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Effects of an artificial hay aroma and compound feed formulation on feed intake pattern, rumen function, and milk production in lactating dairy cows

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Feed intake pattern and rumen function of dairy cows

Abstract

The Kempen system is a dairy feeding system in which diet is provided in the form of a compound feed and hay offered *ad libitum*. *Ad libitum* access to compound feed and hay allows cows in this system to achieved high dry matter intake (DMI). Out of physiological concerns if the voluntary hay intake could be increased and the consumption pattern of compound feed could be manipulated to maintain proper rumen functioning and health. This study investigated the effects of an artificial hay aroma and compound feed formulation on feed intake pattern, rumen function, and milk production in mid to late lactating dairy cows. Twenty Holstein-Friesian cows were assigned to 4 treatments in a 4 × 4 Latin square design. Diet consisted of compound feed and grass hay, fed separately, and both offered *ad libitum*, although compound feed supply was restricted in maximum meal size and speed of supply by an electronic system. Treatments were the combination of two compound feed (CF) formulations: high in starch (CHS) and fiber (CHF), and two grass hays (GH): untreated (UGH) and the same hay treated with an artificial aroma (TGH). Meal criteria were determined using 3-population Gaussian-Gaussian-Weibull density functions. No GH × CF interaction effects on feed intake pattern characteristics were found. Total DMI and CF intake, but not GH intake, were greater ($P < 0.01$) in TGH treatment, and feed intake was not affected by type of CF. Total visits to feeders per day, visits to the GH feeder, visits to the CF feeder, and CF eating time (all $P < 0.01$) were significantly greater in cows fed with TGH. Meal frequency, meal size, and meal duration were unaffected by treatments. Cows fed CHF had a greater milk fat ($P = 0.02$), milk urea content ($P < 0.01$), and a greater milk fat yield ($P < 0.01$). Cows fed TGH had a greater milk lactose content and lactose yield ($P < 0.05$), and milk urea content ($P < 0.01$). Cows fed TGH had smaller molar proportions of acetic acid and greater molar proportions of propionic

acid compared to UGH. In conclusion, treatment of GH with an artificial aroma increased CF intake and total DMI, but did not affect hay intake. Additionally GH treatment increased the frequency of visits to both feeders, and affected rumen VFA profile. Type of CF did not affect meal patterns, ruminal pH nor fermentation profiles.

Keywords: feeding behavior, volatile compounds, sensorial perceptions, satiety signal, fermentation profiles

Implications

Finding optimum strategies to maximize feed intake in dairy cows without negatively affecting animal health and welfare, requires proper understanding of the interrelationship between feed intake pattern and rumen function. In the present experiment, feeding behavior and rumen function were altered by adding an aroma to hay and by varying the level of starch and fiber in compound feed. Application of an artificial hay aroma affected total DM intake, compound feed intake and several aspects of feeding behavior and rumen fermentation profiles, but effects of compound feed formulation were minor.

Introduction

Feed intake is a primary determinant of milk production in dairy cattle. In the so-called Kempen System (Ter Wijlen *et al.*, 2009), diet is provided in the form of a compound feed and hay offered *ad libitum*. *Ad libitum* access to compound feed and forage presents the advantage of allowing for a greater dry matter intake (DMI), but also presents the risk for a variable forage to concentrate ratio. In such a feeding system, voluntary intake of hay is critically important to ensure adequate fiber intake for proper rumen function and health. Animals use their sensorial perceptions (sight, smell and taste) to develop preferences and avoidance for certain feedstuffs (Baumont, 1996). Several flavors and volatile compounds have been applied to improve feed palatability and preference (reviewed by Cannas *et al*., 2009). To ensure adequate effective fiber intake levels, application of odor or taste boosting compounds might help to improve the voluntary intake of hay.

The intake pattern of compound feed is also critical to ruminal function in this feeding system. Cows consume feed in discrete bouts, which can be described as the frequency of bouts consumed in a day (meal frequency), the feed consumed in each bout (meal size), the speed of feed consumption (eating rate), and the distribution of intake throughout the day (Tolkamp *et al.*, 2000). Smaller but more frequent meals may be beneficial for cows as this would reduce daily fluctuation of ruminal pH (González *et al*., 2012). Previous research has shown that feed intake might be influenced by propionate signals coming from the rumen (Allen, 2000). Propionate plays a central role in the hepatic oxidation theory representing the primary satiety signal. Propionate formation in the rumen can be manipulated by starch content and fermentation characteristics in the diet. Besides, the level of effective fiber required to maintain optimal rumen functioning depends on the amount and the rate of fermentation of carbohydrates in the rumen (Zebeli *et al*., 2008), and in this respect dietary carbohydrate characteristics may impact the amount of hay required.

The first objective of this study was to evaluate the efficacy of an artificial aroma in enhancing voluntary intake of grass hay relative to compound feed, and the effect on feed intake pattern, rumen function, and milk production. We hypothesized that the smell and potentially the taste of the artificial aroma could positively influence voluntary intake of grass hay. Secondly, this study aimed to determine the effect of two compound feed formulations (either high in starch or high in fiber) on feed intake and feed intake pattern, rumen function, and milk production. We hypothesized that the high starch feed would be consumed in smaller meals compared to the high fiber feed, mediated by satiety signals from the expected different ruminal propionate production rates.

A fraction of the data presented in this paper was reported by Leen *et al.* (2014), in which the effects of compound feed formulations on feeding behavior were described.

Materials and methods

Animals and experimental design

This experiment was conducted in the Trouw Nutrition Dairy Research Facility (Boxmeer, the Netherlands). Twenty Holstein-Friesian dairy cows (4 primiparous and 16 multiparous) averaging 203 ± 35.4 DIM (mean ± SE), housed in a slatted-floor free-stall barn together with 80 non-trial cows, were used. Cows were blocked according to parity, DIM, and milk yield. One of the blocks consisted of 4 ruminally fistulated cows. The experiment was set up as a 4 × 4 Latin Square, with 4 treatments in a 2 x 2 factorial design. Treatments consisted of two compound feed (CF) formulations (CHS, high in starch; CHF, high in fiber), combined with two differently treated grass hays (GH) (UGH, untreated grass hay; TGH, treated grass hay). The first period started after 3 weeks of gradual adaptation to the feeding system. Each period consisted of 2 weeks of adaptation to the treatment and a 5-day measurement period. Due to metabolic disorders, two non-fistulated cows were removed from the experiment and data generated by these cows were excluded from the final dataset. In period 1 only 3 day feeding visits were available for feed behavioral analyses due to mechanical failure.

Dietary treatments and feeding

The ingredient composition of the CF is provided in Table 1. The CF were formulated to be iso-nitrogenous and iso-energetic, but differing in starch, neutral detergent fiber (NDF) and acid detergent fiber (ADF) content (Table 2). A GH of expected moderate-low palatability was used to study the effects of an artificial aroma (LUCTA SA, Feed Additives Division, Madrid, Spain). The product used was a feed flavor aiming to mimic the sensory properties of a highly palatable hay. This aroma resulted from a series of studies of the effect of naturally present volatiles from hays on intake preference in dairy cows (Trouw Nutrition and LUCTA SA, unpublished). Twenty one samples of ryegrass (genus *Lolium*), 3 samples of oat (*Avena sativa*) and 3 samples of alfalfa (*Medicago sativa*) were screened and ranked by preference in 3 double-choice preference studies. Principal component and cluster analysis were performed on the preference ranking using basic feed analyses including dry matter (DM), crude protein (CP), crude fat, NDF, ADF, sugar, and ash (Masterlab, Boxmeer, the Netherlands), and analysis of volatile components by solid phase micro-extraction, and subsequently quantified by gas chromatography. Positive correlations found among the presence of 150 volatiles analyzed and the preference ranking were used to formulate the artificial aroma combining feed grade approved flavors that included natural and natural-identical compounds. A solution containing the artificial aroma was diluted at a rate of 80 g of additive per liter of water. This solution was evenly sprayed over the hay and mixed at a dose of 54 g of solution per kg fresh weight of hay. Spraying of the hay was performed once a day in a different location than where the animals were housed.

Cows had free access to water and *ad libitum* access to CF offered in 7 automatic CF feeders (Fullwood Packo, Ellersmere, UK) and to GH in 10 Roughage Intake Control (RIC) bins (5 bins for UGH, 5 bins for TGH) (Hokofarm, Marknesse, the

Netherlands). The seven CF feeders were shared with the 80 non-trial cows whereas RIC-bins were reserved only for the trial cows. Each CF feeder can supply either type of CF, and cows were given the proper CF based on their electronic tag. To prevent contamination, the 10 RIC-bins were placed in two groups of 5 adjacent RIC-bins at both sides of the feeding alley and thus the RIC-bins for treated and control hay were separated by an average walking distance of 12 m. The CF feeders were scattered around the barn with an average distance to RIC bins of 17 m. The CF feeders and RIC-bins automatically recognized the individual cows and the system recorded the start and end time of the visit as well as the total feed intake. The CF feeder dispensed 100 g of CF every 33 s until the animal left the feeder. Complete consumption by the cow of the feed supplied was assumed. Maximum daily intake was limited to 25.5 kg CF/cow, with a maximum intake per visit set to 1.5 kg, with a 5-min waiting time before resetting this allowance. Twelve kg fresh matter of GH were filled into the RIC-bin at 0900 and 1600 h to ensure *ad libitum* supply. For individual hay intakes weight change of the RIC-bin (± 0.1 kg), and time at start and end of each visit were recorded.

Sample collection and data recording

The GH was sampled (500 g) in each measurement period. The CF were produced in a single batch and samples were collected at the start of the trial. Cows were milked twice daily and milk yield was recorded during each milking. Milk samples were taken at the milking parlor on Monday evening, Tuesday morning, Wednesday evening, and Thursday morning to estimate weekly milk composition.

Feeding event registrations from RIC-bins were manually checked and corrected for erroneous registrations in 4 steps by excluding (1) registrations of cow visits at wrong RIC-bins, (2) in case end-weight exceeded start-weight, (3) when intake rates exceeded 600 g/min, and (4) visits without feeding. The initial measurement period data set contained 9 150 records of which 0% (1), 1.2% (2), 0.1% (3) and 6% (4) were deleted. The remaining feeding event records were pooled with the records from the CF feeders and used for further processing and data analysis.

Rumen pH was recorded every 2 min with a pH-logger (LRCpH T7 logger; Dascor, Escondido, CA, USA). Data of one fistulated cow in period 4 were removed in analysis of pH, because the ruminal pH observed was very high for all time points (pH > 7), which we deemed biologically impossible. Other pH sensors showed normal pH patterns. Rumen fluid samples (100 ml) were collected from each fistulated cows on Monday, Wednesday, and Friday at 0800, 1100, and 1400 h in each data collection period. Rumen fluid (8 ml) was pipetted into 10 ml tubes containing 0.2 ml 1 M of sulfuric acid (H_2SO_4). All samples were stored at -18°C until further analysis.

Laboratory analyses

The GH was analyzed for DM, ash, CP, ether extract (EE), NDF, ADF, acid detergent lignin (ADL), and sugars using near-infrared spectroscopy (NIRS) (BLGG AgroXpertus, Wageningen, the Netherlands). The CF was analyzed for DM, ash, CP, EE, starch, sugar, NDF, ADF, and ADL (Masterlab, Boxmeer, the Netherlands). DM was determined after drying the samples at 103°C for 4 h and ash by incineration at 550°C (EC 152/2009; EC, 2009). Total N content was determined according to the Dumas method and used to calculate CP (N × 6.25) (ISO, 2008). EE content was determined by treating the sample with hydrochloric acid followed by extraction with petroleum ether (EC 152/2009; EC, 2009). Starch concentration was determined by spectrophotometry after enzymatic conversion using amyloglucosidase (ISO, 2004). Sugar was determined according to the Luff-Schoorl method and expressed as glucose (EC 152/2009; EC, 2009). NDF, ADF, and ADL contents were analyzed according to Van Soest *et al.* (1991) method using heat stable α-amylase and expressed without residual ash. Reported net energy (NE_L) (Table 2) for GH were obtained from equations from CVB (2008) based on composition determined by NIRS and for CF, the values were calculated based on table values and the composition of raw material (CVB, 2008). Milk samples were analyzed for fat, protein, lactose, urea, and somatic cell count using mid-infrared spectroscopy (Qlip, Deventer, the Netherlands). Ruminal VFA analysis was performed through separation and quantification by gas chromatography (Perkin Elmer Autosystem XL, Groningen, The Netherlands; capillary column TR-FFAP of 30 m × 0.53 mm × 1 μm). Ammonia in rumen fluid sample was measured by indophenol colorimetric absorbance using a spectrophotometer (Ultrospec 500 pro, Amersham-Bioscience, Barcelona, Spain) at 625 nm wavelength.

Calculations and statistical analyses

Feeding behavior was analyzed according to Yeates *et al.* (2001). Time interval (in seconds) between two consecutive visits was calculated and transformed with a natural logarithm. The individual transformed time interval was fitted to a 2-population model (Gaussian-Weibull; GW) or a 3-population model (Gaussian-Gaussian-Weibull; GGW) using the PROC FMM (Finite Mixture Models) (SAS Inc., Cary, NC, USA). In this study, the GGW model was chosen based on examination of the graphical fit of the models and a significant lower -2log-likelihood value observed, which indicates that the GGW model improved the goodness of fit to the data. Using the GGW model, a meal criterion (MC; in minutes) was estimated as the interval length where the second Gaussian and the Weibull curve intersected. Cows eat in discrete meals alternated with periods of ruminating and idling, and the MC is the longest length of the nonfeeding interval that is still considered as interval within a meal (Tolkamp *et al.*, 2000). Using those MC, visits separated by intervals shorter than or equal to the MC were clustered into meals. Intake patterns were calculated on a daily basis and on a per meal basis.

Fat and protein corrected milk yield (FPCM; kg/d) was calculated as: milk yield (kg/d) × [0.337 + 0.116 × fat (%) + 0.06 × protein (%)] (CVB, 2012). The cumulative time (min/d) spent below each pH cut-off point, ranging from 5.0 to 7.4 with increments of 0.1, was calculated and the curves fitted using PROC NLIN (SAS Inc., Cary, NC, USA) according to the model of Colman *et al.* (2012):

$$T = 1\,440 / (1 + \exp[-B_0 \times (pH - B_1)])$$

where T is the cumulative time below pH (min/d), B_0 is the slope at the inflection point which reflects the variability of ruminal pH within a day, and B_1 is the inflection point which reflects the median of rumen pH.

Feed intake pattern, milk yield, milk composition, and pH variables were analyzed as repeated measurements with PROC MIXED of SAS 9.4 (SAS Inc., Cary, NC, USA) according to the following model:

$$Y_{ijk} = \mu + CF_i + H_j + (CF \times H)_{ij} + P_l + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, CF_i is the fixed effect of compound feed, H_j is the fixed effect of grass hay, $(CF \times H)_{ij}$ is the interaction of CF and GH, P_l is the repeated effect of period with cow as the random subject, and ε_{ijk} is the residual error. For analysis of VFA, the following model was used:

$$Y_{ijk} = \mu + CF_i + H_j + (CF \times H)_{ij} + T(P_l) + \varepsilon_{ijk}$$

The model was similar as above with addition of the repeated effect of period nested to time, $T(P_l)$. Based on variogram analysis showing an increase in variance with increasing distance in time, the covariance structure chosen was autoregressive (1) for the analysis of feed intake pattern, milk yield and composition, and VFA. Compound symmetry was used for the analysis of ruminal pH and cumulative time below pH. Differences were analyzed using the least squares means method with a simulate adjustment. Significance was declared at $P < 0.05$. A value of $0.05 < P < 0.10$ was considered a trend.

Results

No GH × CF interaction effects on feed intake pattern characteristics were found (Table 3). The estimated MC ranged from 23.8 to 28.1 min. Total DMI ($P < 0.01$) and CF intake ($P < 0.01$) as well as NE intake were greater ($P = 0.01$) for cows fed TGH than UGH. Cows fed CHF tended ($P < 0.09$) to consume more CF compared with cows fed CHS. Intake of hay and number of meals per day were not affected by type of CF or by treatment of GH. Total visits per day ($P < 0.01$), visits to the RIC-bin ($P < 0.01$), and visits to the CF feeder ($P < 0.01$), were significantly greater with TGH, but were not affected by type of CF. Eating time of CF was higher in CHF ($P = 0.04$) and in TGH ($P < 0.01$). When expressed per meal, the number of total visits ($P < 0.01$), visits to the CF feeders ($P = 0.02$), and visits to the RIC-bins ($P < 0.01$) were higher for TGH. The meal duration tended ($P = 0.08$) to be higher for CHF than CHS, and meal size tended ($P = 0.06$) to be higher for TGH than UGH.

No GH × CF interaction effects on milk and milk content yield were found (Table 4), except for milk protein content. CF did not affect milk yield, protein yield, or lactose yield, but milk fat yield was higher ($P < 0.01$) and FPCM yield tended ($P = 0.07$) to be higher for CHF. Milk lactose yield was higher ($P = 0.02$) for cows fed TGH than UGH, whereas milk yield, fat yield, and protein yield where not affected by treatment of GH. Milk fat concentration ($P = 0.02$) and milk urea content ($P < 0.01$) were higher for cows fed CHF. A GH × CF interaction was present (P = 0.02) for milk protein content, with a higher milk protein content with CHS UGH treatment. TGH resulted in increased milk lactose (P = 0.05) and milk urea (P < 0.01) contents. The efficiency of converting feed N to milk N was higher in UGH (P = 0.05) and in CHS (P < 0.01). Feed conversion efficiency (kg FPCM/kg DMI) tended (P = 0.08) to be greater for UGH compared with TGH.

Mean, minimum, maximum pH, and parameter B_1 (inflection point) did not differ between treatments (Table 5). There was a tendency (P = 0.10) for greater fluctuation in pH (as indicated by parameter B_0) with CHS and with UGH. A significant GH × CF interaction for total VFA concentration (P = 0.05) indicated that the increase in VFA concentration for TGH only occurred on CHF but not on CHS (Table 6). Cows fed TGH had a greater molar proportion of propionic acid (P = 0.05) and valeric acid (P = 0.01) than cows fed UGH. The molar proportion of acetic acid (P = 0.03), iso-butyric acid (P = 0.03), and iso-valeric acid (P = 0.01) was lower in cows fed with TGH. Type of CF did not affect VFA molar proportions, except for the proportion of caproic acid (P < 0.01).

Discussion

Meal criteria and meal pattern variables

Meal criteria (MC) analysis has been applied to lactating cows fed TMR (Miron *et al.*, 2004; Abrahamse *et al.*, 2008), but little is known about the suitability of this approach for *ad libitum* systems where forage and compound feed are offered separately. In line with previous results (Yeates *et al*., 2001; Abrahamse *et al*., 2008), the GGW model was found to best fit the present data set. Weibull distributions are thought to be in better agreement with the concept of satiety, in which the probability of animals to start a new meal is expected to increase with time since the last meal (Yeates *et al.,* 2001). In this experiment, MC was estimated by pooling the data per treatment as there was instability in fitting the data when MC was estimated for individual cows. Estimated MC in TMR systems reported by previous studies are 44.7 min (Tolkamp *et al.*, 2000), 16.4 to 18.5 min (Abrahamse *et al.*, 2008), and in our experimental facilities, estimated MC varied between 24.4 and 35.3 min (Doorenbos *et al.*, 2017). Estimated MC depends on the type of animal, the chemical and physical properties of diets, the management system, competition between animals for the feeders, and the way MC are estimated for a given situation (Tolkamp *et al.*, 2000). In a previous study where two different feeds were fed separately, Greter *et al.* (2012) had the ability to estimate separate MC for TMR (33 min) and wheat straw (132 min) as the two feedstuffs were fed during separate time frames. In our study, it was not possible to estimate separate MC since cows had access to both feeders at the same time and, therefore, separating MC would not recognize sequences of CF and GH consumption belonging to the same meal.

Meal frequency, meal size and meal duration did not differ between treatments. Meal frequency (7.0 to 7.3 meals/d) was rather similar to that reported by Abrahamse *et al.* (2008) (7.2 to 7.7 meals/d), but lower than the value of 10.3 to 14.0 meals/d found by Miron *et al.* (2004), and higher than the value of 5.5 to 5.8 meals/d reported by Doorenbos *et al.* (2017). Meal duration in the current study (45 to 49 min/meal) was rather comparable to that found by Doorenbos *et al.* (2017) (45.9 to 50.8 min/meal), but was higher compared with values found by Abrahamse *et al.* (2008) (28 to 37 min/meal) and by Miron *et al.* (2004) (15.6 to 15.9 min/meal). Different methods used to calculate MC attribute to the discrepancies in meal patterns evaluation among studies. Pooling data of GH and CF consumption in this study may have increased the estimated meal durations and affected meal size. Average meal size varied between

3.2 to 3.5 kg DM/meal, somewhat lower than the values obtained by Doorenbos *et al.* (2017) (4.0 to 4.1 kg DM/meal) upon offering a TMR, but somewhat higher than the values found by Miron *et al.* (2004) (1.9 to 2.4 kg DM/meal).

Effects of hay artificial aroma

The objective of using an artificial aroma or flavor is generally to increase intake of feed in choice feeding situations and to improve preference for one feed ingredient over others. We used feed aromas aimed to mimic the sensory properties of a highly palatable hay, based on a range of naturally present volatiles in ryegrass, oat, and alfalfa. Previously, Dohi *et al*. (1996, 1997) extracted flavoring agents from perennial ryegrass and showed that goats and sheep preferred grass hay sprayed with these extracts rather than control hay. Similarly, De Rosa *et al*. (2002) used extracts from perennial ryegrass or white clover, and goats preferred straw pellets using the perennial ryegrass extract but not the clover extract. In the present study, the aroma significantly increased total DMI and increased visits to both feeders. Nevertheless, increased visits to the roughage-bin did not coincide with greater hay intake. Cows were attracted to the smell of the artificial aroma applied to the GH, but other factors might constrain the cow to increase voluntary intake of hay. Gherardi *et al*. (1991) found that increased palatability of hay when sprayed with a mixture of butyric acid and monosodium glutamate had only minor effects on voluntary feed intake when it was the sole feed offered to sheep. Response of animals to odor and / or taste of certain compounds in the short-term (Distel *et al*., 2007) might not be similar in the long term, in which palatability of feeds stimulated by taste and smell could be overruled by post-ingestive feedback mechanisms (Provenza, 1995). Temporal effects of the artificial hay aroma might occur during the adaptation period but the effects may not be sustained during the data recording period. The presence of the volatile compounds in the ingested hay might also affect the taste receptors in the gut (reviewed by Ginane *et al*., 2011) that helps animals to sense the true nutritive value of the hay. Animals develop aversions to nutritional deficiencies and prefer foods that contribute to their energy and protein needs (Provenza, 1995). The fact that GH and CF were fed separately allowed cows to select CF that has a greater NE_L and CP content than GH. In addition, physical characteristics of hay limit intake, which may be attributed to the effect of rumen fill and distension (Blaxter *et al*., 1961). Reasons why cows fed the treated hay went more to CF feeders and spent more time eating CF, which resulted in a higher CF intake, is unknown and requires further investigation.

Increased total DMI for cows fed TGH without associated rise in milk production could be due to the fact that cows that were used in this experiment were in late lactation. In late lactation, a larger proportion of nutrients absorbed at higher intake is directed towards body weight gain. Lower milk N efficiency with TGH coincided with a greater milk urea content, which is in line with the negative relationship between milk urea content and milk N efficiency generally observed (Spek *et al.*, 2013). High milk urea in TGH treatment indicates an excess of rumen degradable protein in relation to fermentable carbohydrate, or an excess of metabolizable protein in relation to metabolizable energy. The CF was formulated to have 230 g CP/kg DM in an attempt to counterbalance the low CP (58 g/kg DM) content in GH. Proportion of hay in the total diet tended to be lower for TGH than UGH and therefore resulted in a higher CP intake. Treatment of hay with aroma also increased milk lactose yield and milk lactose concentration, but the actual differences are not large. There was a slight tendency for less fluctuation in ruminal pH with TGH compared with UGH despite higher total DMI and CF. Thus, higher supply of fermentable substrate due to higher DMI of cows fed

TGH did not affect pH dynamics. Rumen total VFA concentrations and propionic acid molar proportion was higher in TGH which may have been associated with the numerically greater proportion of concentrate in TGH diet compared with UGH diet.

Effects of compound feed formulation

The main objective of feeding a compound feed high in fiber compared to one high in starch was to understand how the nutrient profile of the CF would influence the feed intake pattern of CF and hay. No GH × CF interaction effects on feed intake pattern characteristics were found. Type of CF did not affect mean, minimum or maximum rumen pH, and presumably cows therefore did not need to consume different amounts of hay to provide different levels of effective fiber to maintain optimal rumen functioning. Although CF formulation did not affect total DMI, CHF tended to result in higher concentrate intake than CHS. Similar to current findings, Miron *et al.* (2004) also observed higher DMI of cows fed a high fiber pelleted supplement than a high starch pelleted supplement; however, Abrahamse *et al*. (2008) did not observe differences in DMI in cattle consuming high fiber compared with high starch concentrates. Lower intake of CHS could possibly be due to satiety signals induced through a potentially higher and faster increase of propionate production in the rumen. However, molar proportions of propionate were not affected by type of CF, although ruminal concentrations are not a direct reflection of VFA production, but the resulting balance between production and clearance. Higher consumption of CHF than CHS might also be related to the fact that the present *ad libitum* feeding system allowed cows to self-select feed that may be favorable for their rumen conditions.

Meal size was not affected by compound feed composition, but CHF (elevated soy hulls content) compared with CHS (elevated maize content) tended to result in a greater meal frequency and meal duration. In contrast, Abrahamse *et al.* (2008) did not find differences in meal frequency and meal duration when feeding diets with concentrates high in structural carbohydrates (mainly soyhulls and lupins) compared with concentrates high in non-structural carbohydrates (mainly maize, barley and wheat), but did find increased total eating time and decreased intake rate per meal with the high structural carbohydrate diet. Miron *et al.* (2004) found that the meal size increased while number of meals per day and meal duration decreased in cows fed a high starch diet (containing barley, maize, and soybean meal) compared to a high fiber diet (containing soy hulls and maize gluten feed). The latter authors suggested that the high rate of degradation of starch, high NE_L content and high palatability of the high starch diet are factors that influenced cows to consume more feed per meal than on a high fiber diet. Differences in degradability of starch sources used might explain the discrepancy in meal pattern between these studies. The rate of degradability of maize grain used in the current experiment is lower than that of barley or wheat grain due to a specific protein matrix associated with starch granules (Herrera-Saldana *et al.*, 1990). Maize grain is assumed to have a higher proportion of starch that can by-pass the rumen without being fermented. Changing the site of starch digestion to the small intestine is expected to result in less propionate production and in increased net glucose absorption, and decreased flux of propionate and increased flux of glucose in the portal vein which might stimulate higher feed intake (Allen, 2000). This indicates that the content of rumen bypass starch in CHS was not high enough to mitigate the satiety effect of ruminal propionate production to achieve comparable total DM intakes with CHF, as a trend for lower CHS intake was observed. Larger effects of type of CF on intake and intake pattern could be expected if rapidly degradable starch sources (e.g., barley grain) were used.

Milk fat content and yield was greater in cows fed CHF than CHS. Changes in milk fat content are associated with changes in the acetate to propionate ratio (Ipharraguerre *et al.*, 2002). The CHF was formulated to have more digestible NDF than CHS, which was expected to provide favorable conditions for rumen micro-organisms to synthesize more acetic acid. However, the VFA concentration and molar proportion of acetate were not affected by CF. A low milk fat yield and content in CHS could be related to a decline in ruminal pH which commonly is observed in cows fed with highly fermentable diets. This assumption could not be confirmed as there were no changes in rumen pH between CF treatments. The supplementation of GH might increase saliva production and buffering capacity that helps to stabilize ruminal pH, despite higher intake of CHF. The greater milk urea content at CHF compared with CHS is in line with the lower milk N efficiency, and is likely related to the greater CP intake caused by a smaller hay proportion of the total feed consumed as discussed previously.

Conclusion

The application of an artificial hay aroma did not improve voluntary hay intake, but has significantly increased total DMI and the frequency of visits to both roughage and compound feed feeders independent of type of concentrate fed, and affected total rumen VFA concentration and several individual VFA molar proportions. Compound feed formulation did not significantly alter meal patterns, except for an increased eating time of compound feed high in fiber (elevated soy hulls content) compared with compound feed high in starch (elevated maize content). Cows tended to consume more of the high-fiber than the high-starch compound feed, but compound feed type did not affect rumen pH dynamics and fermentation profiles of the major VFA.

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Declaration of interest

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Ethics statement

Animal handling and procedures were approved by the Animal Care and Use Committee of Utrecht University (DEC number 2013.111.03.031- Utrecht, the Netherlands).

Software and data repository resources

None.

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Table 1 *Ingredient composition (in % as fed) of the high-starch (CHS) and high-fiber (CHF) compound feeds offered to dairy cows (Holstein Friesian)*

Ingredient	CHS	CHF
Maize	30	15
Wheat	10	10
Soybean meal 48	25	25
Soy hulls	9.2	20
Citrus pulp	15	18
Vinasses	8.0	8.0
Limestone	0.6	0.4
Sodium chloride	0.7	0.7
Magnesium oxide	0.3	0.2
Mono-calcium phosphate	0.6	0.7
Vitamins and minerals	0.7	0.7
Hydrogenated palm fatty acids	-	1.4

Table 2 *Chemical composition (g/kg DM, unless otherwise stated) of grass hay (GH) and compound feed high in starch (CHS) and high-fiber (CHF) offered to dairy cows (Holstein Friesian)*

Nutrients	GH	CHS	CHF
Dry matter (g/kg)	867	878	880
Crude protein	58	224	232
Ash	63	66	69
Ether extract	17	31	39
Starch	-[1]	281	177
Sugar	65	82	92
NDF[2]	657	185	236
ADF[2]	365	107	156
ADL[2]	50	-[1]	-[1]
NE_L[3] (MJ/kg DM)	3.8	7.9	7.9

[1] Not determined.

[2] NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin.

[3] Net energy for lactation calculated with the VEM system (CVB, 2008).

Table 3 *Effects of grass hay (GH) and compound feed (CF) formulation and their interactions on meal criterion and feed intake pattern characteristics of dairy cows (Holstein Friesian)*

Item	Treatments				SEM	*P*-value		
	UGH		TGH			GH	CF	GH × CF
	CHS	CHF	CHS	CHF				
Meal criterion (min)	23.8	25.8	26.8	28.1		-	-	-
Per day								
DMI[1] (kg)	22.2	22.1	22.7	24.0	0.6	<0.01	0.20	0.25
DMI hay (kg)	4.8	4.5	4.7	4.8	0.3	0.66	0.47	0.31
DMI CF (kg)	17.4	17.6	18.0	19.2	0.6	<0.01	0.09	0.31
Net energy intake (MJ)	156	156	160	170	5	0.01	0.12	0.30
Meals (n)	7.3	7.1	7.2	7.0	0.3	0.72	0.37	0.98
Hay intake (% total DMI)	22.0	20.3	20.3	20.1	1.3	0.09	0.07	0.37
Visits (n)	26.2	25.5	28.6	29.1	1.5	<0.01	0.85	0.58
Visits hay (n)	11.5	10.6	13.1	12.6	1.1	<0.01	0.23	0.80
Visits CF (n)	14.7	14.8	15.5	16.5	0.6	<0.01	0.20	0.44
Eating time (min)	231	228	233	243	9	0.09	0.44	0.33

Eating time hay (min)	122	117	119	120	7	0.98	0.42	0.57
Eating time CF (min)	109	111	113	123	4	<0.01	0.04	0.28
Per meal								
DMI (kg)	3.2	3.2	3.3	3.5	0.2	0.12	0.22	0.60
DMI hay (kg)	0.7	0.7	0.7	0.7	0.1	0.98	0.99	0.55
DMI CF (kg)	2.5	2.5	2.6	2.8	0.1	0.06	0.13	0.64
Visits (n)	3.7	3.7	4.0	4.2	0.2	<0.01	0.34	0.60
Visits hay (n)	1.6	1.5	1.8	1.8	0.2	<0.01	0.86	0.50
Visits CF (n)	2.1	2.2	2.2	2.4	0.1	0.02	0.10	0.74
Eating time (min)	33	33	33	35	2	0.29	0.19	0.50
Eating time hay (min)	18	17	17	17	1	0.64	0.82	0.40
Eating time CF (min)	15	16	16	18	1	0.06	0.10	0.59
Meal duration (min)	45	47	46	49	2	0.35	0.08	0.63
Intake rate of hay (g/min eating time)	40	39	39	40	2	0.85	0.99	0.59
Intake rate of CF (g/min eating time)	159	158	159	157	1	0.28	0.15	0.68

UGH = untreated grass hay; TGH = treated grass hay; CHS = compound feed high in starch; CHF = compound feed high in fiber.

[1] DMI = dry matter intake.

559 **Table 4** *Effect of grass hay (GH) and compound feed (CF) formulation and their interactions on milk yield, milk composition, and*
560 *efficiency of dairy cows (Holstein Friesian)*

Item	Treatments				SEM	*P*-value		
	UGH		TGH					
	CHS	CHF	CHS	CHF		GH	CF	GH × CF
Yield (kg/d)								
Milk	29.3	29.5	29.8	29.7	1.3	0.42	0.98	0.73
FPCM[1]	28.7	29.7	28.8	29.5	1.1	0.98	0.07	0.76
Milk fat	1.08	1.17	1.06	1.14	0.05	0.53	<0.01	0.81
Milk protein	1.05	1.02	1.06	1.04	0.04	0.49	0.20	0.73
Milk lactose	1.33	1.35	1.38	1.38	0.06	0.02	0.43	0.61
Milk composition (%)								
Fat	3.79	4.02	3.61	3.90	0.19	0.17	0.02	0.78
Protein	3.62	3.47	3.52	3.53	0.06	0.59	0.04	0.02
Lactose	4.54	4.56	4.57	4.64	0.06	0.05	0.12	0.42
Urea (mg/dL)	32.0	36.3	34.8	37.6	1.0	<0.01	<0.01	0.32
SCC[1] (10^3/mL)	112	117	144	100	88	0.78	0.40	0.29

Efficiency								
FPCM/DMI[1] (kg/kg)	1.29	1.34	1.28	1.23	0.04	0.08	0.89	0.14
Milk N[1]/ N intake (%)	24.2	22.4	23.7	21.1	0.8	0.05	<0.01	0.35

UGH = untreated grass hay; TGH = treated grass hay; CHS = compound feed high in starch; CHF = compound feed high in fiber.

[1] FPCM = fat and protein corrected milk; SCC = somatic cell count; DMI = dry matter intake; N = nitrogen.

Table 5 *Effect of grass hay (GH) and compound feed (CF) formulation and their interactions on rumen pH variable of dairy cows (Holstein Friesian)*

Item	Treatments				SEM	*P*-value		
	UGH		TGH					
	CHS	CHF	CHS	CHF		GH	CF	GH × CF
Daily pH values								
pH minimum	5.47	5.70	5.69	5.62	0.16	0.72	0.70	0.44
pH average	6.20	6.27	6.22	6.11	0.15	0.72	0.92	0.62
pH maximum	7.04	6.96	6.92	6.68	0.17	0.28	0.39	0.65
Cumulative pH logistic regression parameters								
B_0 (slope)	3.71	6.88	6.88	7.15	0.75	0.10	0.10	0.15
B_1 (inflection point)	6.20	6.27	6.22	6.11	0.15	0.67	0.93	0.61

UGH = untreated grass hay; TGH = treated grass hay; CHS = compound feed high in starch; CHF = compound feed high in fiber.

Table 6 *Effect of grass hay (GH) and compound feed (CF) formulation and their interactions on volatile fatty acids (VFA) and ammonia nitrogen (NH_3-N) of dairy cows (Holstein Friesian)*

Item	Treatments				SEM	*P*-value		
	UGH		TGH					
	CHS	CHF	CHS	CHF		GH	CF	GH×CF
Total VFA (mM)	121.0	109.6	121.6	131.3	5.2	0.04	0.87	0.05
VFA molar proportions (mol/100 mol)								
Acetic	65.3	66.2	64.8	64.2	1.4	0.03	0.78	0.18
Propionic	17.7	17.2	18.0	19.0	1.9	0.05	0.63	0.16
Butyric	13.7	13.0	13.8	13.7	0.5	0.22	0.31	0.48
Isobutyric	0.74	0.87	0.70	0.67	0.06	0.03	0.32	0.16
Valeric	1.33	1.30	1.37	1.38	0.06	0.01	0.48	0.30
Isovaleric	0.85	1.04	0.80	0.81	0.06	0.02	0.09	0.10
Caproic	0.42	0.34	0.39	0.35	0.06	0.49	<0.01	0.21
NH_3-N (mg/L)	150	184	173	186	14	0.39	0.11	0.48

UGH = untreated grass hay; TGH = treated grass hay; CHS = compound feed high in starch; CHF = compound feed high in fiber.