Adjusting for 30-hour undigested neutral detergent fiber in substitution of wheat straw and beet pulp for alfalfa hay and corn silage in the diet of high-producing cows

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ABSTRACT

This study examined the feeding effects of wheat straw (WS) and beet pulp (BP) substituted for corn silage (CS) and alfalfa hay (AH) based on forage 30-h undigested neutral detergent fiber (uNDF₃₀) on lactation performance in high-producing dairy cows. Twelve multiparous (body weight = 611 ± 31 kg, days in milk = 97 ± 13; 51 ± 3 kg/d of milk; mean ± standard error) Holstein cows were used in a replicated 3 × 3 Latin square design with 28-d periods. Three treatments were established by substituting WS for CS and AH such that the concentration of forage uNDF₃₀ in all diets was the same. The treatments were (1) 0% forage uNDF₃₀ from WS (WS0; control), (2) 50% forage uNDF₃₀ from WS (WS50), and (3) 100% forage uNDF₃₀ from WS (WS100). Beet pulp was added in the straw diets to achieve similar dietary neutral detergent fiber digestibility after 30-h incubation (NDFD₃₀). The 3 diets were similar in forage uNDF₃₀ (14% of dry matter), total uNDF₃₀ (~18.5% of dry matter), and NDFD₃₀ (approximately 42% of neutral detergent fiber). The substitution of WS and BP for AH and CS decreased the proportion of forage (40, 31, and 22.3% of dry matter) and forage neutral detergent fiber (21.2, 19.7, and 18.3% of dry matter) for WS0, WS50, and WS100, respectively, in the diet. However, the substitution linearly increased mean rumen pH (5.90, 6.09, and 6.28 for WS0, WS50, and WS100, respectively), digestibility of nutrients, and selection for long particles of diets without affecting dry matter intake. The substitution also linearly increased cholesterol and blood urea nitrogen concentration in the blood. Milk fat percentage, fat production, fat:protein ratio, and milk urea nitrogen increased linearly when treatments changed from WS0 to WS100, whereas the production of energy-corrected milk (ECM) was not affected by the treatments. Milk yield and milk protein yield were affected in a curvi-

linear manner and were lower in WS100 than other treatments. The efficiency of ECM production linearly increased in the diet with higher inclusion of WS and BP substitution in the diet (1.66, 1.70, and 1.72 for WS0, WS50, and WS100, respectively), but body weight, body weight change, and backfat thickness of cows were not different among treatments. In conclusion, the substitution of WS and BP for CS and AH with fixed uNDF₃₀ improved feed efficiency and rumen pH, decreased milk and protein yield, and did not affect ECM yield.

Key words: undigestible neutral detergent fiber, forage source, wheat straw, beet pulp, dairy cow

INTRODUCTION

Meeting the high-producing dairy cow's requirement for forage fiber is critical to maintain rumen function, milk fat percentage, and overall animal health. Incorporation of highly digestible NDF in corn silage (CS) and alfalfa hay (AH) in the diet has the potential to improve DMI and milk production (Ferraretto et al., 2015). However, availability of high quality forages is sometimes limited because of droughts and insufficient land base on individual farms. Small-grain straw, in particular wheat straw (WS), is an available fiber source that is produced as a byproduct of wheat grain production (~750 million metric tons according to FAOSTAT, 2016). A considerable amount of research has evaluated the effect of replacing AH or CS with WS on a DM (Wang et al., 2014; Ghasemi et al., 2016), NDF (Poore et al., 1991), or forage NDF (Eastridge et al., 2009) basis. Previous research has indicated that high-WS diets generally limit digestibility, DMI, and consequently milk production in high-producing dairy cows (Poore et al., 1991; Eastridge et al., 2009; Wang et al., 2014).

The by-product WS with a high effective fiber content is a poor source of many nutrients that can be easily supplied from concentrate mixtures (NRC, 2001). A primary limitation of WS is that NDF digestibility (NDFD) can be low (McCartney et al., 2006; Raf-

Received September 23, 2018.

Accepted April 15, 2019.

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frenato et al., 2018), which can cause a longer retention time in the rumen and lower DMI for dairy cows (Oba and Allen, 1999). The amount of potentially digestible NDF (**pdNDF**) can be estimated by undigested NDF [measured by after 240-h of in vitro (**uNDF₂₄₀**) or 288-h in situ (**uNDF₂₈₈**) incubation], and its rate of digestion (Bender et al., 2016; Mertens, 2016). Rafrenato et al. (2019) showed that values of slow and fast digestible NDF and **uNDF₂₄₀** varied among WS, AH, and CS. A simple and fast method for evaluation of NDFD has been established as 30 or 48 h in vitro or in situ incubation, and has been shown to have a high coefficient of correlation with true digestibility ($R^2 > 0.90$; Spanghero et al., 2010). The undigested NDF pool after 30-h incubation (**uNDF₃₀**) as well as NDF digestibly after 30-h incubation (**NDFD₃₀**) has been related to fiber digestibility (Oba and Allen, 1999), **pdNDF** digestion rate (Lopes et al., 2015), and intake potential (Nair et al., 2016).

We hypothesized that if the contents of forage **uNDF₃₀** and diet **NDFD₃₀** are properly balanced, WS may be successfully used to replace high-quality forages without affecting DMI in lactating dairy cows. Appropriate balance can be achieved by using WS in combination with a highly digestible fiber sources such as beet pulp (**BP**). Beet pulp is an excellent source of digestible fiber with a rapid digestion rate (NRC, 2001). Furthermore, the use of this by-product with WS can partially compensate the dietary energy deficiency without increasing cereal grain or starch level, which may negatively affect fiber digestibility (Firkins, 1997). Therefore, the objective of the current study was to evaluate the substitution effect of WS and BP for AH and CS with fixed forage **uNDF₃₀** and dietary **NDFD₃₀** on feed intake, ruminal pH, nutrient digestion, blood metabolites, and milk production of lactating dairy cows.

MATERIALS AND METHODS

The experiment was conducted at the dairy facilities of the Lavark Research Station (Isfahan University of Technology, Isfahan, Iran). Animals were cared for according to the guidelines of the Iranian Council of Animal Care (1995) and the experiment was approved by the Institutional Animal Care Committee for Animals Used in Research.

Forages Preparation and Measuring In Situ NDF Digestibility

Alfalfa hay was chopped (Golchin Trasher Hay Co., Isfahan, Iran) with a theoretical length of cut of 15

mm. Corn silage was harvested using a pull-type chopper (Model 965, Claas, Omaha, NE) with a 25 to 30 mm theoretical length-of-cut. To obtain finely chopped WS (Table 1), a threshing machine (designed to separate cereal grains from straw) with a 10-mm theoretical length-of-cut (Golchin Trasher Hay Co., Isfahan, Iran) was used. The WS was reconstituted 24 h before in situ measurement and before feeding by placing the required amount of dry hay into an industrial container without mixing. Water at ambient temperature was added slowly to the WS during mixing to achieve a DM content of 25%, then WS and water was transferred to airtight containers.

An in situ study was conducted to measure undigested NDF and NDFD in forages, BP, and TMR, according to the method described by Bender et al. (2016) and Donnelly et al. (2018; Table 1). Two ruminally cannulated nonlactating Holstein dairy cows were used and fed a high forage TMR diet (25% AH, 25% CS, 25% WS, 4.2% BP, 20.8% concentrate mix; DM basis) as recommended by Krizsan and Huhtanen (2013). Dried samples were ground to pass a 1-mm Wiley mill (Arthur H. Thomas, Philadelphia, PA) screen, and 0.5 g of each sample was weighed into an Ankom F57 bag (Ankom Technology, Macedon, NY) with a pore size of 25 μ m (Bender et al., 2016). This particular bag was selected so that digestibility estimates would be comparable with previously reported methods (Bender et al., 2016; Su et al., 2017; Donnelly et al., 2018) and the result replicable for practice condition. Samples were incubated in the rumen for 30, 240, and 288 h in triplicate along with 3 blank bags for each time point. Blanks were sealed Ankom bags containing 0 g of sample to correct for any infiltration of NDF into the sample bags. After removal, samples were soaked in cold water and then were washed twice in a commercial washing machine with cold water for 12-min cycles to ensure that no residue adhered to the bag. This procedure was then duplicated in a second run. The equation (Bender et al., 2016) for correction by blank for calculation of NDF residue at each time point was

$$\text{NDF (g/g of DM)} = \frac{[(\text{bag weight} + \text{residue}) - (\text{bag weight} \times \text{bag correction factor})]}{[(\text{bag weight} + \text{sample}) - (\text{bag weight})]}. \quad [1]$$

The bag correction factor represents the average fractional weight change of 3 Ankom blank bags following the NDF wash procedure. The **uNDF₃₀**, **uNDF₂₄₀**, and **uNDF₂₈₈** were the NDF residues after 30, 240, and 288 h incubations, respectively, and **NDF0h** was NDF at the start of incubation. The value for **NDFD** at each time point was calculated as

Table 1. Nutrient composition and particle size of forages and beet pulp [mean (SD)]

Item (% of DM, unless otherwise stated)	Alfalfa hay	Corn silage	Wheat straw	Beet pulp
DM (% of as fed)	95.0 (0.82)	26.2 (1.05)	93.0 (0.48)	90.2 (0.52)
OM	88.5 (0.09)	93.6 (0.08)	90.9 (0.93)	92.5 (0.18)
CP	13.3 (0.15)	7.7 (0.12)	2.6 (0.21)	10.3 (0.17)
Ether extract	1.38 (0.07)	2.47 (0.27)	1.37 (0.10)	0.63 (0.07)
NDF	52.0 (0.02)	53.8 (0.04)	82.0 (0.05)	35.1 (0.06)
NFC ¹	21.8 (0.43)	29.6 (0.74)	4.9 (0.25)	46.5 (0.35)
Starch	1.12 (0.16)	23.57 (1.92)	0.405 (0.06)	1.37 (0.40)
Sugar	3.62 (0.62)	1.07 (0.19)	1.03 (0.12)	18.56 (1.40)
Lignin	9.50 (0.73)	3.90 (0.30)	8.40 (0.20)	2.06 (0.50)
uNDF ₃₀ ²	32.0 (0.99)	37.3 (3.70)	62.4 (3.65)	7.7 (3.56)
NDFD ₃₀ ³ (% of NDF)	38.6 (1.92)	30.9 (4.33)	25.9 (2.45)	78.2 (3.55)
uNDF ₂₄₀ ⁴	27.4 (0.62)	12.90 (0.67)	27.4 (0.89)	2.82 (0.41)
uNDF ₂₈₈ ⁵	27.2 (0.57)	12.8 (0.68)	26.6 (0.87)	2.81 (0.39)
pdNDF ₂₈₈ ⁶ (% of NDF)	47.8 (1.10)	76.2 (1.27)	67.6 (1.06)	92.0 (1.12)
pdNDF kd ⁷ (%/h)	4.59 (0.06)	2.08 (0.06)	1.69 (0.07)	7.24 (0.04)
Particle size distribution				
>19 mm	2.00 (0.00)	18.3 (3.30)	1.00 (0.00)	—
8 to 19 mm	33.0 (1.41)	56.8 (2.06)	56.0 (2.24)	—
1.18 to 8 mm	43.3 (0.50)	24.0 (1.41)	37.2 (1.64)	—
<1.18 mm	21.8 (1.26)	0.97 (0.06)	6.20 (1.10)	—
GMPL ⁸ (mm)	4.12 (0.16)	10.6 (0.67)	6.70 (0.28)	—

¹NFC calculated by the difference of 100 – (% NDF + % CP + % fat + % ash).

²uNDF₃₀ = NDF residue after 30-h in situ incubation.

³NDFD₃₀ = in situ NDF digestibility after 30-h in situ incubation.

⁴uNDF₂₄₀ = NDF residue after 240-h in situ incubation.

⁵uNDF₂₈₈ = NDF residue after 288-h in situ incubation.

⁶pdNDF₂₈₈ = potentially digestible NDF after 288-h in situ incubation.

⁷pdNDF kd = potentially digestible NDF fraction digestion rate.

⁸GMPL = geometric mean of particle size.

$$\text{NDFD (g/g of NDF)} = \frac{(\text{NDF0h} - \text{NDF residue})}{(\text{NDF0h})} \quad [2]$$

The potentially digestible NDF fraction (**pdNDF₂₈₈**) was calculated as $\text{NDF} - \text{uNDF}_{288}$ and its rate of degradation (**kd**) was determined using NDF residue measurements at 9 time points (6, 12, 24, 30, 36, 48, 72, 96, and 120 h; Figure 1). Natural logs of the pdNDF residue percentages were calculated. A log-residue value less than –3 was discarded as an invalid result (Bender et al., 2016). A linear regression based on the natural log residual values versus time was then fit to the data, and kd was determined as the slope of the regression (Donnelly et al., 2018).

Animals, Experimental Design, and Treatments

Twelve multiparous Holstein cows (BW = 611 ± 31 kg, DIM = 97 ± 13 d, milk production = 51 ± 3 kg/d, milk fat = 2.93 ± 0.22%, milk protein = 2.88 ± 0.05%, mean ± SD) were used in a replicated 3 × 3 Latin square design with 28-d periods. Each period consisted of 21 d of adaptation followed by 7 d of data collection. Cows were housed in individual stalls (4 × 4 m) within a roofed facility with open sides. Clean wood shavings

and sand were used for bedding and refreshed daily. Three dietary treatments were formulated to replace CS and AH with WS on a forage uNDF₃₀ basis as (1) 0% forage uNDF₃₀ from WS (**WS0**; control); (2) 50% forage uNDF₃₀ from WS (**WS50**); and (3) 100% forage uNDF₃₀ from WS (**WS100**). Then, the appropriate amount of BP was added to make similar NDFD after 30-h incubation (**NDFD₃₀**) in the diets. Diets were formulated using the Cornell Net Carbohydrate and Protein System (**CNCPS**, version 5.0; Fox et al., 2004) to meet nutrient allowance for a lactating dairy cow weighing 650 kg and producing 45 kg/d of milk with 3.0% milk true protein and 3.2% milk fat (Table 2). Feed was supplied twice daily at 1000 and 1800 h in amounts that allowed approximately 10% refusals, which were collected before each morning feeding. Each day, forages, beet pulp, and a concentrate premix were manually mixed and weighed into each cow's feed trough, and refusals were manually removed daily and weighed.

Feed Intake and Chemical Analysis

The TMR amounts delivered and refused were weighed and sampled daily for each cow during d 21

to 28 of each period and daily DMI for each cow was calculated. Representative samples of forages (pooled within period), BP (pooled within period), treatment TMR (pooled by diet within period), and individual refusals (pooled by cow within period) were taken immediately before the morning feeding during the 7-d collection period. All samples were immediately frozen at -20°C until they were analyzed.

After thawing, the DM concentration of forages, TMR, and refusals samples was determined by drying at 60°C in a forced-air oven for 48 h (Townshend, 1995). All samples were ground using a Wiley mill through a 1-mm screen (Arthur H. Thomas, Philadelphia, PA) and analyzed for CP using the Kjeldahl method (Kjeltec 1030 Auto Analyzer, Tecator, Höganäs, Sweden; AOAC International, 2006, method 955.04), ether extract (EE; AOAC International, 2006, method 920.39), ash (AOAC International, 2006; method 942.05), and NDF using heat stable α -amylase and sodium sulfite (Van Soest et al., 1991). The samples of forages, BP, and TMR were also analyzed for the amount of starch (Zhu et al., 2016) and sugar (Dubois et al., 1956). Acid detergent lignin was determined using method (AOAC International, 2006; method 973.18), modified to use 1.0 g per

sample in each Ankom F57 bag (Ankom Technology, Macedon, NY). Percentage of NFC was calculated as

$$100 - (\text{CP} + \text{NDF} + \text{EE} + \text{ash}). \quad [3]$$

Particle Size Measurement and Sorting Activity

For 6 d during d 22 to 27 of the study, samples of forages, TMR, and orts were collected daily for determination of particle size. Particle size was measured in triplicate using the Penn State Particle Separator equipped with 3 sieves and a bottom pan (Kononoff, 2002). After sieving, samples were placed in a forced air oven at 60°C to determine DM of each sieved fraction. Sorting of particles was determined from the actual intake of each fraction compared with the predicted intake of the same fraction had the diet been consumed as formulated (Leonardi and Armentano, 2003). A sorting index score was calculated for each fraction as the percentage of actual intake compared with the predicted intake. Values equal to 100% indicated no sorting, whereas values $<100\%$ indicated selective refusals (sorting against), and $>100\%$ indicated preferential consumption (sorting for).

Figure 1. The in situ NDF digestibility (NDFD) of alfalfa hay, corn silage, wheat straw, and beet pulp incubated in the rumen for 6, 12, 24, 30, 36, 48, 72, 96, 120, 240, and 288 h.

Rumen pH and In Vivo Digestibility Measurements

On d 28 of each period, 4 h after the morning feeding, rumen fluid (approximately 3 mL) from the ventral sac was sampled via rumenocentesis (Nordlund and Garrett, 1994). The pH of the ruminal fluid was immediately determined using a portable digital pH meter (HI 8318, Hanna Instruments, Cluj-Napoca, Romania).

Six fecal grab samples were collected from each cow at 0030, 0830, and 1630 of d 25 and 26 to represent the 24-h feeding cycle. The 6 samples were composited for each cow in each period and analyzed for nutrient digestibility. Apparent total-tract digestibility of nutrients was determined using uNDF_{288} as an internal marker.

Blood Sampling and Analyses

On d 27 of each period, 4 h after the morning feeding, blood samples (7 mL) were collected from the coccygeal vein using an evacuated tube without anticoagulant. Blood samples were placed on ice immediately after collection and centrifuged at $3,000 \times g$ for 15 min at 4°C. Serum was separated and stored in plastic tubes and frozen at -10°C until analysis. The concentrations

of serum glucose, cholesterol, BUN, high-density lipoprotein cholesterol, triglyceride, total protein, albumin, creatinine, aspartate aminotransferase, and alkaline phosphatase were measured by an autoanalyzer (Abbott Alcyon 300, Abbott Laboratories, Chicago, IL) using commercial kits (Pars Azmoon Co., Tehran, Iran) according to the manufacturer's instructions. The analyzer was calibrated and controls assayed daily according to the manufacturer's instructions to ensure acceptable assay performance. Serum BHB, fatty acids, and total antioxidant capacity were determined by commercial colorimetric kits (Randox Laboratories Ltd., Ardmore, UK) using the same autoanalyzer. The concentration of serum malondialdehyde (MDA) was determined by the thiobarbituric acid reacting substances method, in which the absorbance of a colored complex that is formed from the reaction of MDA with 2-thiobarbituric acid in acid is measured at 532 nm (Wullepit et al., 2012). Globulin concentrations were calculated by subtracting albumin concentrations from total protein.

Milk Yield and Components, BW, and BFT

Cows were milked 3 times daily at 0100, 0900, and 1700 h in a herringbone milking parlor. Milk yield for all cows was recorded and sampled at each milking during d 21 to 28 of each period. Milk samples were preserved with potassium dichromate, stored at 4°C, and submitted to the Isfahan University of Technology Central Milk Testing Laboratory (Isfahan, Iran) for fat, protein, and lactose analyses using an infrared analyzer (MilkoScan 134 BN, Foss Electric, Hillerød, Denmark; AOAC International, 2002; method 972.16). The MUN content was also determined by enzymatic assay (Wilson et al., 1998). Milk was centrifuged at $1,200 \times g$ for 15 min at 4°C, and the fat was removed. The defatted milk was then thoroughly mixed and deproteinized by mixing 0.2 mL of defatted milk with 1.8 mL of cold trichloroacetic acid and allowing the mixture to stand for 5 min. The sample was then centrifuged at $1,200 \times g$ for 5 min at 20°C, and 0.2 mL of the clear supernatant was analyzed for MUN using a colorimetric diacetyl monoxine procedure. Milk yield was corrected for milk components as ECM (Tyrrell and Reid, 1965):

$$\text{ECM} = 0.3246 \text{ milk yield} + 12.96 \text{ fat yield} \\ + 7.04 \text{ protein yield.} \quad [4]$$

Cows were weighed at the beginning (d 1) and the end (d 28) of each period. Backfat thickness (BFT) was measured on d 28 according to Schroder and Staufenbiel (2006) using a portable B-mode ultrasound generator

Table 2. Ingredient composition of dietary treatments as % of DM [values in parentheses are % of forage 30-h undigested neutral detergent fiber (uNDF_{30})]

Item	Diet ¹		
	WS0	WS50	WS100
Wheat straw	0.0	11.16 (50)	22.32 (100)
Alfalfa hay	18.0 (41)	7.08 (16)	0.0
Corn silage	22.0 (59)	12.68 (34)	0.0
Beet pulp	2.00	7.60	12.40
Barley grain	16.0	16.0	16.0
Corn dry finely ground	20.0	20.0	20.0
Meat meal	3.80	3.80	3.80
Soybean meal	11.2	14.0	16.84
Soybean extruded	2.00	2.00	2.00
Canola meal	0.40	0.40	0.40
Sodium bicarbonate	1.00	1.00	1.00
Calcium carbonate	0.52	0.56	0.52
Dicalcium phosphate	0.08	0.16	0.40
Magnesium oxide	0.16	0.20	0.24
Minvit ²	0.80	0.80	0.80
Salt	0.20	0.20	0.20
Energy Booster	1.84	2.36	3.08

¹Experimental diets were different uNDF_{30} adjusted levels of wheat straw (WS): (1) WS0 = 0% forage uNDF_{30} of WS, (2) WS50 = 50% forage uNDF_{30} from WS, and (3) WS100 = 100% forage uNDF_{30} from WS.

²Contained 2.5 g/kg of Fe, 1.6 g/kg of Cu, 3 g/kg of Mn, 0.1 g/kg of Co, 20 g/kg of Mg, 5 g/kg of Zn, 0.04 g/kg of Se, 0.10 g/kg of I, 3 g/kg of monensin, 50 g/kg of Mycosorb, 10,000,000 IU/kg of vitamin A, 250,000 IU/kg of vitamin D, and 5,000 IU/kg of vitamin E. Minvit: vetaque, Sirjan, Iran; mycosorb: Alltech Inc., Nicholasville, KY; energy booster: Energizer RP-10, IFFCO, Johor, Malaysia.

(SonoVet 600V, BCF Technology Ltd., West Lothian, Scotland, UK) with a linear transducer and frequency between 5.0 and 6.5 MHz.

Statistical Analyses

Data were analyzed using the mixed model procedure of SAS (Proc Mixed; SAS Institute, 2002) to account for effects of square, cow within square, and treatment. The treatment, square, and period were considered as fixed effects; cow within square was considered a random effect. The mathematical expression of the model can be as below:

$$Y_{ijkl} = \mu + P_i + S_j + C(S)_{kj} + T_l + e_{ijkl}, \quad [5]$$

where Y_{ijkl} = the dependent variable, μ = the population mean, P_i = the fixed effect of period i , S_j = the fixed effect of square j , $C(S)_{kj}$ = the random effect of cow k within square j , T_l = the fixed effect of treatment l , and e_{ijkl} = the random residual error, assumed to be normally distributed. Estimation method was REML and the degrees of freedom method was Kenward-Rogers. Polynomial orthogonal contrasts were used to test the linear and quadratic models. Additionally, the differences among the treatments were evaluated using a multiple comparison test following the Tukey-Kramer method. Statistical significance of any main effect was declared at $P \leq 0.05$, and tendencies were discussed at $0.05 \leq P \leq 0.10$.

RESULTS

Forages and Diets

Nutrient composition, in situ NDFD, and particle size distribution of forages and diets are shown in Tables 1 and 3. Wheat straw had the greatest amount of $uNDF_{30}$ (62.4% of DM), followed by CS (37.3%), AH (32.0%), and BP (7.7%). Inversely, $NDFD_{30}$ (% of NDF) measured 25.9, 30.9, 38.6, and 78.2 for WS, CS, AH, and BP, respectively. The amount of $uNDF_{288}$ (DM basis) was lower in CS (12.8%) than AH (27.2%) with no difference between AH and WS. Moreover, notably, the finely chopped WS produced a geometric mean particle size (**GMPL**) of 6.70 mm that was higher than AH (4.12 mm) and lower than CS (10.6 mm).

As intended, the 3 dietary treatments were designed to have a same forage $uNDF_{30}$ of $13.9 \pm 0.05\%$, but were different in forage NDF with amounts of 21.2, 19.7, and 18.3% for WS0, WS50 and WS100, respectively (Table 3). Additionally, measured CP and predicted NE_L contents were similar among the 3 dietary treatments. The treatments resulted in a similar dietary $uNDF_{30}$

(~18.5% of DM) and $NDFD_{30}$ (~42% of NDF), whereas the content of starch (from 29.0 to 23.5%), ADL (2.4 to 1.5%), and $uNDF_{288}$ (10.1 to 8.5%) were decreased as level of WS inclusion elevated. The percentage of particles >19 mm (4.02, 2.20, and 0.50) and GMPL (3.84, 3.72, and 3.54 mm) of the TMR was also decreased as WS was increased in the diet.

Intake, Sorting Activity, Rumen pH, and Apparent Digestibility

The substitution of WS and BP for CS and AH in the diet did not affect the intake of DM, NDF, and $uNDF_{30}$, but decreased the intake of $uNDF_{240}$ and $uNDF_{288}$, and increased intake of $pdNDF_{288}$ (Table 4). However, sorting in favor of long particles (particles ≤ 19 mm and 8–19 mm) increased as the amount of WS and BP increased in the diet. Also, ruminal pH was 5.90, 6.09, and 6.28 for WS0, WS50, and WS100, respectively ($P < 0.01$; Table 4). Furthermore, increased inclusions of WS and BP curvilinearly increased the digestibility of DM (62.8, 63.4, and 68.0%) and OM, and linearly increased the digestibility of EE, NDF (32.9, 37.1, and 45.1), $pdNDF$ and CP ($P < 0.01$), but did not affect the digestibility of NFC (Table 4). The digestibility of $pdNDF$ was 11 units (0.95 kg/d) greater in WS100 (59%) than in WS0 (48%).

Blood Metabolites

Treatments did not affect glucose, high-density lipoprotein, triglyceride, or fatty acid concentration in the blood, but increased inclusions of WS and BP linearly increased cholesterol (250, 259, and 283 ± 11.7 mg/dL; $P = 0.02$), BHB (0.55, 0.64, 0.72 ± 0.06 mg/dL; $P = 0.03$), and BUN (16.7, 18.4, and 22.8 ± 0.76 mg/dL; $P < 0.01$) concentrations in blood for WS0, WS50, and WS100, respectively. The concentration of BUN was 36.5% greater in WS100 than WS0. The concentration of albumin was linearly increased (3.35, 3.33, and 3.57 ± 0.07 mg/dL for WS0, WS50, and WS100, respectively; $P = 0.05$) with increased inclusions of WS and BP, whereas the concentration of globulin remained unaffected. The activity of aspartate aminotransferase and alkaline phosphatase enzymes in the plasma, as well as the concentration of total antioxidant capacity and MDA, were not affected by treatments.

Milk Yield and Component, Feed Efficiency, and BW Change

Milk yield was affected in a curvilinear manner, with the WS100 diet lower than other treatments (Table 5), but the yield of ECM was not affected by the treat-

ments. The fat yield, fat percentage, MUN, and fat:protein ratio increased linearly with increasing WS and BP, but protein and lactose contents were decreased. Similar to milk yield, protein and lactose yield were affected curvilinearly, and were lower in WS100 than other treatments.

The efficiency of milk yield was not affected by the treatments, but the efficiency of ECM linearly increased as the level of WS and BP increased in the diet. A 0.06 unit improvement occurred in feed efficiency [ECM (DMI)⁻¹] when WS0 (1.66) was compared with WS100 (1.72). Body weight, BFT, and BW change were unaffected with the change in uNDF₃₀ adjusted level of WS.

DISCUSSION

The present study attempts to investigate the outcome of using uNDF₃₀ and NDFD₃₀ for balancing ratios of high- or low-quality forages and by products in dairy cow diets. Wheat straw contained both the

highest concentration of uNDF₃₀ and lowest NDFD₃₀. On the other hand, BP as a nonforage fiber source had the lowest amount of uNDF₃₀ and the highest NDFD₃₀. Thus, feeding WS along with BP not only resulted in the same forage uNDF₃₀, but also in the same dietary uNDF₃₀ and NDFD₃₀. Nevertheless, concentration of dietary uNDF₂₈₈ linearly decreased and pdNDF₂₈₈ increased as the level of BP and WS increased. The performance results presented herein indicate that WS and BP substitution for CS and AH formulated on the basis of the method did not have a negative effect on lactation performance, but improved production efficiency.

Dry matter intake and the intake of uNDF₃₀ were not different among treatments, which was expected because the diets were formulated for similar levels of forage uNDF₃₀. However, intake of uNDF₂₈₈ or uNDF₂₄₀ were decreased, and this relates to a lower proportion in the formulated diets. Replacing forage with WS on a DM or NDF basis has not been found to affect DMI in previous studies (Poore et al., 1991; Eastridge et al.,

Table 3. Nutrient composition and particle size of dietary treatments [mean (SD)]

Item (% of DM, unless otherwise stated)	Diet ¹		
	WS0	WS50	WS100
DM (% of as fed)	46.6 (1.86)	46.8 (2.71)	44.8 (2.91)
OM	91.4 (0.15)	91.9 (0.14)	91.8 (0.09)
CP	16.3 (0.18)	16.1 (0.07)	16.5 (0.50)
Ether extract	4.57 (0.28)	5.06 (0.18)	5.47 (0.11)
NDF	31.4 (1.00)	32.1 (1.52)	32.5 (1.43)
NFC ²	39.1 (0.84)	38.6 (1.54)	37.4 (2.12)
Starch	29.02 (1.47)	26.78 (1.22)	23.58 (1.08)
Sugar	3.12 (0.45)	3.95 (0.49)	4.68 (0.63)
Lignin	2.41 (0.13)	2.08 (0.14)	1.51 (0.10)
Forage NDF	21.2 (0.21)	19.7 (0.10)	18.3 (0.09)
NE _L ³ (Mcal/kg of DM)	1.65	1.66	1.65
Forage uNDF ₃₀	14.0 (0.63)	13.9 (0.46)	13.9 (0.66)
uNDF ₃₀ ⁴	18.2 (1.32)	18.3 (1.41)	19.0 (1.17)
NDFD ₃₀ ⁵ (% of NDF)	42.2 (1.76)	43.0 (3.23)	41.7 (2.38)
uNDF ₂₄₀ ⁶	10.21 (0.40)	9.85 (0.46)	8.75 (0.54)
uNDF ₂₈₈ ⁷	10.1 (0.45)	9.52 (0.43)	8.48 (0.67)
pdNDF ₂₈₈ ⁸ (% of NDF)	67.8 (1.99)	70.3 (2.01)	73.9 (1.60)
pdNDF kd ⁹ (%/h)	2.42 (0.11)	2.60 (0.14)	3.10 (0.14)
>19 mm	4.02 (0.86)	2.20 (0.20)	0.50 (0.20)
19 to 8 mm	23.1 (1.11)	23.7 (1.52)	21.1 (2.14)
1.18 to 8 mm	51.5 (0.47)	52.7 (1.27)	58.2 (1.50)
<1.18 mm	21.4 (2.28)	21.4 (0.08)	20.3 (2.48)
GMPL ¹⁰ (mm)	3.84 (0.18)	3.72 (0.08)	3.54 (0.08)

¹Experimental diets were different uNDF₃₀ adjusted levels of wheat straw (WS): (1) WS0 = 0% forage uNDF₃₀ from WS, (2) WS50 = 50% forage uNDF₃₀ from WS, and (3) WS100 = 100% forage uNDF₃₀ from WS.

²NFC calculated by the difference of 100 - (% NDF + % CP + % fat + % ash).

³Based on tabular values (Cornell Net Carbohydrate and Protein System; Fox et al., 2004).

⁴uNDF₃₀ = NDF residue after 30-h in situ incubation.

⁵NDFD₃₀ = in situ NDF digestibility after 30-h in situ incubation.

⁶uNDF₂₄₀ = NDF residue after 240-h in situ incubation.

⁷uNDF₂₈₈ = NDF residue after 288-h in situ incubation.

⁸pdNDF₂₈₈ = potentially digestible NDF after 288-h in situ incubation.

⁹pdNDF kd = potentially digestible NDF fraction digestion rate.

¹⁰GMPL = geometric mean of particle size.

Table 4. Intake, rumen pH, and nutrient digestibility of dairy cows fed different forage 30-h undigested neutral detergent fiber (uNDF₃₀) from wheat straw (WS) or alfalfa hay and corn silage

Item	Diet ¹			SEM	P-value	
	WS0	WS50	WS100		Linear	Quadratic
Intake (kg/d)						
DM	24.9	25.0	24.2	0.65	0.09	0.27
NDF	7.73	7.96	7.95	0.25	0.87	0.16
uNDF ₃₀ ²	4.47	4.53	4.62	0.09	0.12	0.85
uNDF ₂₄₀ ³	2.51	2.44	2.14	0.06	<0.01	0.01
uNDF ₂₈₈ ⁴	2.48	2.36	2.07	0.06	<0.01	0.03
pdNDF ₂₈₈ ⁵	5.24	5.59	5.87	0.14	<0.01	0.65
Sorting index of different feed particles (%)						
>19 mm	85.6	92.4	98.8	3.34	<0.01	0.95
19 to 8 mm	99.3	100.7	100.8	0.48	<0.01	0.09
1.18 to 8 mm	100.9	100.4	100.4	0.25	0.01	0.08
<1.18 mm	101.1	98.2	96.5	0.92	<0.01	0.47
pH	5.90	6.09	6.28	0.10	<0.01	0.82
Digestibility (%)						
DM	62.8	63.4	68.0	0.63	<0.01	<0.01
OM	65.6	66.4	70.7	0.57	<0.01	<0.01
NDF	32.9	37.1	45.1	1.15	<0.01	<0.12
pdNDF ₂₈₈	48.2	51.7	59.2	1.56	<0.01	0.22
Ether extract	78.4	80.3	84.3	1.15	<0.01	0.43
CP	66.9	68.4	72.0	0.87	<0.01	0.30
NFC	90.3	88.5	89.8	1.28	0.68	0.13

¹Experimental diets were different uNDF₃₀ adjusted levels of WS: (1) WS0 = 0% forage uNDF₃₀ from WS, (2) WS50 = 50% forage uNDF₃₀ from WS, and (3) WS100 = 100% forage uNDF₃₀ from WS.

²uNDF₃₀ = NDF residue after 30-h in situ incubation.

³uNDF₂₄₀ = NDF residue after 240-h in situ incubation.

⁴uNDF₂₈₈ = NDF residue after 288-h in situ incubation.

⁵pdNDF₂₈₈ = potentially digestible NDF after 288-h in situ incubation.

Table 5. Milk production and composition, feed efficiency, and BW change of dairy cows fed adjusted amount of forage 30-h undigested neutral detergent fiber (uNDF₃₀) from wheat straw (WS) or alfalfa hay and corn silage

Item	Diet ¹			SEM	P-value	
	WS0	WS50	WS100		Linear	Quadratic
Yield (kg/d)						
Milk yield	46.3	46.7	44.4	0.76	<0.01	0.01
ECM ²	41.7	42.9	42.1	0.99	0.58	0.13
Fat	1.32	1.39	1.43	0.05	<0.01	0.68
Protein	1.36	1.37	1.28	0.02	<0.01	<0.01
Lactose	2.21	2.21	2.08	0.04	<0.01	<0.01
Composition (%)						
Fat	2.84	2.97	3.25	0.11	<0.01	0.24
Protein	2.94	2.93	2.90	0.02	0.02	0.37
Lactose	4.78	4.75	4.69	0.04	<0.01	0.63
MUN (mg/dL)	14.44	13.82	16.50	0.52	0.01	0.01
Fat:protein	0.96	1.00	1.11	0.03	<0.01	0.12
Feed efficiency						
Milk yield/DMI	1.85	1.87	1.82	0.05	0.25	0.20
ECM/DMI	1.66	1.70	1.72	0.03	0.01	0.67
BW (kg)	617	616	612	11.72	0.18	0.69
BW change (kg)	2.66	0.16	0.15	3.85	0.65	0.79
BFT ³ (mm)	29.1	28.8	28.6	0.96	0.62	1.00

¹Experimental diets were different uNDF₃₀ adjusted levels of WS: (1) WS0 = 0% forage uNDF₃₀ from WS, (2) WS50 = 50% forage uNDF₃₀ from WS, and (3) WS100 = 100% forage uNDF₃₀ from WS.

²ECM = 0.3246 milk yield + 12.96 fat yield + 7.04 protein yield (Tyrrell and Reid, 1965).

³Backfat thickness (BFT) was measured using the ultrasonographic method (Schroder and Staufienbiel, 2006).

2009; Wang et al., 2014). These authors hypothesized that finely chopped WS does not increase rumen fill, and that energy demand is the primary regulator of intake. However, Fustini et al. (2017) indicated that DMI was higher in cows fed a highly digestible forage compared with cows fed a less digestible forage, despite similar uNDF₂₄₀ levels. Ferraretto et al. (2015) also reported greater DMI and milk yields by cows fed CS with higher 30-h *in vitro* NDFD.

In the present study, cows fed WS0 sorted the ration, ingesting a ration smaller in particle length than offered, and this behavior linearly decreased as level of WS increased. Cows have previously been observed to sort rations against longer particles and to a greater degree when poorly digestible forage is offered (Suarez-Mena et al., 2013; Miller-Cushon and DeVries, 2017). Adequate forage chopping (Fustini et al., 2016) and reconstituting (Hooper and Welch, 1985; Pasha et al., 1994) have been suggested approaches to decrease sorting when poorly digestible forage is required in dairy cow diets. Fine chopping and reconstituting of WS for 24 h before feeding in the present study also decreased sorting against long particles. This result agrees with the predicted consequences of optimal specific gravity, adhesion of fine concentrate particles to larger fiber particles, and uniform particle size distribution of ingested feed (Hooper and Welch, 1985; Teimouri Yansari et al., 2004; Fustini et al., 2016). Also, in agreement with these observations, Suarez-Mena et al. (2013) reported that as particle length of low-quality forage decreased, sorting against longer particles decreased linearly, and Coon et al. (2018) reported that decreasing particle size of WS can prevent sorting against long particles in dairy cows. The importance of this discussion is that dietary effective fiber is required by dairy cows to stimulate chewing, maintain optimal rumen environment, and regulate peristalsis of the gastrointestinal tract (Van Soest, 1994). Ruminant pH at 4-h postfeeding increased with inclusion of WS and BP in the diet. The pH data might not represent the time-course changes of rumen pH throughout a 24-h period. However, cows fed the WS diet selected for long particles and the starch level in the respective diet was lower than in the control diet. These observations agree with our observations of rumen pH.

In the current study, the substitution of WS and BP increased digestibility of DM and most other nutrients, including NDF and pdNDF. Increased intake of pdNDF, decreased dietary particle size, and decreased lignin as well as balancing of NDFD₃₀ of diets with BP can be possible reasons for enhanced fiber digestibility (Van Soest, 1994; De Veth and Kolver, 2001). However, several studies found decreased (Poore et al., 1991; Farmer et al., 2014; Wang et al., 2014) or unaffected

(Eastridge et al., 2009) nutrient digestibility with feeding WS diets substituted based on DM or NDF, and it is possible that uncontrolled uNDF₂₈₈ and lignin have caused these responses. One additional difference between the present study and previous works is that inert fat, rather than grain, was used to correct for energy in WS-supplemented diets. Suppression of NDFD in low rumen pH condition has been well documented (Mertens, 1993; Nasrollahi et al., 2015, 2016). Of interest, the present study identified increased digestibility of pdNDF as treatment changed from WS0 to WS100. The important difference between the present study and previous works, in this case, is that BP was used to adjust the level of NDFD₃₀ in WS diets. Inclusion of BP was perhaps a primary reason for increased intake of pdNDF in WS diets. The uniform ingestion of WS and BP particles, as discussed previously in this report, also perhaps contributed to retention of digestible fiber in the rumen. The extent of fiber digestibility depends on the size of the indigestible fraction and the competition between the rate of ruminal passage and degradation (Oba and Allen, 1999). We hypothesized that increased fiber digestibility was due to the increased inclusion of highly digestible BP, and also to the increased inclusion of WS that promoted greater rumen pH and particle retention.

Although feeding WS as the sole forage source lowered milk yield, but did not affect the production of ECM as cows fed WS100 had higher milk fat percentage than cows fed WS0 (3.25 vs. 2.84%). Moreover, the feed efficiency calculated as ECM/DMI improved by feeding WS in combination with BP. The substitution of WS and BP for forages was associated with greater ingestion of long particles, increased ruminal pH, and perhaps a greater molar percentage of butyrate from feeding BP. These effects (Voelker and Allen, 2003; Nasrollahi et al., 2015, 2016), and the effects of increasing the level of dietary supplemented fat (mainly palmitic acid; Lock et al., 2013) might supply cows fed WS with greater substrates that promote milk fat production. Previous studies have generally reported that feeding high level of WS based on NDF or forage NDF decreased milk yield with no effect or a negative effect on FCM or fat production (Poore et al., 1991; Eastridge et al., 2009; Farmer et al., 2014; Wang et al., 2014). Therefore, the use of digestibility or uNDF₃₀ index would be an advancement over using forage NDF in formulating diets with different forage qualities. Diet digestibility represents an important target for improving the efficiency of energy utilization in dairy cows (Reynolds et al., 2011). The higher nutrient digestibility partially explains the higher efficiency with WS diets compared with the control diet. Furthermore, the highly digestible sugar and soluble fiber that was sup-

plied with BP in WS diets likely improved the supply of energy in these diets.

Blood urea nitrogen increased and milk protein production decreased with increasing levels of WS. The high concentration of BUN has been associated with reduced fertility in dairy cows (Nasrollahi, 2011), and as an indicator of low N efficiency (Broderick, 2006). In the present study, starch was 5.6 percentage units lower in WS100 than in the control diet (23.6 vs. 29.0%). With similar DMI, total starch intake was 1.35 kg/d lower in the WS100 diet than in the control diet. This low level of starch could have limited rumen microbial protein synthesis and decreased the amount of absorbed propionate from the rumen. If this conjecture is accurate, these effects could have decreased the effective insulin response to cause a decrease in milk protein synthesis (Hall and Herejk, 2001; Mahjoubi et al., 2009; Piccioli-Cappelli et al., 2014). The imbalance of rumen fermentable starch and protein in the rumen could also explain increased BUN in the WS diets. One observation in previous works that supports this hypothesis is that when an administration of WS in the diet was balanced for NFC or starch, the concentration of MUN or milk protein was unaffected (Eastridge et al., 2009), or even increased (Poore et al., 1991). On the other hand, when substituted WS was added without providing sufficient NFC or starch, the concentration of MUN increased (Farmer et al., 2014; Wang et al., 2014) and milk protein production decreased (Wang et al., 2014; Ghasemi et al., 2016). Further work seems to be needed to improve N efficiency and increase milk protein production from poorly digestible fiber sources such as WS by considering better synchronization of energy, total N, and AA supply.

Finally, it should be stated that the result of the present study was dependent on specific values of uNDF₃₀ and NDFD₃₀ in forages and BP. The variety, growing conditions, and harvesting conditions can all have affected these values even in the same forage types. An example of the effect of variety is that the CS used in the current study was similar in NDFD₃₀ to WS. This observation likely is not consistent among all CS. Therefore, when applying of the results of the present study, it is highly recommended to measure uNDF₃₀ and NDFD₃₀ for each forage and byproduct to be used.

CONCLUSIONS

Forage fiber along with other factors plays an important role in determining fiber requirements of the lactating cow. The formulation of diets based on forage fiber digestibility (i.e., uNDF₃₀ and NDFD₃₀) is proposed as an alternative marker to forage NDF for the control of rumen fill, DMI, and performance of dairy

cows. Measures of uNDF₃₀ and NDFD₃₀ can be used for the selection and formulation of forages with different qualities. The provision of WS and BP in place of AH and CS with fixed uNDF₃₀ and NDFD₃₀ had no negative effects on DMI and ECM in dairy cows. However, the substitution improved ruminal pH, nutrient digestibility, milk fat yield, and ECM efficiency. These beneficial effects were possibly the results of a good combination of WS and BP. Therefore, BP served as an alternative to high-quality forages when WS was added to maintain uNDF₃₀. However, feeding WS as the sole forage source, increased MUN, and decreased milk protein yield. Further studies on the supplementation of rumen available carbohydrate or RDP with WS diets are needed to optimize N efficiency.

ACKNOWLEDGMENTS

This manuscript is contribution no. 95816047 from the Iran National Science Foundation (Tehran, Iran). The authors are grateful to David E. Cook (BoviSync, Eden, WI) for his kindly helpful assistance in calculation of digestibility parameters and editions on the final version of this paper. We are also grateful to Lucas Krueger (Agri-King Inc., Fulton, IL) for his kindly and helpful editions on the final version of this paper. The authors also thank A. Piadeh, H. Kahyani (Isfahan, Iran), and Feed Processing Co. of Kabileh (Isfahan, Iran) for their technical assistance.

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