feeding (Change); the overall *P*-value for the duration in weeks within the high grain feeding (Dur); the *P*-value for the effect of phytogenic supplementation (S); and the *P*-value for the interaction of phytogenic supplementation × weeks of high grain feeding (I).
[a,b]Within week, means with different superscripts indicate a significant difference ($P < 0.05$) between CON and PHY.

Table 5
Effect of changing from forage to a high grain diet on salivation and feed bolus size of non-lactating Holstein cows supplemented with or with no phytogenic feed additive[1].

	Forage diet Week 0		High grain diet Week 4			*P*-values[3]		
Item	CON	PHY	CON	PHY	SE[2]	Diet	S	I
Feed bolus size (as is), g	270.6	242.4	284.2	303.1	23.14	< 0.05	0.86	0.20
Feed bolus size (DM), g	35.7	31.8	64.1	75.5	6.71	< 0.01	0.62	0.15
Saliva in bolus, g	160.9	144.4	143.7	144.8	8.92	0.31	0.40	0.28
Feed ensalivation, g saliva/g feed DM	5.01	5.37	3.31	2.39	0.486	< 0.01	0.61	0.10
Saliva flow, g saliva/min	80.4	72.2	71.8	72.4	4.46	0.31	0.40	0.28

[1]CON: A control diet with no phytogenic supplementation; PHY: supplementation with 0.4 g/kg DM of a phytogenic feed additive based on a combination of menthol, thymol and eugenol.
[2]The largest standard error of the mean.
[3]*P*-values for the effect of diet (Diet), phytogenic feed additive supplement (S) and the diet × phytogenic feed additive interaction (I).

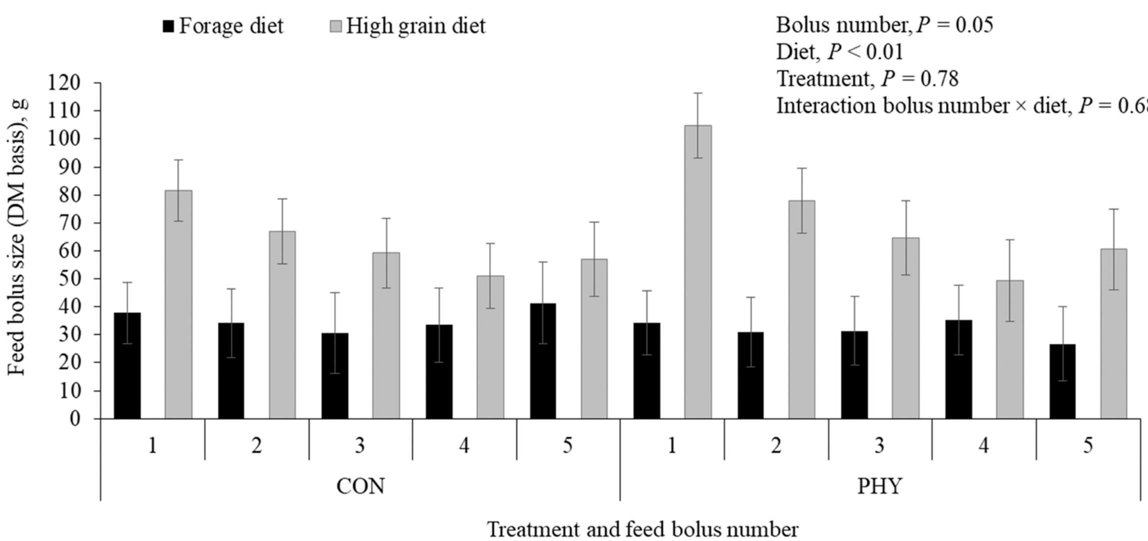

Fig. 1. Effect of feeding forage or a high grain diet on feed bolus size evaluated over a 30-min interval in non-lactating Holstein cows not supplemented or supplemented with a phytogenic feed additive containing a mixture of menthol, thymol and eugenol.

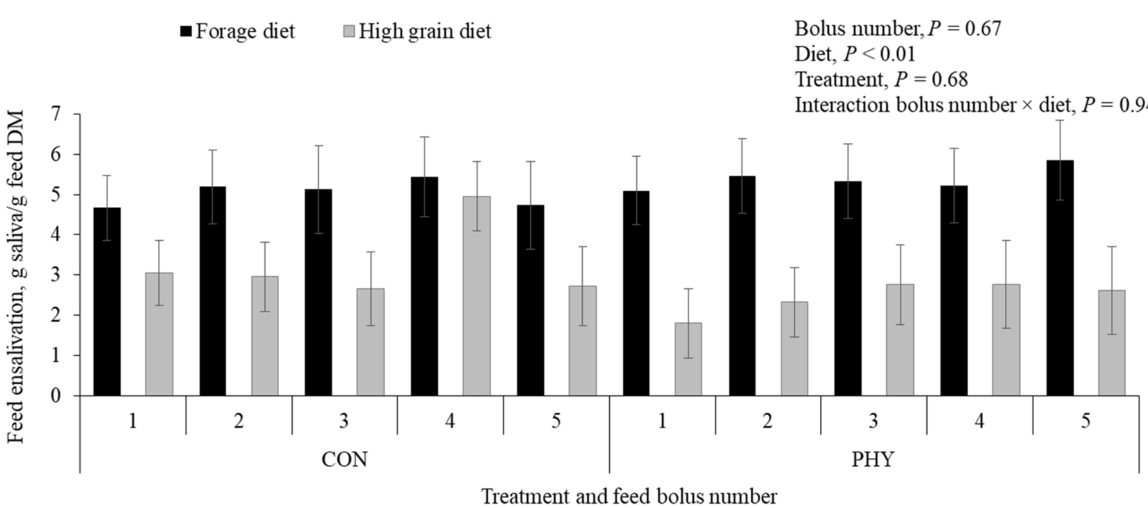

Fig. 2. Effect of feeding forage or a high grain diet on feed ensalivation evaluated over a 30-min interval in non-lactating Holstein cows not supplemented or supplemented with a phytogenic feed additive containing a mixture of menthol, thymol and eugenol.

studies in dairy cows using adaptation periods of 2 weeks have found a strong impairment of chewing activity due to a decrease in dietary forage (Jiang et al., 2017) or shorter forage particle size (Kammes and Allen, 2012). Another study using 3 weeks of adaptation reported that cattle supplemented with hay or corn silage did not differ in rumination time (Graf et al., 2005). Although these reports are reasonable, given the recovery of chewing activity we observed 4 weeks after the diet switch, it is feasible to suggest that cattle may need longer adaptation periods typically used in switch-over designs for a more reliable evaluation of the response variables, especially when assessing chewing activity. The tendency for longer rumination and chewing times in week 4 on high grain for the PHY-supplemented cows compared to CON hints at potential beneficial effects of the PHY feed additive, but further research is warranted to fully understand the magnitude of these changes.

The increase in rumination time and sorting for long fibrous feed particles in week 3 and 4 support our previous reports (Castillo-Lopez et al., 2022) as well as research from other investigators (Lechartier and Peyraud, 2010; Farmer et al., 2014; Ranathunga et al., 2019), showing that greater fiber intake is accompanied by increased proportion of acetate in the rumen fluid. This is because dietary fiber promotes chewing activity and stimulates acetate synthesis by fibrolytic bacteria (Beckett et al., 2020). We also observed that the change in acetate in weeks 3 and 4 on high grain was at the expense of ruminal butyrate. This observation may be because advanced duration of high grain feeding could downregulate the butyryl CoA-acetyl CoA transferase pathway, one of the main pathways for short chain fatty acid synthesis. In this metabolic route, there is utilization of acetate for the synthesis of butyrate (Duncan et al., 2002), which was found to be greater for PHY cows, as presented in the companion paper.

Another interesting observation shows that compared to the week of forage feeding, peaks for rumination activity post feeding were delayed from the second week on high grain onwards. This increment in the elapsed time for maximum rumination peaks may be due to the decreased DMI observed in weeks 3 and 4 on high grain. Rumination is usually triggered by ruminal stretch and mechanical stimuli (Ruckebusch, 1988). Thus, with a less bulky high grain diet and lower DMI in those weeks compared to the start of the high grain feeding, cows probably required more time and feed intake to trigger rumination. The faster eating rate revealed especially by the greater first feed bolus during high grain feeding compared to the forage diet, was likely due to increased feed palatability, which may have led to greater number of short visits to the automatic feeders. These results support previous reports from Cao et al. (2020) indicating that a low NDF diet is associated with greater number of meals per day. In this regard, the low peNDF-diets used in this study can be considered as acidogenic (Khorrami et al., 2021). Thus, the greater DMI and eating rate, particularly at the start to the high grain feeding, are in agreement with our previous observations showing rapid acidification not only in the rumen, but also in the hindgut (Castillo-Lopez et al., 2022).

We further found that lying time of cows increased mostly in week 3 of consuming the high grain diet. In this study, we did not evaluate claw health and laminitis in cows. However, it is plausible that the observed increase in lying time reflected claw damage and discomfort due to advanced length of time on high grain, because increased lying time has been associated with claw lesions, with cows being more reluctant to stand up (Peterse et al., 1984; Cook et al., 2004; Omontese et al., 2020). The lesions in claws have been related to the production of proinflammatory molecules in the rumen and systemically when cattle are fed high grain rations (Guo et al., 2021). In another study, severe lameness in dairy cows was strongly associated with increased lying time as well as with longer bout duration (Ito et al., 2010), especially when cows were housed in deep-bedded stalls. Thus, future studies should consider the evaluation of claw health due to prolonged exposure to high grain ration in dairy cows and its association with lying behavior.

There is limited research on salivary properties in cattle (Bailey, 1961; Bailey and Balch, 1961). Results revealed a reduction in salivary bicarbonate mostly in week 4 on high grain, which could have contributed to the greater time that ruminal pH was below 5.8 in week 4 compared to week 2 on high grain (Castillo-Lopez et al., 2022). Additionally, the immediate effect of high grain feeding on salivary osmolality could be due to increased rumen fluid osmolality, which draws water from circulation (Owens et al., 1999; Silanikove and Tadmor, 1989). This finding agrees with the increment in drinking time due to diet change, which likely reflected greater thirst. The increased salivary lysozyme activity for CON in week 4 on high grain may be due to a change in the oral microbiome (Oliver and Wells, 2015) with advanced duration on high grain. Most importantly, findings indicate that cattle experience adaptive changes even 3 and 4 weeks after the dietary change.

The lower rumination times with the high grain diet resulted in reduced amount of saliva per unit of DMI, because of the role of chewing in the stimulation of salivation. In addition, the increase in feed bolus size was associated with reduced feed ensalivation. Our results agree with previous research showing that a reduction in the dietary forage leads to a reduction in feed ensalivation in cattle (Chibisa et al., 2016). This may be due to the known stimulating effects of dietary fiber on chewing and saliva production. Other studies have also reported that duration of exposure to a high grain diet may influence salivation (Schwaiger et al., 2013), which agrees with previous findings from our group showing a reduction of feed ensalivation after 3 weeks of high grain feeding (Castillo-Lopez et a, 2021a). Results also showed that salivation rate and feed ensalivation behaved independently of PHY. This finding contrasts previous reports showing that phytogenic additives like menthol can enhance salivation in monogastrics (Eccles, 1994; Haahr et al., 2004) and dairy cows (Ricci et al., 2021). These effects had been attributed to the taste and smell, which stimulate the salivary glands (Liston et al., 2004; De Sousa Barros et al., 2015; Proctor, 2016) and the olfactory-salivary reflex (Eccles, 1994; Haahr et al., 2004). Discrepancies between our observations and previous reports could be due to potential antagonistic interactions among phytogenic compounds when combined.

5. Conclusions

Overall, results from the present experiment show that dairy cows still experience adaptive changes 3 and 4 weeks after the diet change. The negatives effects of grain-rich diets on chewing behavior are observed mainly in week 2 after the diet change, which may be due the lowest amount of long feed particles consumed by cows in that week, factors that decreased the stimulus that triggers rumination. However, there was a recovery in rumination time in week 3, likely due to increased sorting and greater amount of intake of fibrous feed particles, suggesting adaptation of cows to the diets with advance duration on high grain, but without having an influence on the time that ruminal pH < 5.8 previously reported. Lying time was not affected immediately after the diet change. However, lying time significantly increased in week 3 on high grain, possibly reflecting claw damage. On the other hand, salivary bicarbonate was strongly negatively affected with advanced duration on high grain, which may have contributed to the greater time that ruminal pH < 5.8 in week 4 compared to previous weeks. Therefore, results suggest that in experimental designs involving a change between diets with different chemical composition, cows need at least 4 weeks of adaptation to the new diet in order to minimize potential carry-over effects. More research is warranted regarding the positive role of PHY supplementation on feed sorting, salivary characteristics and chewing, and how this will influence health, metabolic health status and welfare of high-producing dairy cows, which suffer from high grain feeding.

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CRediT authorship contribution statement

Acquisition of funding and leader of the project: QZ. Conception and design of the study: QZ, ECL, SR, RR-C. Experimental performance and acquisition of data: ECL, SR, RR-C, BK. Analysis and interpretation of data: ECL, RR-C, AH. Original drafting of the manuscript: ECL. Revising the manuscript critically for intellectual content: QZ, SR, RR-C, BK, AH, NR, ECL.

Conflict of Interest

Nicole Reisinger is employed by BIOMIN Holding GmbH, which is part of DSM. However, this circumstance did not influence the design of the study or bias the presentation and interpretation of results.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.105877.

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