Adjusting for 30-hour undigested neutral detergent fiber in substitution of wheat straw and beet pulp for alfalfa hay and corn silage in the dairy cow diet: Chewing activities, diurnal feed intake, and ruminal fermentation

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Graphical Abstract

Holstein cows (n = 12)
Energy-corrected milk yield = 42.2 kg/d
Days-in-milk = 97

Experimental diets

| Diet | Description | Forage uNDF30 | Diet uNDF | Time spent eating | Acetate:propionate | Ammonia-N (mg/dL) |
|------|-------------|--------------|-----------|-------------------|-------------------|------------------|
| W50  | 0% forage uNDF from wheat straw – Forage uNDF = 14.0% of diet DM (22.0% corn silage + 18.0% alfalfa hay + 2.0% beet pulp + 0% wheat straw) | 5.0% | 13.0% | 0.01 | 2.0 | 6.0 |
| W550 | 50% forage uNDF from wheat straw – Forage uNDF = 13.9% of diet DM (12.7% corn silage + 7.08% alfalfa hay + 7.60% beet pulp + 11.2% wheat straw) | 25.0% | 31.0% | 0.01 | 2.5 | 8.0 |
| W5100| 100% forage uNDF from wheat straw – Forage uNDF = 13.9% of diet DM (0.0% corn silage + 0.0% alfalfa hay + 12.4% beet pulp + 22.3% wheat straw) | 50.0% | 60.0% | 0.01 | 3.0 | 10.0 |

Summary

Feeding behavior and ruminal fermentation characteristics of lactating cows were investigated when offered rations supplying similar forage undigested neutral detergent fiber (NDF) and dietary NDF digestibility (both measured after 30 hours of in situ incubation). Diets contained wheat straw (WS) and beet pulp (BP), which replaced corn silage and alfalfa hay. Time spent eating decreased linearly and rumination activity remained unaffected as the proportion of WS and BP increased. This resulted in a linear reduction in chewing behavior.

Highlights

• WS and BP were substituted for corn silage and alfalfa hay in diets with similar forage undigested NDF and dietary NDF digestibility.
• As the proportion of WS and BP increased in the diet, time spent eating decreased.
• As the proportion of WS and BP increased in the diet, acetate:propionate and ammonia-N concentration in the rumen increased.

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Adjusting for 30-hour undigested neutral detergent fiber in substitution of wheat straw and beet pulp for alfalfa hay and corn silage in the dairy cow diet: Chewing activities, diurnal feed intake, and ruminal fermentation

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Abstract: This experiment investigated the effects of replacing alfalfa hay (AH) and corn silage (CS) with wheat straw (WS) and beet pulp (BP) in diets with similar forage 30-h undigested neutral detergent fiber (uNDF$_{30}$) on chewing behavior and ruminal fermentation of lactating cows. Twelve multiparous Holstein cows (51 ± 3 kg/d of milk; days in milk = 97 ± 13; mean ± standard error) were housed in individual stalls and used in a replicated 3 × 3 Latin square design with 28-d periods. Experimental diets were (1) 0% forage uNDF$_{30}$ from WS (WS0, contained 2% BP); (2) 50% forage uNDF$_{30}$ from WS (WS50, contained 7.6% BP); and (3) 100% forage uNDF$_{30}$ from WS (WS100, contained 12.4% BP). From 0 to 2 h after the morning feeding, there was a tendency observed for a quadratic effect on dry matter intake (DMI), with cows fed WS50 consuming the greatest amount of DM (9.19 kg). Later DMI (4 to 6 h and 6 to 24 h postfeeding) decreased as dietary proportion of WS and BP increased. Increasing WS and BP decreased eating behavior, but had no detected effect on rumination time (455 min/d), which resulted in a linear reduction in chewing time (the sum of eating and rumination activities). As WS and BP inclusion increased, the number of meals decreased linearly, whereas time between meals, eating rate, and meal size per kilogram of DM increased linearly. Increased dietary inclusion of WS and BP tended to decrease total ruminal VFA and resulted in a linear decrease in proportion but an increase in acetate proportion and ammonia-N concentration in the rumen. Overall, the substitution had no effect on rumination activity, possibly suggesting that a combination of WS and BP could be used in dairy cow rations as substitutes for high-quality forages when WS was added to maintain the uNDF$_{30}$ level.

Providing adequate fiber to dairy cows is critical for maintaining rumen function and chewing behavior. Intake of dietary forage is important as it primarily contributes to the physiologically effective neutral detergent fiber (peNDF) portion of the diet, which is responsible for stimulating rumination and improving fiber digestibility (Zebeli et al., 2012). Corn silage (CS) and alfalfa hay (AH) are 2 main forage sources commonly used in dairy cow diets. However, these forages are sometimes limited, and low-quality forages such as wheat straw (WS) are occasionally used in dairy rations. Wheat straw is usually used in dairy cow rations as a peNDF source to promote rumination (Eastridge et al., 2009). Poor fiber digestibility in low-quality forages such as WS is associated with a longer retention time in the rumen and a slower outflow, which may decrease DMI (Oba and Allen, 1999) and compromise milk production of high-producing dairy cows.

Feeding and rumination behavior, ruminal fill, DMI, and milk production could be influenced by neutral detergent fiber digestibility (NDFD). Forage NDF digestibility has a large impact on rumen turnover and fill, and thus voluntary feed intake and performance, particularly in high-producing cows (Oba and Allen, 1999; Mertens, 2016). We theorized that in dairy cow rations formulated to supply a similar forage 30-h undigested neutral detergent fiber (uNDF$_{30}$) and dietary NDFD$_{30}$, a combination of WS, a low-quality forage source, and a highly digestible source of fiber such as beet pulp (BP, in vitro NDFD of 76–90% after 30 and 48 h incubations; Hoffman and Combs, 2003) could replace high-quality forages without affecting diurnal feed intake patterns, chewing activities, or ruminal fermentation in high-producing cows. Furthermore, the use of BP with WS can partially compensate for the dietary energy deficiency without increasing cereal grain or starch level, which may negatively affect fiber digestibility (Firkins, 1997). However, nonforage fiber sources do not stimulate chewing activity due to their small particle size and high digestibility (Zebeli et al., 2012).

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Kahyani et al. (2019) reported that WS, BP, CS, and AH differ widely in the content of undigested NDF (at 30, 240, and 288 h of incubation), NDFD, as well as the digestion rate of potentially digestible neutral detergent fiber fraction (pdNDF). Regardless of NDF content, CS typically contains a higher proportion of pdNDF with a lower digestion rate than AH (Van Soest, 1994). Moreover, WS has a lower concentration of pdNDF with a slower rate of digestion than BP (Kahyani et al., 2019). Therefore, formulating dairy rations on the basis of NDF concentration alone would result in diets having variable content of pdNDF, uNDF, and digestion rates.

The simultaneous replacement of CS and AH with WS and BP is a nutritional strategy that could maintain feed intake and productivity of high-producing dairy cows (Kahyani et al., 2019). However, their effects on feeding behavior and ruminal fermentation characteristics are not clearly understood. Therefore, the objective of this study was to evaluate the substitution effect of WS and BP for AH and CS with fixed forage uNDF30 and dietary NDFD30 on feeding behavior and ruminal fermentation of high-producing dairy cows.

The Institutional Animal Care Committee for Animals Used in Research approved the experimental protocols. Care for animals was based on guidelines set by the Iranian Council for Animal Care (1995). This research is a continuation of a larger project that was performed using 12 multiparous Holstein cows and data on DMI, lactation performance, and nutrient digestibility were previously reported (Kahyani et al., 2019). In brief, cows were housed in individual 4 × 4 m stalls, and the total amount of feed was offered twice daily (1000 and 1800 h) and adjusted daily to ensure approximately 10% feed refusal per cow. Actual feed refusal averaged 9.75 ± 3.08% (mean ± SD) of the feed offered (on a DM basis) over the course of the experiment, and was not different from diet; and 0.0, 0.0, 12.4, and 22.3 in the WS50 diet; and 12.7, 7.08, 7.60, and 11.2 in the WS50 diet; and 0.0, 0.0, 12.4, and 22.3 in the WS100 diet, respectively. Chop length of CS at harvest was 25 to 30 mm. Alfalfa hay and WS were chopped at the length cut of 15 and 10 mm, respectively. Wheat straw was chopped using a threshing machine that was designed to separate cereal grains from straw (Golchin Trasher Hay Co.). Twenty-four hours before feeding, the chopped, dry WS was reconstituted in airtight containers to achieve a DM content of 25%, according to the procedure explained earlier (Kahyani et al., 2019). Undigested NDF30 concentration (% of DM) in AH, CS, WS, and BP averaged 32.0, 37.3, 62.4, and 7.70, respectively (Kahyani et al., 2019), which was determined according to the in situ procedure described in the companion paper (Kahyani et al., 2019). Diets were formulated to have similar energy density (NE3 = 1.65 Mcal/kg of DM) and CP concentration (16.3 ± 0.25% of DM). Forage-to-concentrate ratio was 40:60, 31:69, and 22:78 for WS0, WS50, and WS100, respectively, but diet DM, forage uNDF30, and dietary NDFD30 contents were similar across diets, averaging 46.1% DM, 13.9% of DM, and 42.3% of NDF, respectively. Dietary starch concentration averaged 29.0, 26.8, and 23.6% of DM for WS0, WS50, and WS100, respectively (Kahyani et al., 2019).

The delivered and remaining amounts of the diets in form of TMR were weighed and sampled daily for each cow from d 22 to 28 of each period 2, 4, 6, and 24 h after feed delivery in the morning, to determine diurnal pattern of DMI. All samples were immediately frozen at −20°C pending analysis.

On d 26 and 27 of each period, behavioral activities were monitored visually for each cow over a 24-h period using 4 trained observers. The observation was based on instantaneous recording, and scanning all 12 cows took around 1 min. Eating and ruminating activities were noted at 5-min intervals, and each activity was assumed to persist for the entire 5-min interval. The 5-min interval was selected based on the validated methodology of Endres et al. (2005), identifying a high correlation (r > 0.95) between the 1-min video observations and scan intervals of 10 min or less, confirming that the 5-min scans are capable of capturing information on feeding behavior in an efficient and adequate manner. Chewing activity was defined as the sum of eating and rumination activities (Maekawa et al., 2002). A period of rumination was defined as at least one observation of rumination occurring after at least 5 min without rumination (Maekawa et al., 2002). Feeding activity was separated into meals using a meal criterion of 30 min, which is close to the validated value of 27.7 min, as reported by DeVries et al. (2003). A meal criterion is the minimum time interval between 2 meals and is used to determine meal frequency and meal duration, and a meal consisted of eating and interval times or intervals between feeding visits within a meal (Black et al., 2016). The time spent eating, ruminating, and chewing per kilogram of DM, NDF, or uNDF30 intake, as well as the number of meals per day, number

### Table 1. Effects of substitution of wheat straw and beet pulp for corn silage and alfalfa hay on feed consumption pattern of mid-lactation Holstein cows (n = 12)

| Item                                | Diet1 | Diet2 | Diet3 | Diet4 | Diet5 |
|-------------------------------------|-------|-------|-------|-------|-------|
|                                     | WS0   | WS50  | WS100 | SEM   | P-value |
| Diurnal DMI, kg                     |       |       |       |       | Linear  |
| 0–2                                 | 7.74  | 9.19  | 8.66  | 0.46  | 0.15    |
| 2–4                                 | 1.78  | 2.19  | 2.43  | 0.35  | 0.09    |
| 4–6                                 | 1.27  | 0.68  | 0.55  | 0.25  | 0.02    |
| 6–24                                | 14.5  | 13.6  | 11.9  | 0.74  | <0.01   |
| Refusals, % of total feed offered (DM basis) | 8.54  | 10.2  | 10.5  | 0.81  | 0.17    |

1) WS0 = 0% forage 30-h undigested neutral detergent fiber (uNDF30) from wheat straw (WS); WS50 = 50% forage uNDF30 from WS; WS100 = 100% forage uNDF30 from WS.
of ruminations per day, and eating rate (total daily DMI/total eating time; g of DM/min) were averaged by cow within the period (Kahyani et al., 2013).

On the last day of each period, rumen fluid samples (approximately 3 mL) were taken 4 h after morning feed delivery from the ventral sac via rumenocentesis (Nordlund and Garrett, 1994). A 2-mL portion was acidified with 200 µL of 25% metaphosphoric acid/mL and kept frozen at −20°C for VFA analysis. Another 1-mL portion was acidified with 200 µL of sulfuric acid (1% wt/vol)/mL and kept frozen at −20°C for ammonia-N analysis. After thawing, ruminal fluid samples for ammonia-N analysis were centrifuged at 30,000 × g for 20 min at 4°C. The supernatant was harvested for ammonia-N assay using the method of Broderick and Kang (1980). For VFA analysis, the acidified samples were thawed and centrifuged at 10,000 × g for 20 min and analysis was undertaken using GC (0.25 × 0.32, 0.3 μm i.d. fused silica capillary, model no. CP-9002 Vulcanusweg 259 a.m., Chrompack).

Data were analyzed using Proc Mixed of SAS (2002, SAS Institute Inc.). The model included fixed effects of square, period, and treatment. Cow within square was included in the Random statement. Normality of distribution and homogeneity of variance for the residuals were tested using Proc Univariate (SAS Institute Inc.). If the Shapiro-Wilk value (W) was 0.98 or greater, polynomial orthogonal contrasts were used to identify the linear and quadratic effects. Significance was declared at P ≤ 0.05, and tendencies were noted if 0.05 < P ≤ 0.10.

Data of DMI during the day are reported in Table 1. From 0 to 2 h, there was a tendency observed for a quadratic effect (P = 0.07) on DMI, with cows fed WS50 consuming the greatest amount of DM (9.19 kg). From 2 to 4 h after feeding, there was a tendency observed for a linear increase (P = 0.09) on DMI as WS and BP increased. Later DMI (4 to 6 h and 6 to 24 h after feeding) decreased linearly as WS and BP increased.

Data of chewing activities and meal patterns are presented in Table 2. There was a linear decrease in time spent for eating (P = 0.01) and total chewing (P = 0.04) as the dietary proportion of WS and BP increased. Rumination time was not different across treatments, averaging 455 ± 21.2 min/d (P = 0.22). Eating and chewing time expressed per kilogram of DM, NDF, and uNDF30 decreased linearly as WS and BP inclusion increased in the diets. The incremental inclusion of WS and BP in the diets resulted in a linear decrease in number of meals from 10.3 to 8.8 meals/d (P = 0.01), and a linear increase in time between meals, eating rate, as well as meal size per kilogram of DM (from 2.49 to 3.02; P < 0.01). The rumination patterns were not different across treatments, but the number of bouts/day was the greatest in WS50-fed cows, averaging 13.0 bouts/d (P₂ quadratic = 0.06).

The incremental addition of WS and BP to the diets resulted in a linear decrease in propionate concentrations but linear increases in acetate and ruminal ammonia-N concentrations (P = 0.01; Table 3).

The present study investigated the effects of diets containing different forage qualities but formulated to supply similar levels of forage uNDF30 and dietary NDFD30 on diurnal feed intake patterns.

### Table 2. Effects of substitution of wheat straw and beet pulp for corn silage and alfalfa hay on chewing activities and meal patterns of mid-lactation Holstein cows (n = 12)

| Item                      | Diet¹ | SEM | Linear P-value | Quadratic P-value |
|---------------------------|-------|-----|----------------|-------------------|
| **Eating**                |       |     |                |                   |
| Min/d                     | 303   | 300 | 272            | 10.0              |
| Min/kg of DM              | 12.3  | 12.0| 11.2           | 0.61              |
| Min/kg of NDF             | 39.3  | 37.8| 35.0           | 1.95              |
| Min/kg uNDF₃₀             | 68.0  | 66.4| 58.3           | 2.75              |
| **Rumination**            |       |     |                |                   |
| Min/d                     | 461   | 473 | 432            | 21.2              |
| Min/kg of DM              | 18.8  | 19.4| 18.0           | 1.14              |
| Min/kg of NDF             | 60.3  | 61.1| 56.2           | 3.66              |
| Min/kg uNDF₃₀             | 104.1 | 107.1| 93.8           | 6.30              |
| **Total chewing**         |       |     |                |                   |
| Min/d                     | 764   | 773 | 704            | 21.9              |
| Min/kg of DM              | 31.1  | 31.5| 29.2           | 1.45              |
| Min/kg of NDF             | 99.6  | 98.9| 91.2           | 4.66              |
| Min/kg uNDF₃₀             | 177   | 166 | 155            | 7.34              |
| **Meal pattern**          |       |     |                |                   |
| **Eating**                |       |     |                |                   |
| Number of meals/d         | 10.3  | 9.5 | 8.8            | 0.53              |
| Length, min/meal          | 27.1  | 30.2| 29.8           | 2.50              |
| Time between meals, min   | 109   | 129 | 134            | 7.51              |
| Rate, g of DM/min         | 84.2  | 85.2| 90.2           | 4.17              |
| Meal size, kg of DM       | 2.5   | 2.7 | 3.0            | 0.15              |
| **Rumination**            |       |     |                |                   |
| Bouts/d                  | 12.2  | 13.0| 11.3           | 0.55              |
| Min/bout               | 38.6  | 36.9| 39.4           | 1.62              |
| Time between bouts, min   | 72.8  | 71.5| 81.1           | 5.54              |
| Rate, g of DM/min        | 56.3  | 55.4| 58.6           | 3.51              |

¹WS0 = 0% forage 30-hour undigested neutral detergent fiber (uNDF₃₀) from wheat straw (WS); WS50 = 50% forage uNDF₃₀ from WS; WS100 = 100% forage uNDF₃₀ from WS.
and increased digestion rate. As dietary uNDF concentration escape of fiber from the rumen mainly because of size reduction increased in the diet. The increased chewing activity facilitates the from 2.48 to 2.07 kg/d (Kahyani et al., 2019) as WS and BP level increases, a greater proportion of the fiber is reliant upon chew- ing action for passage (Smith, 2019). Therefore, the decrease in chewing time as feeding the denser diets (higher concentrate in acetate molar concentration (Table 3). The replacement of slowly fermentable carbohydrates with rapidly fermentable carbohydrates causes the acetate-to-propionate ratio to decrease, a factor contributing to milk fat reduction in dairy cows (Zebeli et al., 2006). The WSO diet had a higher proportion of forage-to-concentrate with greater concentration of starch and NFC (Kahyani et al., 2019). The greater fermentability of the carbohydrate component in WSO compared with WSO50 and WSO100 diets is possibly the main con- tributor to the lower acetate-to-propionate ratio in the rumen, and thus milk fat depression. Beet pulp and WS substitution for CS and AH resulted in increase of ammonia-N concentration in the rumen, which is suggestive of the less efficient protein utilization, and a better synchronization of energy and nitrogen for microbial protein synthesis in WSO-fed cows. Increased availability of fermentable carbohydrates improves the utilization of ammonia in the rumen (Hristov et al., 2005). The greater starch intake in WSO than in WSO50 and WSO100 (Kahyani et al., 2019) is linked to the increased availability of fermentable carbohydrates in the rumen, which possibly improved the microbial utilization of dietary N and decreased ammonia accumulation in the rumen. Eastridge et al. (2009) reported that nitrogen efficiency expressed as milk urea nitrogen or milk protein was not different between cows fed diets containing straw but formulated with fixed forage uNDF30 and dietary NDFD30 levels had no negative effects on total feed intake and rumination activity but the substitution increased ruminal pH and acetate-to-

Table 3. Effects of substitution of wheat straw and beet pulp for corn silage and alfalfa hay on ruminal fermentation of mid-lactation Holstein cows (n = 12)

| Item                  | Diet1          | P-value          |
|-----------------------|----------------|------------------|
|                       | WSO | WSO50 | WSO100 | SEM | Linear | Quadratic |
| Total VFA, mM         | 108 | 112   | 91.9   | 5.58 | 0.06   | 0.08      |
| Individual VFA, mM/100 mM |      |       |        |      |        |
| Acetate               | 68.8 | 67.8  | 71.6   | 1.07 | 0.05   | 0.12      |
| Propionate            | 22.8 | 21.7  | 19.6   | 0.73 | 0.01   | 0.34      |
| Butyrate              | 6.88 | 7.60  | 6.41   | 0.41 | 0.42   | 0.06      |
| Isobutyrate           | 0.38 | 0.42  | 0.36   | 0.04 | 0.72   | 0.27      |
| Valerate              | 0.85 | 0.93  | 0.64   | 0.11 | 0.15   | 0.13      |
| Isovalerate           | 1.33 | 1.53  | 1.42   | 0.10 | 0.58   | 0.26      |
| Acetate:propionate    | 3.14 | 3.21  | 3.68   | 0.14 | 0.01   | 0.24      |
| Ammonia-N, mg/dL      | 10.1 | 10.4  | 12.4   | 0.76 | 0.01   | 0.25      |

1WS0 = 0% forage 30-h undigested neutral detergent fiber (uNDF30) from WS; WSO50 = 50% forage uNDF30 from WS; WSO100 = 100% forage uNDF30 from WS.
propionate ratio. These beneficial effects could have resulted from a good combination of WS and BP, and possibly the role of WS fiber in maintaining rumination activity and increasing ruminal pH. Therefore, BP could be considered as an alternative to high-quality forages when WS was added to maintain uNDF_30. However, feeding WS as the sole forage source increased ruminal ammonia-N concentration while decreasing eating and chewing time. Further research on the supplementation of readily available sources of carbohydrates or degradable proteins at the ruminal level with WS-containing diets is required to optimize nitrogen efficiency.

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