**Effect of non-forage roughage replacement on feeding behaviour and milk production in dairy cows**

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**Abstract**

The objective of this study was to determine whether the partial replacement of roughage from forage with non-forage fiber sources, in a total mixed ration (TMR), could reduce feed sorting by dairy cows without modifying behaviour and milk production. Twelve Holstein cows were fed two TMR maize silage based diets in a cross-over experiment. Compared to the control diet (C-diet), experimental diet (E-diet) was formulated by replacing 8% neutral detergent fibre (NDF) from straw and alfalfa hay with soybean hulls and wheat bran. E-diet had a lower physical effectiveness factor (pef) (0.37 vs 0.34; P<0.001) and physical effective NDF (peNDF) (15.5 vs 14.6%; P<0.01). Feeding and sorting behaviour, ruminal activity, milk yield and composition and blood metabolites were measured. The results indicated that dry matter intake was not affected by diet. Cows fed the experimental TMR spent less time eating (192 vs 178 min/d; P<0.05) but showed the same number of meals per day. C-diet fed cows sorted against peNDF in a greater extent (98.3 vs 100.9%; P<0.05). Treatments did not affect cows time budget of general behaviours, with particular regard to ruminating activity. Despite different forage sources in TMR, no significant differences in milk yield and quality were detected.

**Introduction**

The chemical composition and physical characteristics of a dairy cow diet should be evaluated to achieve optimal utilisation. Fibre content and quality are specific requirements for dairy cows; thus, animals consuming diets where the minimum neutral detergent fibre (NDF) requirement (NRC, 2001) is not met develop metabolic disorders that influence milk production and health status, leading to displaced abomasum, ruminal parakeratosis, laminitis, acidosis and fat cow syndrome (Keunen et al., 2002). Along with NDF content, adequate physical characteristics of fibre, such as particle size and density, are necessary for proper ruminal fermentation, utilisation, metabolism and milk fat production (Mertens, 1997). Reduced forage particle size and non-forage fibre sources have been shown to decrease chewing time and saliva production, which buffers the rumen. Thus, reduced forage particle size and non-forage fibre sources lead to a low ruminal pH (<5.8) (Dohme et al., 2007). However, when fibre is provided in total mixed ration (TMR), and the proportion of particles longer than 19 mm is high, cows are more likely to sort the ration and the diet that is actually consumed by them is very different than the one originally formulated (Leonardi and Armentano, 2007). If cows are allowed to sort their ration, ruminal pH may fluctuate and affect feed intake, fermentation and overall digestion (DeVries et al., 2008). To achieve optimal diet utilisation leading to maximal rumen functionality, health status and milk yield, adequate particle length/size of forage should be maintained (Lammers et al., 1996), and feed sorting by cows should be minimised. In spite of different ration compositions and particle size distributions, many authors found that dairy cows were able to sort out the fibrous component of the feed (Kolonoff et al., 2003b; DeVries et al., 2007). Replacing a portion of high-roughage forage in the TMR and a portion of concentrates with short, readily digestible NDF-rich by-products may reduce sorting by animals. Moreover, dry matter (DM) and NDF intake may be more homogenous and improve milk yield through a simultaneous utilisation of energy and proteins by rumen bacteria (Halachmi et al., 2004).

The objective of this study was to characterise feeding, sorting, ruminal function, behaviour, milk yield and quality of dairy cows fed a ration with a roughage component based on both forage and readily digestible NDF-rich by-products.

**Materials and methods**

**Animals, experimental design and dietary intake**

During a trial lasting 8 weeks, twelve Holstein Friesian cows were assigned to two experimental groups that were balanced by milk yield (31.7±5.8 kg/d), days in milk (223±140 d) and parity (2.0±1.2). The groups were exposed to one of two dietary treatments in a 2×2 cross-over design (2 diets x 2 periods) with a period of 28 days (21 days of an adjustment phase followed by 7 days of data collection). Cows were offered one of two isoenergetic, isonitrogenous and isofibrous dietary treatments, formulated as shown in Table 1. The TMR in the experimental diet (E-diet) included a fibrous blend of wheat bran and soybean hulls, and was characterised by a smaller amount of cereal mix, hay and straw and by lower values of physical effectiveness factor (pef) and physical effective NDF (peNDF) (Tables 1 and 2). The cows were kept in loose-housing conditions in two symmetrical 88 squared meter pens, each equipped with six feeding stations and two waterers: animals were on straw bedding (replaced every day), were fed ad libitum daily at 09.00 h, were milked twice at 05.00 h and 16.00 h and were continuously provided water. The mangers were cleaned out daily just before the TMR distribution. Cows wore identification collars and had access to each of the feeding stations of their pen: dry matter intake (DMI), feeding time and the number of visits to the manger were individually and continuously recorded through an automated feeding control system (Biocontrol A/S, Rakkestad, Norway).
visits were clustered into bouts called meals. A meal was defined by estimating a meal criterion on based on visits data (Melin et al., 2007). The meal criterion was considered as the longest non-feeding interval between two consecutive visits which is accepted as part of a meal. To calculate meal criterion, all feeding intervals between visits of each diet (n=3356 and n=3305 for C-diet and E-diet, respectively) were LN-transformed and then grouped in class widths of 0.5 LN units. A double Gaussian (Normal) was fitted (Gaussian mixture of Statistica, 2010) to determine the meal criterion, represented by the point that could be assigned to one of two distributions with equal probabilities. The estimated meal criterion resulted very similar between treatments and it was equal to 20.1 and 22.1 min for C- and E-diet, respectively.

**Sampling and chemical analyses**

Samples of TMR were collected and their chemical composition was determined according to the AOAC methods (AOAC, 2000) and Van Soest et al. (1991). Moreover, TMR and ort samples of the two dietary treatments were collected from all the feeding stations four times each day at 09:00 (t1), 12:00 (t2), 15:00 (t2) and 21:00 (t3) h. Sampling was performed three times in each experimental week, resulting in a total of 288 TMR and ort samples. Before collection the TMR of each feeding station was thoroughly mixed to have an unbiased feed sample. The estimation of particle size distribution was based on prompt fractionation of complete diets by the separator developed by Pennsylvania State University and composed of two screens with nominal diameters of 19.0 mm (S1) and 8.0 mm (S2) and a bottom pan (B) (Lammers et al., 1996). A fresh TMR sample was placed on the upper sieve, and the apparatus was shaken horizontally according to the protocol described by Kononoff et al. (2003a). The amount of material collected in each sieve and in the bottom pan was weighed and expressed as a percent of the total weight. The values of DM and NDF were determined for each sample fraction (S1, S2 and B). To define the ability of feed to stimulate mastication and saliva production, the physical effectiveness factor (pef) and physical effective NDF (peNDF) of each diet were calculated (Beauchemin and Yang, 2005). Sorting activity at each sampling time (t1, t2 and t3) for pef, peNDF and NDF and for each fraction (S1, S2 and B) was calculated as the actual intake (t1, t2 and t3) and expressed as a percentage of predicted intake (Miller-Cushon and DeVries, 2009). The predicted intake of an individual variable was calculated as the product of the total diet DMI and the percent DMI in the TMR for the specific variable at t0 (DeVries et al., 2007). Values <100% indicated selective refusals, >100% suggested preferential consumption and equal to 100% indicated that sorting did not occur.

Sorting activity was determined 3 (t1), 6 (t2) and 12 (t3) h after feeding to better understand time dependent sorting activity, because eating patterns vary throughout the day (Tolkamp et al., 2000). Cows were milked twice a day with an automated milking plant, and milk yield was recorded during the final five days of each period. A milk sample was collected in the morning and afternoon of each experimental period and were combined and refrigerated at 4°C. The samples were analysed for fat, protein and lactose by a Milk-o-Scan 4000 infrared analyser (Fossomatic, Foss electric, Hillerød, Denmark). The milk urea content was determined using differential pH-metry (EUROCHEM CL 10 plus, Microlab EFA). After the morning milking, blood samples from the jugular vein were taken twice during each experimental week and were centrifuged (1500 g, 15 min, 4°C) for plasma separation. The plasma was analysed for the following haematological parameters: total protein, albumin, globulin, total bilirubin, aspartate aminotransferase (AST), γ-glutamyl transferase (γGT), creatine kinase (CK), urea, glucose, triglycerides, non-esterified fatty acids (NEFA), cholesterol, calcium (Ca), phosphorus (P) and magnesium (Mg). The haematological parameters were measured with reagents supplied by Roche Diagnostics and Randox Laboratories Ltd. for the Roche Hitachi 912 Plus automatic analyser (Roche Diagnostics, Indianapolis, IN, USA).

**Behaviour monitoring**

Animal behaviour was observed by scan

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**Table 1. Formulation and composition of C-diet (roughage from forage) and E-diet (roughage from forage, soybean hulls and wheat bran).**

| Ingredients                     | C-diet (%) | E-diet (%) |
|---------------------------------|------------|------------|
| Maize silage, % DM              | 53.9       | 53.7       |
| Permanent meadow 1st crop, % DM | 10.6       | 10.1       |
| Cereal mix, % DM                | 14.4       | 10.4       |
| Soybean based blend, % DM       | 11.3       | 11.2       |
| Soybean hulls, % DM             | -          | 4.3        |
| Wheat bran, % DM                | -          | 4.3        |
| Alfalfa hay 2nd crop, % DM      | 6.4        | 3.8        |
| Straw, % DM                     | 2.5        | 1.3        |
| Crushed linseed, % DM           | 0.9        | 0.9        |
| Diet composition                |            |            |
| Dry matter, %                   | 55.4±0.7   | 55.3±0.7   |
| Crude protein, % DM             | 15.9±0.2   | 15.6±0.6   |
| Ether extract, % DM             | 3.6±0.3    | 3.3±0.1    |
| Crude ash, % DM                 | 6.8±0.1    | 6.8±0.1    |
| NDF, % DM                      | 34.1±1.6   | 35.1±1.8   |
| Non-fibre carbohydrates, % DM   | 39.6±1.5   | 39.2±1.2   |
| NEL, Mcal/kg DM                 | 1.63       | 1.64       |

+NDF was calculated based on NRC (2001) equations.

**Table 2. Particle distribution of C-diet (roughage from forage) and E-diet (roughage from forage, soybean hulls and wheat bran) and values of pef and peNDF.**

| Dietary treatment | SEM | P       |
|-------------------|-----|---------|
|                   | C-diet | E-diet |
| S1, %             | 9.1    | 7.4     | 0.2    | *** |
| S2, %             | 31.2   | 29.6    | 0.2    | **  |
| B, %              | 59.7   | 63.0    | 0.2    | *** |
| pef               | 0.37   | 0.34    | 0.01   | *** |
| peNDF, %          | 15.5   | 14.6    | 0.2    | **  |

+Sieves: S1 19.0 mm; S2 8.0 mm; B, bottom pan; pef, physical effectiveness factor [calculated as ratio between (S1 + S2) and (S1 + S2 + B) on dry matter basis]; peNDF, physical effective neutral detergent fibre (NDF), it was the ratio between the sum of NDF of S1 and S2 on the total dry matter. **P<0.01, ***P<0.001.
Sampling every 5 min for 22 h a day on two days per each diet (Mitlohon et al., 2001; Cooper et al., 2007). During milking and movement to the milking parlour (2 h per day, from 05.00 to 06.00 and from 16.00 to 17.00), cow behaviour was not taken into account because it was biased by interaction with farm personnel and by difficulties in observing animals (Kononoff et al., 2002). During observations feeding, ruminating, resting and posture (standing or lying) were recorded for each animal. Other actions including moving, drinking, and grooming, were recorded as other activities. The data for each activity and posture are presented as the percentage of total daily observations. To evaluate the effect of treatments on ruminal and feeding activities, time spent ruminating and chewing per kg of DM, NDF and peNDF ingested were calculated (Beauchemin and Yang, 2005). Chewing time is the sum of time spent eating and ruminating (Kononoff et al., 2003b).

Statistical analysis

According to a 2×2 cross-over design, a two-way ANOVA (PROC GLM) was performed on particle distribution, pef and peNDF data to test the fixed effects of dietary treatment and period, along with their interaction. A mixed model procedure (PROC MIXED) was performed to evaluate data on feed intake (feeding time, rate, visits and meals), behaviour, productivity, milk quality and metabolic profile. The linear random model included the fixed effects of dietary treatment and period along with their interaction, the random effect of cow and the residual error. To test whether sorting of each diet occurred, sorting activity for each fraction, pef, NDF, and peNDF was tested, for a difference from 100% by using t-tests. Moreover, to verify whether sorting activity differed between dietary treatments and over time, sorting data were analyzed using a MIXED procedure (PROC MIXED) with a CS (compound symmetry) structure with repeated measurements. This model contained the fixed effects of dietary treatment and period, the repeated effect of time (3 levels including 3, 6 and 12 h after TMR distribution), the random effect of feeding station, the interaction between dietary treatment and time and the random residual error. Compound symmetric covariance structure generally resulted in the best fit of the data. The assumption of normality of the residual plot of all the variables included in the dataset was tested by using the Shapiro-Wilk test (PROC UNIVARIATE). Before analyzing the behavioural activities, those frequency variables were transformed (square root-arcsine transformation) to better approximate the normal distribution. However, they were presented as back-transformed LSmeans. When the interaction between dietary treatment and time was significant (P<0.05), LSMeans were separated using the PDIF option along with a Bonferroni adjustment as multiple comparison. All statistical analyses were carried out by SAS (2002).

Results

As reported in Table 1, diets had a similar DM concentration and chemical composition but were different with respect to pef and peNDF due to different forage concentrations (Table 2). E-diet was characterised by significantly lower amounts of particles in S1, S2 and by a higher percentage in B (Table 2).

Intake of DM and NDF were not affected by dietary treatment as reported in Table 3, but the intake of peNDF was lower in the E-diet. Cows fed experimental TMR spent less time eating and made significantly less visits to the manger per day than cows fed the C-diet, even though no difference was found when visits are grouped into meals (Table 3). Regardless of dietary treatment cows showed the same feeding rate and DMI per meal (Table 3). As reported in Table 4, the extent of selection by animals was very low but significantly (P<0.01) different from the reference point (100%) for all the parameters with the exception of pef (P>0.10) and NDF (P>0.10). All cows sorted for long particles, avoided medium length particles and selected for the finest fraction, but no difference between diets was evident (Table 4). No sorting was found for NDF and pef. Cows exhibited different sorting behaviour for peNDF, which was avoided in the C-diet and selected in the E-diet. Time affected the sorting of the smallest fraction of feed that was selected to a greater extent after 12 h. Moreover, cows showed a tendency to sort against pef after 12 h from TMR distribution. Sorting behavior showed significant diet x time interaction; in the E-diet, peNDF was pro-

| Table 3. Feed intake behaviour and ruminating time of C-diet (roughage from forage) and E-diet (roughage from forage, soybean hulls and wheat bran) fed cows. |
|----------------------------------------|--------|--------|
| Dietary treatment | C-diet | E-diet |
| Intake, day−1 cow−1 |          |        |
| DM, kg | 22.7 | 21.7 |
| NDF, kg | 7.7 | 7.6 |
| peNDF, kg | 3.5 | 3.2 |
| Feeding time, min | 192 | 178 |
| Ruminating time°, min | 455 | 436 |
| Feeding rate, g DM/min | 121 | 125 |
| Visit to the manger, n/d | 61.5 | 56.6 |
| Meal duration, min | 7.9 | 7.5 |
| DM/meal, g | 2863 | 2886 |

Time percentage spent ruminating multiplied by 1320 min (22 h of observation); DM, dry matter; NDF, neutral detergent fibre; peNDF, physical effective NDF; ns, not significant. *P<0.05; **P<0.01.

| Table 4. Effect of C-diet (roughage from forage) and E-diet (roughage from forage, soybean hulls and wheat bran) on dry matter (DM) basis. |
|----------------------------------------|--------|--------|
| Dietary treatment | C-diet | E-diet |
| Intake (g DM/meal) |          |        |
| Diet | SEM | P |
| C-diet | E-diet |
| S1 | 101.0 | 101.3 |
| S2 | 99.4 | 99.4 |
| B | 101.2 | 101.3 |
| NDF | 100.1 | 100.3 |
| peNDF | 98.3 | 100.9 |

Sorting (≥ +100 active selection; = 100 no selection; <100 active avoidance); S1: 19.0 mm; S2: 8.0 mm; B: <8.0 mm; pef, physical effectiveness factor; NDF, neutral detergent fibre; peNDF, physical effective NDF (for pef and peNDF see Material and Methods); ns, not significant. *P<0.05; **P<0.01.
gressively selected after 3, 6 and 12 h, and peNDF was progressively avoided in the C-diet. After 12 h, sorting activity values of 95.9% and 101.8% were observed for the C- and E-diet, respectively.

There was no effect of diet on milk yield and milk composition (Table 5). Regardless of treatment, cows had a similar feeding efficiency, measured as the ratio between the mean FCM (kg) production and mean DMI (kg), with values of 1.22 and 1.25 for the C- and E-diet, respectively. E-diet did not modified blood parameters with the exception of urea (5.2 vs 5.7 mmol/L; P<0.05) and AST content (103 vs 95 U/L; P<0.05), which were higher and lower respectively.

Dietary treatments did not affect cow behaviour, especially ruminating and chewing time. They spent on average 33.8% of time ruminating (90.6% lying down), 18.1% feeding, 34.9% resting (including lying down and standing), and 13.1% engaged in different activities such as moving, drinking, grooming, etc. With no statistically differences, cows ruminated and chewed for approximately 24 and 29 min per kg of DM, for 59 and 83 min per kg of NDF, and for 135 and 190 min per kg of peNDF, respectively.

### Discussion

TMR particle distribution is a result of forage, grain and non-forage roughage composition. Thus, the C-diet possessed a higher hay and straw content and had more material retained on the sieves compared to the E-diet, which contained more fine particles retained on the bottom pan, including concentrates, soybean hulls and wheat bran. The results of this study, according to Halachmi et al. (2004), indicate that replacing a portion of forage with soy hulls and wheat bran did not affect DMI; however, a decrease in particles greater than 8 mm led to a reduction in peNDF consumption (Yang and Beauchemin, 2006). On average, cows fed the E-diet spent approximately 15 minutes less each day eating (8%) than animals fed the control diet and had a lower number of visits to the feeding trough on a daily basis; however the number of meals per day, that are reported to be the biologically relevant unit of short-term feeding behaviour (Melin et al., 2007), resulted to be the same. Differences between dietary treatments in feeding time could be related to the forage concentration, pef value and TMR particle distribution of diets. As stated by other authors, a higher forage concentration (DeVries et al., 2007) and a greater proportion of long fibrous particles (Voelker et al., 2002; Galli et al., 2006) could increase the filling effect of the diet and lead to a longer feeding time, as found in C-diet. The results on sorting behaviour clearly indicated that cows sorted to a lesser extent compared to other studies (Leonardi and Armentano, 2003), regardless of dietary treatment. This could be partially related to the procedure of mixing and sampling the feed throughout the day that, as suggested by Maulfair and Heinrichs (2010), could have reduced the degree of sorting. Both diets led to a minimal amount of sorting of all fractions, especially particles retained by the S2 sieve (99.4%). This degree of sorting is in line with the results of DeVries et al. (2007), who reported that cows did not sort for or against medium length particles (<19 mm, >8 mm). Miller-Cushon and DeVries (2009) also observed low sorting activity by cows fed a diet based on corn silage (57.6 of DM), which was similar to ours. Moreover both diets in this study were characterised by a high forage concentration (73.4 vs 68.9% DM), which reduces sorting activity, as reported by DeVries et al. (2007). We assumed that cows would be highly motivated to sort for the smaller particles of the TMR (B) and against longer ones (S1), as reported in other studies (Kononoff et al., 2003b; DeVries et al., 2007), especially when the availability of the finest fraction was limited, as in the C-diet. Regardless of dietary treatment, cows sorted both for the finest particles and the longer fraction. This amount and type of sorting is unusual, but a similar selection of S1 particles (100.7%) was found by Miller-Cushon and DeVries (2009). The shape of feeding troughs could have made sorting against long forage particles more difficult because sorted forage tended to fall down and cover fine particles on the floor of the manger, especially during the first few hours after TMR distribution when the feeding trough was full. Cows fed the E-diet did not sort against peNDF, whereas animals fed the C-diet actively avoided it. This is likely due to the lower peNDF content in the E-diet and this result confirmed that the addition of soybean hulls and wheat bran, together with a reduction of long hay, could assure a physical effective fiber intake similar to the one predicted.

As observed by other authors (Kononoff et al., 2003b), cows sorted for fine particles and against pef throughout the day regardless of diet, and performed the highest level of particle selection 12 h after TMR distribution because it was easier to select small particles from the feeding trough when it was partially empty. As expected, animals fed on C-diet actively avoided peNDF after 12 h (95.5%), whereas cows feeding soybean hulls and wheat bran sorted for peNDF (101.8%), even after 12 h. Results showed that the replacement of approximately 8% of NDF from forage with NDF from soybean hulls and wheat bran did not compromise rumination time that was around 445±10 min a day, according to data reported by Kononoff et al., (2003b); moreover, the time spent lying down by both groups (63%), complied with dairy cow comfort, that is often measured in terms of time spent lying.

According to the results of feeding intake, cows fed the low forage diet provided the same milk yields and fat content as the control group. Concerning the metabolic profile, the differences of AST and urea values were not biologically relevant and were in agreement with values related to healthy lactating dairy cows (Radostis et al., 2000). The limited importance of the slightly higher value of plasma urea in E-diet is confirmed by the lack of variation of milk urea content.

### Table 5. Effect of C-diet (roughage from forage) and E-diet (roughage from forage, soybean hulls and wheat bran) on milk yield, milk composition and traits related to cheese production.

| Dietary treatment | SEM | P |
|-------------------|-----|---|
| Yield, day⁻¹ cow⁻¹ |     |   |
| Milk, kg          | 27.5| 27.5|
| FCM 3.5% kg       | 27.6| 27.2|
| Milk composition  |     |   |
| Protein, %        | 3.38| 3.36|
| Fat, %            | 3.53| 3.43|
| Lactose, %        | 4.87| 4.86|
| Milk urea, mg/100 mL | 27.5| 26.6|

FCM, fat corrected milk (3.5%); ns, not significant.
Conclusions

The replacement of approximately 8% of NDF from forage with NDF from soybean hulls and wheat bran did not change total feed intake and number of meals per day, but led to a lower feeding time. The experimental diet reduced the motivation to avoid long and fibrous particles, resulting in reduced sorting against peNDF, especially 12 h after TMR distribution. Additionally, the experimental diet did not compromise ruminal function and behaviour: parameters such as time spent ruminating and lying down were unaltered by the diets. Moreover, animals fed the experimental diet displayed the same milk yield and fat content as the control group. Therefore, we concluded that replacing part of forage with soybean hulls and wheat bran could reduce feed sorting and guarantee the minimum fiber intake in mid lactating dairy cows.

Further research is required to verify whether the same effects could be obtained also at the beginning of lactation.

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