Total replacement of corn silage with sorghum silage improves milk fatty acid profile and antioxidant capacity of Holstein dairy cows

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ABSTRACT

Total mixed rations containing corn silage (CS) or forage sorghum silage (SS) were fed to mid-lactation Holstein cows to determine the effects on feed intake, lactation performance, milk composition and fatty acid profile, nutrient digestibility, blood metabolites, rumen microbial N synthesis, and antioxidant status. The experiment was designed as a 2-period change-over (two 28-d periods) trial with 2 diets including CS diet or SS diet and 12 cows. Total replacement of CS with SS had no significant influence on dry matter intake. Substituting CS with SS had no effect on milk production, feed efficiency, and milk concentrations of fat, protein, lactose, and solids-not-fat, whereas yields of milk fat, protein, and lactose were greater for cows fed the CS diet. Blood parameters including glucose, albumin, cholesterol, triglyceride, total protein, urea N, and fatty acids were not affected by the dietary treatments. Apparent digestibility coefficients of dry matter, organic matter, crude protein, ether extract, neutral detergent fiber, and acid detergent fiber were not significantly influenced by the diets. Replacing CS with SS had no effect on total saturated fatty acids and total monounsaturated fatty acids, whereas total polyunsaturated fatty acid percentage was greater with the SS diet. Proportions of C20:0, C18:3n-3, and C18:3n-6 were affected by feeding SS. Cows fed CS had a greater amount of urinary purine derivatives. Feeding SS had a positive effect on total antioxidant capacity of blood and milk. In conclusion, SS can be fed to lactating Holstein cows as a total replacement for CS without undesirable effects on animal performance, but with positive effects on antioxidant capacity and polyunsaturated fatty acids of milk. This forage can be an excellent choice for dairy farms in areas where cultivation of corn is difficult due to water shortage.

Key words: sorghum silage, performance, milk fatty acid, antioxidant capacity, Holstein cow

INTRODUCTION

Global changes in climate (e.g., lower levels of rainfall particularly in arid and semi-arid areas) underline the need for forages adapted to drier conditions compared with conventional forage (e.g., corn, a major source of forage for dairy farms). Sorghum is an alternative forage because it uses water more efficiently than corn and has a greater biomass yield (Peerzada et al., 2017). Experiments conducted on lactating cows have proven that the total replacement of corn silage (CS) with sorghum silage (SS), properly supplemented with starch sources, did not affect milk yield or milk composition (Colombini et al., 2010, 2012; Cattani et al., 2017). Some sorghum varieties contain phenolic compounds that can be associated with adverse effects as antinutritional factors, causing a lower DMI and milk yield and reduced digestion of protein (Oliveira et al., 2007). Nevertheless, some newer sorghum varieties are characterized by the presence of low or moderate levels of phenolics and these offer an opportunity to optimize the nutrient supply for ruminant production (Zhang et al., 2015). Numerically higher in vitro DM and OM digestibility of silage mixtures (made with sorghum and alfalfa) with a lower sorghum content (20:80 SS: alfalfa silage) were observed. The effect of moderate levels of phenolics on milk yield and milk composition is in agreement with Abarghuei et al. (2013, 2014) and Alipour and Rouzbehan (2010), who reported a positive effect on the efficiency of ruminal protein metabolism (i.e., a decrease in $\text{NH}_3\text{-N}$ losses), resulting in increased dairy cow performance and contributing to a decrease in environmental pollution, as well as improving milk fatty acid composition (i.e., higher in PUFA). High concentrations of PUFA in milk negatively affect the stability of fat, which may oxidize and develop off-flavors in milk (Zhao et al., 2013), reducing shelf life compared with milk with a common fat profile. For this reason, efforts have been made to enrich milk with

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antioxidant compounds (e.g., phenolics) in a natural way by altering the diet of cows (Gagnon et al., 2009; Aguiar et al., 2014; Santos et al., 2014).

Little research seems to have been done into changes in fatty acid profile and antioxidant capacity in the milk of dairy cows when dietary CS is replaced with SS. Therefore, the aim of this study was to assess the effects of total dietary replacement of CS with SS on nutrient digestibility, milk yield, composition and fatty acid profile, urinary purine derivatives (**PD**), blood metabolites, and antioxidant capacity in the blood and milk of lactating Holstein cows.

MATERIALS AND METHODS

The care and use of all the cows and the experimental protocols and procedures were approved by the Animal Science Committee of Tarbiat Modares University (Tehran, Iran).

Crops and Silages

Corn was sown on May 21, 2016, at Gavdasht Farm located in Babol, the capital of Babol County, Mazandaran Province, Iran. Forage sorghum was sown on an adjacent field on the same farm on the same date. Weed control was applied to the sorghum and corn forages through row cultivation during the first weeks after plant sowing. On July 2016, the corn forage was harvested at the two-thirds milk line stage of maturity, and sorghum forage at the beginning of the early bloom stage. After harvest, sorghum and corn were ensiled into permanent horizontal silos (one 60-t silo per sorghum and one 60-t silo per corn), and kept for 60 d. The materials were compacted by driving over them with a compaction machine (a tractor). Silage samples were taken from 4 different points from each silage. The silage samples were oven-dried at 55°C to a constant weight, mixed thoroughly, and ground using a hammer mill with a 1-mm sieve (standard model 4, Arthur H. Thomas, Philadelphia, PA).

Chemical Analysis and Fermentation Characteristics of Silage

The CP ($N \times 6.25$, method no. 984.13), DM (method no. 930.15), and ash (method no. 942.05) were determined by the standard methods of AOAC International (1998). Ash-free NDF (**NDFom**) was determined according to the method of Mertens (2002). The ADF (method no. 973.18; AOAC International, 1998) was determined and expressed exclusive of residual ash (ash-free ADF; **ADFom**). The ADL was determined using the sulfuric acid method (AOAC International,

1998). Water-soluble carbohydrates (**WSC**) were measured by the anthrone method (MAFF, 1982) and nitrate using colorimetry (Singh, 1988). Starch content of the CS and SS was analyzed according to AOAC (method 996.11; AOAC, 1990).

A 50-g sample of silage was mixed with 125 mL of distilled water and left for 1 h at 25°C with occasional stirring, then pH was recorded by a digital pH meter (Sartorius PT-10, Göttingen, Germany). Nine milliliters of silage extract was mixed with 1 mL of 7.2 *N* sulfuric acid, and ammonia-N was measured spectrophotometrically using a phenol hypochlorite technique (Galyean, 1997). Two milliliters of silage juice was mixed with 0.5 mL of an acid solution (containing 20% orthophosphoric acid and 20 mM 2-ethyl butyric acid, as the internal standard), and centrifuged at $15,000 \times g$ for 15 min at 4°C. The supernatant was used to quantify VFA using a gas chromatograph (UNICAM 4600; SB Analytical, Cambridge, UK) with a capillary column (Agilent J&W HP-FFAP, 10 m by 0.535 mm by 1 μ m, 19095F-121; Agilent, Santa Clara, CA).

Total phenolics (**TP**) were measured using the Folin-Ciocalteu method described by Makkar (2000). Two hundred milligrams of dried plant material was extracted with acetone:water (10 mL; 70:30, vol/vol) in an ultrasonic bath for 20 min. The contents were centrifuged (4°C, 10 min, $3,000 \times g$) and the supernatant was kept on ice until analysis. Extractable total tannins were determined after adding insoluble polyvinylpyrrolidone, and reacting with Folin-Ciocalteu reagent to assess the difference between TP and nontannin phenolics (Makkar, 2000). The chemical composition, fermentative characteristics, and the anti-nutrients of 2 silages are given in Table 1.

Experimental Cows and Diets

Twelve mid-lactation Holstein cows with mean ($\pm$ SD) BW of 550 kg (± 40 kg), parity 2 ± 1.2 , and an average yield of 30 ± 3.0 kg of milk/d (121 ± 10 DIM) at the start of the trial were housed individually in a tiestall commercial dairy farm belonging to the farm described above (Gavdasht farm, Babol). The experiment was designed as a 2-period change-over trial with 2 diets including CS diet (**CSD**) or SS diet (**SSD**) and 12 cows. Thus, each group of cows received a TMR, formulated according to the Nutrient Requirements of Dairy Cattle (NRC, 2001), in amounts to ensure 10% orts, based on either on CS or SS in 2 periods each lasting 28 d (Table 2). The first 21 d served as an adaptation period, and the last 7 d served as the data collection period. During the experiment, all cows had free access to water. The animals were milked 3 times a day and fed 3 times after each milking. To compensate for

Table 1. Chemical composition, fermentative characteristics, and anti-nutrients (% of DM unless otherwise stated) of corn silage (CS) and sorghum silage (SS; n = 10)

Item ¹	CS	SS	SEM	P-value
Chemical composition				
DM	27.22	28.20	0.20	0.45
NDF	42.26	49.53	1.23	0.04
ADF	26.94	38.09	1.10	0.04
ADL	2.11	4.13	0.18	0.04
CP	8.32	10.8	0.15	0.31
WSC	1.73	2.94	0.03	0.12
Starch	20.10	2.50	0.84	<0.001
Ash	4.81	9.06	0.60	0.04
Fermentative characteristic				
pH	3.91	4.02	0.04	0.40
Ammonia-N, g/kg of total N	64.93	45.56	1.44	0.01
Lactic acid	4.39	3.35	0.22	0.81
Acetic acid	0.92	1.65	0.03	0.64
Propionic acid	0.23	0.015	0.02	0.02
Butyric acid	0.00	0.01	0.008	0.001
Anti-nutrient				
Nitrate, g/kg of DM	3.42	2.95	0.12	0.06
TP, g/kg of DM	0.53	16.98	0.19	0.03
TT, g/kg of DM	0.44	13.53	0.43	0.007
Hydrocyanic acid	0.00	0.067	0.003	0.01

¹WSC = water-soluble carbohydrates; TP = total phenolics; TT = total tannins.

the lower starch content of SS compared with CS, the SSD contained a greater concentration of corn meal. The 2 TMR were prepared using a total mixer wagon.

Feed Intake and Diet Digestibility

The amount of diet distributed individually to each experimental cow and correspondingorts were recorded daily to estimate voluntary feed intake. The representative samples of 2 diets and orts were collected at each period, and kept frozen at −20°C, to analyze chemical composition. For digestibility determination, fecal grab samples were collected from the rectum of each cow about 4 h pre-feeding (a.m. sampling) and 4 h postfeeding (p.m. sampling) on d 21 to 28 of each period. The samples of diets, orts, and feces were oven-dried at 60°C to a constant weight to determine DM concentration, and then ground to pass through a 1-mm sieve (standard model 4, Arthur H. Thomas, Philadelphia, PA). After grinding the fecal samples, a single composite was prepared for each cow within the treatment and stored for later analysis. Samples were analyzed for OM, ether extract, NDF, and ADF as described earlier. Acid detergent insoluble ash content of the diets and feces was used as an internal marker to determine apparent total-tract nutrient digestibility (McGeough et al., 2010). The ME (MJ/kg of DM) content for each diet was calculated as $0.0157 \times \text{digestible OM in the DM (g/kg of DM)}$, after in vivo determination of digestible OM in the DM (Alderman et al., 1993).

Table 2. Ingredients and chemical analysis (% of DM) of the experimental diets [corn silage diet (CSD) and sorghum silage diet (SSD)]

Item	CSD	SSD
Alfalfa hay	9.44	9.44
Wheat straw	1.50	—
Corn silage	25.00	—
Sorghum silage	—	25.00
Corn grain	10.24	13.01
Barley grain	13.09	13.02
Soybean meal	14.44	14.44
Cottonseed meal	2.41	2.41
Wheat bran	12.45	11.00
Sugar beet pulp	7.00	7.00
Fat supplement	1.02	1.88
Calcium carbonate	0.97	0.75
Salt ¹	0.38	0.30
Bentonite ²	0.38	0.38
Vitamin-mineral premix ³	0.71	0.70
Sodium bicarbonate	0.97	0.70
Chemical composition ⁴		
CP	15.05	15.06
ADF	20.58	21.13
NDF	37.03	37.15
ADL	2.87	3.21
Ash	9.01	10.04
Starch	24.22	21.39
ME, ⁵ Mcal/kg	2.51	2.51
NE _L , Mcal/kg	1.61	1.59

¹Calcium salts of soybean fatty acids (Pehparvaran Nami Naghshe Jahan Co., Isfahan, Iran).

²Zarin Binder, South Khorasan, Ghaen, Iran.

³Contained (per kg): 196 g of Ca, 96 g of P, 71 g of So, 3 g of Mg, 0.3 g of Fe, 2 g of Cu, 3 g of Mn, 0.1 g of Zn, 0.1 g of Co, 0.001 g of I, 500,000 IU of vitamin A, 100,000 IU of vitamin D, and 100 mg of vitamin E.

⁴Calculated from each feed composition, which were analyzed.

⁵Estimated using a gas production technique, as described by Menke et al. (1979).

Milk Production, Composition, and Fatty Acid Profile

Cows were milked 3 times daily and the amount of milk produced for each cow at each milking was recorded. During the last week of each period, milk samples were taken from each cow at each milking for the whole week. The samples from the whole week were pooled for each animal within the treatment and stored at 4°C to analyze for fat, protein, and lactose, and a sub-sample was stored at -80°C for determination of fatty acid composition. Fatty acid analyses were carried out with a gas chromatograph (HP 5890A Series II, Hewlett Packard, Palo Alto, CA) equipped with a 100-m CP-Sil 88 capillary column (i.d., 0.25 mm; film thickness, 0.20 µm; Chrompack, Middelburg, the Netherlands) and a flame ionization detector (Farnworth et al., 2007).

Blood Sampling and Analyses

On d 27 of each period of the experiment, blood was taken from the coccygeal vein of each cow into tubes containing clot activator (MediPlus, Sunphoria Co. Ltd., Taiwan). The blood samples were collected 3 h after morning feeding. Samples were allowed to clot at room temperature and then placed in a refrigerator within 30 min. The tubes were centrifuged within 6 h of collection, and serum was harvested and frozen at -20°C until analysis. The concentrations of glucose, triglyceride, cholesterol, urea N, total protein, and albumin were measured using commercial assay kits from Pars Azmon Diagnostics (Tehran, Iran) by a spectrophotometer (Genova; Jenway, Barloworld Scientific Ltd., Dunmow, Essex, UK).

Excreted and Absorbed Purine Derivatives

The urine samples were collected on d 23 to 28 of each period of the experiment. Each cow was manually stimulated to urinate and spot samples of urine were taken from all of the cows. Urine samples were acidified immediately after collection by diluting 1 volume of urine with 4 volumes of 0.072 N H₂SO₄, and stored at -20°C until analysis. The samples were thawed at room temperature and filtered through Whatman no. 1 filter paper. Filtrates were analyzed for creatinine, and then daily urine volume was estimated as BW × 29/urinary creatinine concentration (mg/L; Valadares et al., 1999). Urinary PD were estimated by spectrophotometric methods (Chen and Gomes, 1995). Urinary allantoin was measured by a colorimetric method at 522 nm after its conversion to phenylhydrazone. Uric acid was measured from the reduction in optical density at 293 nm after degradation of uric acid to allantoin with uricase (Product No. U-9375, Sigma-Aldrich, Darmstadt, Ger-

many). Daily excreted urinary PD was used to estimate the amount of exogenous purines absorbed as described by Chen and Gomes (1995):

$$X = (Y - 0.385 W^{0.75}) / 0.85,$$

where X is the absorbed exogenous purine as mmol/d, Y is the urinary PD excretion as mmol/d, and W is the live weight (Chen and Gomes, 1995).

Antioxidant Capacity of Blood and Milk

Total antioxidant capacity (TAC) of the blood and milk samples was evaluated by measuring the ferric reducing antioxidant power (Benzie and Strain, 1996). The method is based on the reduction of ferric tripyridyltriazine (Fe³⁺-TPTZ) complex to ferrous (Fe²⁺) form in the presence of antioxidants, which develops an intense blue color with absorbance at 593 nm. A calibration curve was prepared using ferrous sulfate solution and the results were expressed in mmol Fe²⁺ formed per liter of the sample.

Statistical Analysis

Data were analyzed using the PROC MIXED of SAS (version 9.1, SAS Institute Inc., Cary, NC). Data of intake, in vivo digestibility, blood metabolites, milk composition and fatty acid profiles, and PD were analyzed in a change-over design using the statistical model

$$Y_{ijk} = \mu + T_i + C_j + P_k + e_{ijk},$$

where Y_{ijk} is observation, μ is the overall mean, T_i ($i = 1-2$) is the fixed effect of treatment, C_j ($j = 1-12$) is the random effect of cows, P_k ($k = 1-2$) is the fixed effect of period, and e_{ijk} is the residual error. P -values and standard errors of the mean were reported.

RESULTS AND DISCUSSION

Composition and Fermentation Quality of Experimental Silages

As seen in Table 1, no difference was observed between the silages for DM concentration, and DM was within the range of medium quality silage according to McDonald et al. (1991). Miron et al. (2007) reported a similar concentration of DM for SS (29% of DM), whereas Oliver et al. (2004) showed greater DM concentrations for both SS and CS compared with our results (30 vs. 34.4% of DM, respectively). The differences in DM concentration among the studies are probably due to factors such as soil, climate, planting date, crop

management, stage of maturity, and season (McDonald et al., 2011; Rezaei et al., 2014).

In agreement with NRC (2001) tables, the NDF, ADF, and ADL concentrations for SS were greater ($P < 0.05$) than those of CS (Table 1). Harper et al. (2017) reported the greater NDF and ADF concentrations in SS compared with CS (62.7 and 40.8 vs. 40.2 and 25.9% of DM, respectively). The SS CP was greater than that reported by Oliver et al. (2004); however, the CP content in the SS was lower than that reported by Harper et al. (2017), and this inconsistency is probably due to differences in some factors such as plant (sorghum) species, stage of maturity, climate, soil conditions, and fertilization (McDonald et al., 2011).

Forage WSC content is an important factor in reducing the pH to achieve a well-preserved silage. In our study, the concentration of WSC was low in both of the experimental silages (Table 2), which is probably due to the consumption of these compounds by *Lactobacillus* bacteria producing lactic acid (McDonald et al., 1991). Ash concentration of both CS and SS was close to the values reported by NRC (2001). Starch is the main source of energy in CS and is a quantitatively important nutrient for the performance of dairy cows (Jensen et al., 2005). Rezaei et al. (2014) noted that CS produced in warm climate regions tended to present lower starch concentrations, which is similar to that reported in current study (20.1% DM basis, Table 1), but is lower than those stated in NRC (2001) tables (i.e., 32% DM basis).

The pH values for both silages were within the range of 3.5 to 4.5, indicating good fermentation quality (Jones et al., 2004). Although the concentration of ammonia-N was significantly ($P < 0.05$) greater for CS compared with SS (Table 2), the ammonia-N in both silages was less than the guidelines of McDonald et al. (1991; i.e., 65 g/kg of total N) that indicate good fermentation quality. In all the silages, the concentrations of acetic, propionic, and butyric acids were low, suggesting a good fermentation (McDonald et al., 1991). Nitrate concentrations of the silages were lower than the toxic level (i.e., >10–30 g/kg of DM) reported by Adams et al. (1992). Total phenolics, total tannins, and hydrocyanic acid concentrations of SS were greater ($P < 0.05$) compared with those of CS.

Total-Tract Nutrient Digestibility

According to Table 3, replacing CS with SS in the diet had no effect on the DM and nutrients (i.e., OM, CP, NDFom, and ADFom) digestibility, which could be related to the similar lignin content in the diets as shown in Table 2. Furthermore, the NRC (2001) tables reported higher starch content in CS (i.e., 32% DM

Table 3. Nutrient digestibility (%) in lactating Holstein cows fed diets including corn silage (CSD) or sorghum silage (SSD)

Item ¹	CSD	SSD	SEM	P-value
DM	63.09	62.26	0.57	0.34
OM	66.29	66.78	0.77	0.67
CP	63.35	63.57	1.70	0.79
NDF	53.30	52.05	0.89	0.52
ADF	55.08	53.04	1.42	0.13

¹Average of fecal samples collected from each cow on d 23 to 28.

basis) than those obtained in the current study (20.1% DM basis), which probably was not sufficient to improve the nutrient digestibility in comparison to SSD (Ferraretto et al., 2013). The TP content (16.98 g/kg of diet DM) in SSD had no apparent negative effect on the digestibility of DM and nutrients. Abarghuei et al. (2013) observed no effect of the dietary inclusion of TP (up to 65 g/kg of DM diet) on the digestibility of DM, OM, CP, and ADF in lactating Holstein cows. Colombini et al. (2012), moreover, reported that digestibility of CP, NDF, and ADF showed no difference between sorghum and corn diets. In a study by Oliver et al. (2004), the digestibility of CP was similar for cows fed either on a diet containing ensiled sorghum or diet containing CS. In contrast, Harper et al. (2017) observed that digestibility of CP and DM was higher in cows fed CS compared with SS, which may have been due to the greater lignin content in the SS (4.89 vs. 3.7% of DM, respectively), since lignin is considered the main limiting factor of the digestion of nutrients in the rumen (Van Soest, 1994). However, recently Raffrenato et al. (2017) indicated that the content of lignin is not directly responsible for digestibility; more likely, the degree of cross-linking of phenolics within the cell wall carbohydrate directly influences the nutrient digestibility.

Dry Matter Intake, Milk Yield, and Composition

The DMI of the experimental cows was not affected by the experimental diets ($P > 0.05$), which could be explained by the similar diet digestibility among the animals (Table 4). Oliver et al. (2004) and Colombini et al. (2012) reported that DMI in cows receiving diet containing SS was not significantly different from those offered CS. However, in a study conducted by Harper et al. (2017), cows fed with a diet containing CS had a greater DMI compared with those offered a diet containing SS, which was probably due to the higher NDF, ADF, and ADL content in the SSD leading to slower digestion and passage rate and increased rumen fill.

Milk yield among the cows fed either CSD or SSD were not different ($P > 0.05$). The similarity in milk yield and FCM was related to the comparable values

Table 4. Effect of feeding corn silage diet (CSD) or sorghum silage diet (SSD) on intake, milk production and composition, and feed efficiency in lactating Holstein cows

Item	CSD	SSD	SEM	P-value
DMI, kg/d	23.00	22.72	0.90	0.37
ME intake, ¹ Mcal/d	57.76	57.04	0.53	0.36
NDF intake, kg/d	8.51	8.42	0.07	0.45
Milk production, kg/d	30.63	30.02	0.49	0.42
4% FCM, kg/d	27.50	26.94	0.69	0.63
Milk/DMI	1.34	1.33	0.02	0.75
Milk fat, %	3.33	3.32	0.14	0.97
Milk fat, ¹ kg/d	1.01	0.99	0.04	0.74
Milk protein, %	2.76	2.60	0.06	0.07
Milk protein, ¹ kg/d	0.84	0.78	0.02	0.06
Milk lactose, %	4.74	4.53	0.09	0.28
Milk lactose, ² kg/d	1.44	1.37	0.04	0.19
MUN, mg/dL	10.85	11.42	0.27	0.17

¹The ME (MJ/kg of DM) was calculated as $0.0157 \times \text{digestible OM in the DM (DOMD, g/kg of DM)}$, after in vivo determination of DOMD (Alderman et al., 1993).

²Calculated using the milk yield of the 2 consecutive milk sampling days.

for MN (Oba and Allen, 2003), diet digestibility, and DMI in these animals. Oliver et al. (2004) and Bernard and Tao (2015) found no difference in DMI and milk yield between dairy cows fed diets containing SS or CS. In contrast, Harper et al. (2017) reported a lower milk production in the cows offered ensiled sorghum than those animals fed with CS. Data reported by these authors suggested that the reduction of DMI in cows receiving SS has led to a decrease in milk yield. In our study, no difference was observed in feed efficiency between the experimental groups as there was no difference between DMI or milk yield (Table 4) between animals fed with SSD instead of CSD. Colombini et al. (2012), also, reported a similar feed efficiency between animals fed with diets containing SS or CS. Milk fat, protein, and lactose concentrations and yields were not affected by the dietary treatments. The same milk fat content among the experimental cows could be explained by a similar NDF intake between CSD and SSD groups. A similar content of milk protein between the experimental groups shows that feeding CSD or SSD had no negative effect on supplying postruminal microbial protein, as demonstrated by Chibisa et al. (2012). Oliver et al. (2004) and Colombini et al. (2012) reported that cows fed with SS did not show different contents of fat, protein, and lactose in their milk compared with those animals that received CS. The MUN is a fraction of milk N that is greatly correlated with BUN, and is a useful tool in assessing the protein and energy balance status of a group of dairy cows (Moore and Verga, 1996). In the current study, the MUN concentrations of the animals (11.4 vs. 10.8 mg/dL for SSD and CSD, respectively) were within the normal ranges (11–14 mg/dL) reported for dairy cows (Moore and

Verga, 1996), indicating substitution of CS with SS in the diet had no effect on protein and energy balance of the lactating cows.

Blood Biochemistry Metabolites

The concentrations of glucose, cholesterol, total protein, albumin, globulin, and urea N in the blood serum of the animals were within the typical ranges reported for cattle (Radostits et al., 2007). The effects of experimental diets on serum glucose, cholesterol, triglyceride, albumin, globulin, total protein, and urea N were similar (Table 5). Also, the fatty acid concentration showed no significant difference between the diets ($P > 0.05$). Absence of difference in blood parameters among the experimental cows was probably due to the similar nutrient digestibility and DMI. Blood concentration of total protein is an indicator of the long-term protein status of dairy cows (Topps and Thompson, 1984), and the lack of effect of the silages on blood total protein in the current study denotes the normal protein status. Blood albumin decreases in unhealthy animals (Solaiman et al., 2010). In our study, the similar serum albumin concentration and albumin-to-globulin ratio among the experimental animals indicate that no health problems are affecting the cows' performance as reported by Radostits et al. (2007) and Solaiman et al. (2010). The BUN is a key indicator of protein status of an animal and ruminal performance (Hess et al., 2000), which in the present study was not influenced by the diets. Blood glucose and fatty acid concentrations are important indicators of energy metabolism in dairy cows (Yang et al., 2017). Researchers reported that serum fatty acids correlate negatively with energy balance in dairy cows (Butler et al., 2006). In present study, concentrations of fatty acids and glucose in 2 animal groups were within normal ranges (Drackley et al., 2001). Lack of any differences in blood fatty acids and glucose among the treatments was probably due to similar intakes and digestibility.

Table 5. Effect of feeding corn silage diet (CSD) or sorghum silage diet (SSD) on blood metabolites in lactating Holstein cows

Item	CSD	SSD	SEM	P-value
Glucose, mg/dL	53.64	51.14	1.20	0.13
Globulin, g/dL	2.92	2.72	0.23	0.54
Albumin, g/dL	4.31	4.55	0.13	0.15
Cholesterol, mg/dL	244.40	222.32	10.12	0.15
Total protein, g/dL	7.23	7.33	0.15	0.64
Triglyceride, mg/dL	13.18	14.15	0.97	0.51
Albumin-to-globulin ratio	1.69	1.88	0.20	0.51
BUN, mg/dL	15.73	18.83	1.59	0.18
Fatty acid, mmol/L	0.02	0.04	0.01	0.14

Table 6. Effect of feeding corn silage diet (CSD) or sorghum silage diet (SSD) on milk fatty acid (FA) composition (g/100 g of FA) of lactating Holstein cows

Fatty acid ¹	CSD	SSD	SEM	P-value
C10:0	4.10	3.77	0.21	0.76
C12:0	2.98	2.69	0.18	0.38
C14:0	12.27	10.93	0.46	0.31
C14:1 <i>cis</i> -9	1.18	1.05	0.035	0.23
C15:1	0.20	0.16	0.022	0.49
C16:0	34.99	34.34	1.64	0.47
C16:1 <i>cis</i> -9	1.38	1.51	0.18	0.39
C17:0	0.75	0.65	0.064	0.60
C17:1	0.72	0.62	0.055	0.052
C18:0	11.84	13.01	0.72	0.14
C18:1 <i>cis</i>	24.92	25.59	1.33	0.38
C18:1 <i>trans</i>	0.52	0.62	0.028	0.051
C18:2n-6 ²	2.06	2.75	0.289	0.053
C18:3n-3 ³	0.14	0.11	0.029	0.037
C18:3n-3 ³	0.23	0.38	0.035	0.011
C20:0	0.49	0.65	0.026	0.023
C20:3	1.12	1.02	0.006	0.37
C20:4	0.11	0.16	0.011	0.045
n-6/n-3	8.84	8.11	0.32	0.17
Total SFA	67.42	66.04	3.02	0.58
Total MUFA	28.91	29.54	1.59	0.36
Total PUFA	3.67	4.42	0.166	0.037

¹Average of milk FA profile, milk collected of each cow in 2 periods of the trial, in d 23 to 28 of the data collection period.

²n-6 = n-6 FA including C18:2 + C20:3 + C20:4.

³n-3 = n-3 FA including C18:3.

Milk Fatty Acid Composition

Results of this study highlighted that milk fatty acid profile was influenced by the diets, with changes in the proportions of the individual fatty acids. Milk was greater in PUFA proportion when the cows were fed with SSD ($P < 0.05$), whereas the total proportions of SFA and MUFA were not influenced by the dietary treatments (Table 6).

Compared with CSD, feeding SSD resulted in an increased proportion of PUFA in the milk. The inclusion of SS in the diet, moreover, led to the increased linoleic and α -linolenic acids in the milk. The percentage of n-3 fatty acids in the milk of the cows fed with SSD was significantly greater compared with those receiving CSD. This difference may be based on the greater concentration of plant active ingredients, especially tannins, in SS and these inhibit growth and metabolism of bacteria that are capable of biohydrogenation (Mapiye et al., 2011), resulting in increasing PUFA concentrations. Therefore, protection of fatty acids from ruminal biohydrogenation or inhibition of ruminal bacteria activity in SSD could have produced higher escape of PUFA from rumen to tissue and milk. Furthermore, fatty acids such as C18:3 *cis* (n-3) is converted by desaturase and elongase enzymes into nutritionally important long-chain PUFA such as eicosatrienoic acid (C20:3) and eicosatetraenoic acid (C20:4; Brenna et al.,

2009; Harris et al., 2009). Thus, apart from ruminal production, the higher amount of eicosatetraenoic acid (C20:4) obtained in milk fat of cows fed the SSD might have arisen from increased endogenous biosynthesis of this fatty acid from C18:3 *cis* (n-3) in the mammary gland tissue. It has been reported that n-6:n-3 has a valuable influence on the healthiness of dairy products (Cabbidu et al., 2009), but this ratio was not significantly different between the dietary treatments in our study. The milk of cows fed with SSD had a significantly greater C20:4 concentration compared with the milk from CSD-fed cows.

Excreted and Absorbed Purine Derivatives

In this experiment, the urinary allantoin and uric acid excretion, and total PD excreted and absorbed were not influenced by the diets ($P > 0.05$; Table 7). The concentration of allantoin in the urine of dairy cows is an important factor in estimating the amount of microbial protein being utilized (Braga et al., 2012). This similarity in the urinary excreted PD between the 2 groups of experimental cows may be attributed to the similar amounts of nutrient digestibility and DMI with feeding both diets. In another study, Harper et al. (2017) reported that urinary excretions of allantoin, uric acid, and total PD were not significantly different between dairy cows fed with diets containing SS or CS.

Total Antioxidant Capacity of Blood and Milk

Antioxidant capacity is associated with a decreased risk of various diseases and mortality (Huxley and Neil, 2003; Whitley et al., 2003). A positive correlation between oxidative stress and illness is widely documented in animals (Miller et al., 1993; Chirase et al., 2004). Phenolic compounds attain their active antioxidant power through the scavenging activity of free radicals (Zheng et al., 2009), transition-metal-chelating activity (Andjelković et al., 2006), singlet-oxygen quenching capacity (Mukai et al., 2005), or a combination of these. There was a significant ($P = 0.01$) effect on blood an-

Table 7. Effect of feeding corn silage diet (CSD) or sorghum silage diet (SSD) on purine derivatives of lactating Holstein cows

Urinary excretion, mmol/d ¹	CSD	SSD	SEM	P-value
Allantoin	224.74	215.37	21.02	0.94
Uric acid	55.05	42.28	3.85	0.15
TPD ² excretion	273.46	257.72	16.97	0.48
TPD absorbed	230.94	215.07	16.90	0.52

¹Average of sampling of urine collected from 12 animals assigned to each treatment in d 23 to 28 of the data collection period.

²TPD = total purine derivatives.

Table 8. Effect of feeding corn silage diet (CSD) or sorghum silage diet (SSD) on total antioxidant capacity (TAC; mmol Fe²⁺/L) of the blood serum and milk in lactating Holstein cows

Item ¹	CSD	SSD	SEM	P-value
Blood serum TAC	2,201	2,360	39.67	0.01
Milk TAC	2,320	2,677	100.74	0.03

¹Average of sampling of blood and milk collected from 12 animals assigned to each treatment on d 23 to 28 of the data collection period.

tioxidant capacity when the cows were fed with SSD compared with CSD, and TAC in milk was significantly ($P = 0.03$) greater in cows fed with SSD compared with CSD (Table 8). Therefore, it was hypothesized that feeding SSD may be an inexpensive and natural way to increase the antioxidant content in milk.

CONCLUSIONS

Sorghum silage can be fed to lactating Holstein cows as a total replacement for CS without undesirable effects on animal performance, but positive effects on antioxidant capacity and PUFA of milk. This forage can be an excellent choice for dairy farms in areas where cultivation of corn is difficult due to water shortage.

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