Livestock Science 141 (2011) 95–103

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effects of the inclusion of dried molassed sugar beet pulp in a low-forage diet on the digestive process and blood biochemical parameters of Holstein steers

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ARTICLE INFO

Article history:
Received 15 July 2010
Received in revised form 10 May 2011
Accepted 16 May 2011

Keywords:
Sugar beet pulp
Barley grain
Rumen fermentation
Digestibility
Steer

ABSTRACT

We evaluated the effects of substituting various concentrations of dried molassed sugar beet pulp (SBP) for barley grain in low-forage diets on chewing behavior, ruminal fermentation, nutrient digestibilities and blood biochemical parameters using four ruminally cannulated Holstein steers in a 4×4 Latin square design over 28-day periods. Steers (368±8 kg initial body weight) were fed 9.5 kg of dietary dry matter (DM) as a total mixed ration (TMR) (containing 350 g forage and 650 g concentrate per kg DM) twice daily at 0800 and 1600 h. The diets were formulated to supply approximately 2.3 times the maintenance requirements of the animals so that there was no refusal. Barley grain in the basal experimental diet (330 g/kg DM) was replaced with 0, 110, 220 and 330 g SBP on a DM basis to create the experimental diets SBP0, SBP110, SBP220 and SBP330, respectively. Ruminal fluid was collected by suction through the rumen cannula from before the morning feeding (0.0 h) to 8 h after feeding at 30-min intervals. Eating, ruminating and total chewing time linearly ($P<0.01$) increased with the proportion of SBP in the diet. Moreover, mean ruminal pH showed linear and quadratic increases ($P<0.05$) with the inclusion of SBP. In contrast, the substitution of SBP for barley grain resulted in a linear and quadratic decrease ($P<0.01$) in the mean ruminal ammonia concentration, which was highest in steers fed SBP0. In addition inclusion of SBP gave significantly ($P<0.05$) higher acetate and butyrate molar proportions and lower propionate and total volatile fatty acids (VFA) concentrations in the rumen fluid. Total tract apparent digestibility of DM and neutral detergent fiber (NDF) increased quadratically with the proportion of SBP in the diet, but crude protein (CP) and acid detergent fiber (ADF) digestibilities were similar among treatments. Plasma urea nitrogen (PUN) before the morning feeding decreased linearly ($P<0.01$) with SBP inclusion and was highest and lowest for SBP0 and SBP330, respectively (21 *vs.* 16.26 mg/dl). Other blood biochemical parameters and venous blood gasses (including plasma glucose, blood pH, CO_2 pressure, O_2 pressure, oxygen saturation, base excess of extracellular fluid, base excess of blood and bicarbonate) were not affected by the treatments ($P>0.05$). These results suggest that partial replacement of barley grain with SBP at low and moderate inclusion rates might improve the chewing behavior, ruminal environment and nutrient digestibility of Holstein steers fed low-forage diets.

1. Introduction

Because of low pasture availability, ruminant diets in arid and semiarid regions are usually based on concentrates. However, high-grain diets can lead to digestive disorders such as latent rumen acidosis, reduced rumination and reduced saliva secretion, eventually impacting performance (Enemark et al., 2002). For many years, Ionosphere antibiotics have been used to reduce the risk of ruminal acidosis and feedlot bloat (Cheng et al., 1998) by regulating rumen microbial fermentation and by

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doi:10.1016/j.livsci.2011.05.009

directly inhibiting lactate-producing bacteria (Galyean and Rivera, 2003). In recent years, however, the use of these feed additives has been banned in many countries due to the risk of spreading antibiotic resistance (Bedford, 2000). These regulations, combined with current public concerns about the use of additives in animal production, make it necessary to develop alternative means of obtaining similar production benefits to maintain profitability and competitiveness.

One way to reduce the use of cereals in ruminant rations is to replace them with high-energy non-cereal by-products, such as molassed sugar beet pulp (SBP). This feedstuff contains approximately 400 g/kg neutral detergent fiber (NDF) and is unique in its high concentration of neutral detergent soluble fiber, especially pectic substances [~250 g/kg of dry matter (DM)]. Previous studies indicate that the NDF in SBP can be digested more quickly than forage NDF (Bhatti and Firkins, 1995) and that pectin is degraded more rapidly than cellulose and hemicellulose (Marounek et al., 1985). Pectin fermentation produces less lactate and propionate than starch fermentation and does not inhibit cellulose and hemicellulose digestion, primarily because pectinolytic bacteria are also inhibited at low pH (Marounek et al., 1985). Although the effects of substituting SBP for starch sources have been investigated previously (Alamouti et al., 2009; Mahjoubi et al., 2009; Voelker and Allen, 2003a), the responses of animals (including DMI, chewing behavior and ruminal fermentation) to the substitution of SBP for barley grain have not been consistent (Bodas et al., 2007; Mandebvu and Galbraith, 1999). Thus, the objective of the present study was to evaluate the effects of substituting SBP for barley grain on the chewing behavior, ruminal fermentation characteristics, total tract nutrient digestibilities and blood biochemical parameters of Holstein steers given low-forage diets.

2. Materials and methods

2.1. Animals, diets, and experimental design

The research was carried out at the experimental farm of the Ferdowsi University of Mashhad, Mashhad, Iran. Four ruminally cannulated Holstein steers (initial BW = 368 ± 8 kg and age = 420 ± 12 days), were randomly assigned to treatments in a 4 × 4 Latin square design over 28-day periods. Each experimental period consisted of 21 days of acclimation followed by 7 days of data collection. Diets consisted of 200 g/kg corn silage, 150 g/kg alfalfa hay and 650 g/kg concentrate on a DM basis. Barley grain in the basal experimental diet (330 g/kg DM) was replaced with 0, 110, 220 and 330 g SBP on a DM basis to create the experimental diets SBP0, SBP110, SBP220 and SBP330, respectively (Table 1 and Table 2). Steers were fed 9.5 kg of dietary DM as a total mixed ration (TMR) twice daily at 0800 and 1600 h. The diets were formulated to supply approximately 2.3 times the maintenance requirements of the animals so that there was no refusal. The animals were housed in individual 12-m^2 concrete-floor pens, each of which had a separate feed bunk and watering point. Animals were cared for according to the Iranian Council of Animal Care guidelines.

Table 1
Chemical composition (g/kg of DM unless stated) and distribution of particle size fractions of barley grain and sugar beet pulp used in the experimental total mixed rations (N = 4).

	Barley grain	Sugar beet pulp
Dry matter (g/kg as is)	903	894
Crude protein	105	112
Neutral detergent fiber	208	350
Acid detergent fiber	72	231
Starch	60	590
Lignin	19	16
Ash	76	24
Ether extract	22	11
g/kg of particles retained on		
19 mm	0	0
8 mm	0	138
Pan	1000	862
pef[a]	0	0.14

[a] pef = physical effectiveness factor, calculated as the cumulative proportion of particles retained on two sieves (Lammers et al., 1996) of a Penn State Particle Separator.

2.2. Sampling and chemical analysis

2.2.1. Food and fecal analysis, and total tract nutrient digestibility

Samples of the TMR were obtained daily throughout the collection period. Feed samples were composited by period, weighed, oven dried at 60 °C for 48 h and ground in a Wiley mill (standard model 4; Arthur H. Thomas Co., Philadelphia, PA, USA) to pass a 1-mm screen. During each collection period, fecal grab samples were obtained from each steer at 1200 h and stored frozen at −20 °C. After thawing, samples were weighed, oven dried at 60 °C for 72 h and ground to pass

Table 2
Ingredients and chemical composition of the experimental diets.

Item	Diets[a]			
	SBP0	SBP110	SBP220	SBP330
Ingredients (g/kg DM)				
Alfalfa hay	200	200	200	200
Corn silage	150	150	150	150
Barley grain, rolled	330	220	110	–
Sugar beet pulp, dried molassed	–	110	220	330
Soybean meal	170	170	170	170
Wheat bran	138	138	138	138
Calcium carbonate	5	5	5	5
Salt	2	2	2	2
Mineral–vitamin premix[b]	5	5	5	5
Nutrient (g/kg DM unless stated otherwise)				
Crude protein	169	169	168	168
Neutral detergent fiber	325	336	350	369
Acid detergent fiber	180	197	215	232
Starch	305	247	188	130
ash	62	67	72	78
Non-fiber carbohydrates	439	424	410	395
Total digestible nutrients[c]	72	71	70	69
Net energy for growth (Mcal/kg DM)[c]	1.69	1.66	1.63	1.6
Net energy for maintenance (Mcal/kg DM)[c]	1.13	1.10	1.07	1.04

[a] Barley grain was replaced with SBP at rates of 0.0 g/kg (SBP0), 110 g/kg (SBP110), 220 g/kg (SBP220) or 330 g/kg (SBP330).
[b] Guaranteed analysis: 20 g/kg Mg, 50 g/kg K, 30 g/kg Zn, 20 g/kg Mn, 30 g/kg Fe, 3 g/kg Cu, 0.01 g/kg Se, 0.1 g/kg Co, 0.1 g/kg I, 500 IU/g of vitamin A, 100 IU/g of vitamin D, and 1 IU/g of vitamin E.
[c] Estimated using NRC (2001).

a 1-mm screen. The samples were then proportionately composited for each animal during each of the four collection periods. Ground feed and fecal samples were analyzed for nutrient components.

Crude protein (CP) was determined using an automated Kjeldahl system (Kjeltec Auto 1030 Analyzer Tecator, Sweden) with Na_2SO_4 and $CuSO_4$ as catalysts. The distillate was collected in a boric acid solution (AOAC, 2000; 984.13). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the Fibertec System (1010 Heat Extractor, Tecator, Sweden) using the procedure of Van Soest et al. (1991) and were corrected for ash. Sodium sulfite and heat-stable α-amylase (Sigma A3306; Sigma-Aldrich, Steinheim, Germany) were used during NDF analysis. Starch was determined following the method of Hall et al. (1999).

Acid insoluble ash (AIA) was used as an internal marker to estimate the apparent total tract digestibility of DM, CP, NDF and ADF (Van Keulen and Young, 1977). Apparent digestibility was calculated based on the relative concentrations of these nutrients and of AIA in the feed and feces.

2.2.2. Particle size distribution and chewing behavior

The particle size distribution of the diets as TMR was determined on as-fed samples using a Penn State Particle Separator (PSPS) with two sieves (Lammers et al., 1996). Particles retained on each fraction were oven dried at 60 °C for 48 h. The physical effectiveness factor (pef) was calculated as the cumulative proportions of feed DM retained on the sieves. The factor physical effective NDF (peNDF) was calculated as the ration NDF multiplied by the pef.

The eating and ruminating behaviors of individual animals were visually observed and recorded at 5-min intervals for 24 h on day 27 of each period. We assumed that a particular chewing episode persisted for the entire 5-min period between successive visual observations. Total chewing time was the sum of eating and ruminating time. Chewing behavior was expressed as the total minutes during a 24-h period or on the basis of each nutrient ingested (DM, NDF and peNDF) by dividing the minutes of eating, ruminating and chewing by intake.

2.2.3. Ruminal fermentation and blood parameters

Ruminal fluid was collected by suction through the rumen cannula from before the morning feeding (0.0 h) to 8 hours after feeding at 30-min intervals on days 24 to 25 of each period. The pH of each rumen fluid sample was measured immediately with a portable pH meter (Metrohm 744, Herisau, Switzerland). The rumen fluid was then strained through four layers of cheesecloth and prepared for subsequent ammonia-N and volatile fatty acids (VFA) analysis. For ammonia-N determination, 5-mL of rumen fluid from each collection point (except 4.5, 5.5, 6.5 and 7.5 h) was acidified with 5 ml of 0.2 N HCl and then analyzed for ammonia concentration using the distillation method (Kjeltec Auto Analyzer, Model 1030, Tecator Co., Sweden). For VFA analysis, 5 ml of rumen fluid (collected 4 h after the morning feeding) was mixed with 1 ml of 250 g/l meta-phosphoric acid. The concentrations of VFA were determined by gas chromatography (Chrompack, Model CP-9002, Chrompack, EA Middelburg, Netherlands) with a 50-m (0.32 mm ID) silica-fused column (CP-Wax Chrompack Capillary Column, Varian, Palo Alto, CA, USA). Helium was used as carrier gas and oven initial and final temperatures were 55 and 195 °C, respectively, and detector and injector temperatures were set at 250 °C. Crotonic acid (1:7 v/v) was used as internal standard.

On the last day of each experimental period, blood samples were collected in tubes containing sodium heparin by jugular venipuncture at 0.0 and 4 h after the morning feeding. Blood samples were centrifuged (3000 g for 15 min at 5 °C); plasma was harvested and frozen at −20 °C for later analysis. Concentrations of plasma glucose (Trinder, 1969) and plasma urea nitrogen (PUN) (Talke and Schubert, 1965) were determined using commercial kits according to the manufacturer's instructions (Zist-Shimi Co., Tehran, Iran). For blood pH and venous blood gas analyses, whole blood samples were taken at 4 h after the morning feeding using heparinized vacutainers. Samples were immediately analyzed using a Stat Profile pHOx Plus blood analyzer (Nova Biomedical, USA) for pH, CO_2 pressure (pCO_2), O_2 pressure (pO_2), oxygen saturation (O_2Ct), base excess of extracellular fluid (BEecf), base excess of blood (BEb) and bicarbonate (HCO_3^-).

2.3. Statistical analysis

Data were analyzed as a 4×4 Latin square design using the MIXED procedure of SAS (2001) according to the following model: $Y_{ijk}=\mu+T_i+P_j+S_k+e_{ijk}$, where Y_{ijk} is the dependent variable, μ is the overall mean, T_i is the fixed effect of treatment ($i=1$ to 4), P_j is the fixed effect of period ($j=1$ to 4), S_k is the random effect of steer ($k=1$ to 4) and e_{ijk} is the residual error. For ruminal pH and ammonia-N concentration, for which sampling was repeated over time, the effects of time and time×treatment were included in the REPEATED statement of the model. A compound symmetric covariance structure was selected based on the Akaike Information Criterion (AIC). The particle size distributions and peNDF contents of the experimental diets were analyzed using the GLM procedure as a completely randomized design with four replicates.

The significance of differences among treatments was tested using Duncan's multiple range tests, and the treatment effects were further divided into linear and quadratic effects using orthogonal polynomial contrasts. In this study, the effects of the factors were declared significant at $P<0.05$, and trends were recognized at $P<0.10$.

3. Results and discussion

3.1. Particle size distribution and chewing behavior

Replacement of barley grain with SBP resulted in a linear increase ($P=0.01$) in the proportion of DM particles retained on the 8-mm sieve (Table 3). In addition, the proportion of DM particles smaller than the 8-mm sieve decreased linearly ($P=0.01$) with the addition of SBP. Inclusion of SBP, especially at higher inclusion rates, caused a linear increase ($P<0.05$) in pef and peNDF contents.

Just as dietary peNDF increased with added SBP, eating time per day, per kg of dry matter intake (DMI), per kg of NDF intake and per kg of peNDF intake increased linearly ($P<0.01$) at all levels of SBP inclusion but were similar for SBP110 and SBP220. Similarly, Voelker and Allen (2003a) reported that eating time per day and per kg of DMI increased linearly when pelleted SBP replaced high-moisture corn in the diet of lactating dairy cows, but eating time per kg of NDF decreased with the inclusion of

Table 3
Particle size distribution, physical effective NDF content of total mixed rations and chewing behavior of steers fed diets containing molassed dried sugar beet pulp and/or barley.

Item	Diets[e]					*P*-value[f]		
	SBP0	SBP110	SBP220	SBP330	SEM	Trt	L	Q
g/kg of particles retained on								
19 mm	70	55	66	65	5.70	0.42	0.29	0.91
8 mm	165[b]	200[b]	227[ab]	257[a]	16.59	0.06	0.01	0.86
Pan	765[a]	745[a]	707[b]	678[b]	15.16	0.05	0.01	0.80
pef[g]	0.23[b]	0.25[b]	0.29[ab]	0.32[a]	0.015	0.05	0.01	0.81
peNDF[h]	76[c]	86[bc]	103[ab]	119[a]	5.21	0.01	0.015	0.58
Eating time (min)								
per day	111.2[c]	123.7[b]	128.8[b]	143.7[a]	3.15	<0.01	<0.01	0.70
per kg of DMI	11.7[c]	13.0[b]	13.5[b]	15.1[a]	0.33	<0.01	<0.01	0.71
per kg of NDF intake	33.7[c]	37.4[b]	39.1[b]	43.6[a]	0.93	<0.01	<0.01	0.69
per kg of peNDF intake	123.0[c]	136.7[b]	143.6[b]	160.4[a]	3.35	<0.01	<0.01	0.66
Ruminating time (min)								
per day	232.5[b]	267.5[b]	365.0[a]	391.2[a]	14.5	<0.01	<0.01	0.77
per kg of DMI	24.5[b]	28.1[b]	38.4[a]	41.2[a]	1.52	<0.01	<0.01	0.77
per kg of NDF intake	71.6[b]	82.3[b]	112.1[a]	120.1[a]	4.07	<0.01	<0.01	0.74
per kg of peNDF intake	271.4[b]	312.2[b]	423.2[a]	452.6[a]	12.11	<0.01	<0.01	0.65
Total chewing time (min)								
per day	343.7[b]	391.2[b]	493.7[a]	535.0[a]	14.24	<0.01	<0.01	0.83
per kg of DMI	36.2[b]	41.2[b]	51.9[a]	56.3[a]	1.5	<0.01	<0.01	0.82
per kg of NDF intake	105.2[c]	119.8[b]	151.2[a]	163.7[a]	4	<0.01	<0.01	0.81
per kg of peNDF intake	394.5[d]	449.0[c]	566.9[b]	613.0[a]	11.9	<0.01	<0.01	0.74

[a, b, c, d] Means with different superscript letters within rows are significantly different.
[e] Barley was replaced with SBP at rates of 0.0 g/kg (SBP0), 110 g/kg (SBP110), 220 g/kg (SBP220) or 330 g/kg (SBP330).
[f] Trt: treatment effect, L: linear effect, Q: quadratic effect.
[g] pef = physical effectiveness factor, calculated as the cumulative proportion of particles retained on two sieves (Lammers et al., 1996) of a Penn State Particle Separator.
[h] peNDF = physical effective NDF (g/kg DM), calculated as ration NDF multiplied by pef.

SBP in the diet, in contrast to our results. Alamouti et al. (2009) reported that eating time per kg of NDF and peNDF intake was greater for high *vs* low neutral detergent soluble fiber from pelleted SBP substituted for barley and corn in the diet of lactating dairy cows. However, Clark and Armentano (1997) reported no effect of SBP substitution for corn on eating time per day or per kg of DMI. In this study, longer eating time in steers fed SBP containing diets could be attributed to lower density of SBP *vs.* barley grain (data not shown), that might decrease the eating rate.

Like eating time, ruminating and total chewing (eating + ruminating) time increased linearly according to all evaluating indices ($P<0.01$) with the inclusion of SBP in the diet. However, no difference was found between SBP0 and SBP110, probably because peNDF did not differ significantly between these treatments. Voelker and Allen (2003a) found that the inclusion of SBP resulted in a linear increase in ruminating and total chewing time per kg of DMI ($P=0.09$ and $P=0.01$, respectively), but ruminating and total chewing time per kg of NDF intake decreased linearly ($P=0.05$, $P=0.07$, respectively). Our results are consistent with those of Beauchemin et al. (1991), who reported that ruminating time per unit of NDF intake increased when non-forage fiber sources were incorporated into low-forage diets. Grant (1997) also suggested that cows might possess an adaptive mechanism whereby they ruminate more effectively under conditions of limited effective forage NDF. On the other hand, our results are not consistent with those of a previous study that reported no effect of SBP on ruminating and total chewing time (Alamouti et al., 2009). Chewing time generally increases with increasing dietary peNDF content. Because other factors (such as the animal, level of intake and type of feed) also affect chewing behavior, dietary peNDF may have variable effects on chewing among different cows and studies. Yang and Beauchemin (2006b) reported that chewing behavior (min/d) was correlated with particles remaining on the 8-mm sieve ($r=0.52$) and peNDF ($r=0.52$). The positive correlation of dietary peNDF and chewing time is consistent with increased chewing time and increasing dietary peNDF levels in the present study (Table 3).

In our experiment, the increase in total chewing time due to increased dietary peNDF resulted from both eating and ruminating time, consistent with previous reports (Beauchemin and Yang, 2005; Kononoff and Heinrichs, 2003a). However, this pattern does not confirm the findings of Kononoff and Heinrichs (2003b) and Yang and Beauchemin (2006a) that increased chewing time resulted from increased ruminating time rather than eating time. Kononoff and Heinrichs (2003a) reported that particles larger than 19 mm primarily stimulate chewing, whereas other reports (Alamouti et al., 2009; Yang and Beauchemin, 2006b) found a stronger correlation between chewing behavior and the proportion of particles larger than 8 mm. Apparently, the relative contribution of each particle fraction to the pef may determine which fraction has a prominent role in promoting chewing behavior (Yang and Beauchemin, 2006b). In our study, the proportion of particles larger than 8 mm increased linearly ($P=0.01$), but no differences were found in the proportion of particles larger than 19 mm. This result, combined with the increase in chewing time with the addition of SBP, indicates that particles larger than 8 mm primarily affected chewing behavior in our study.

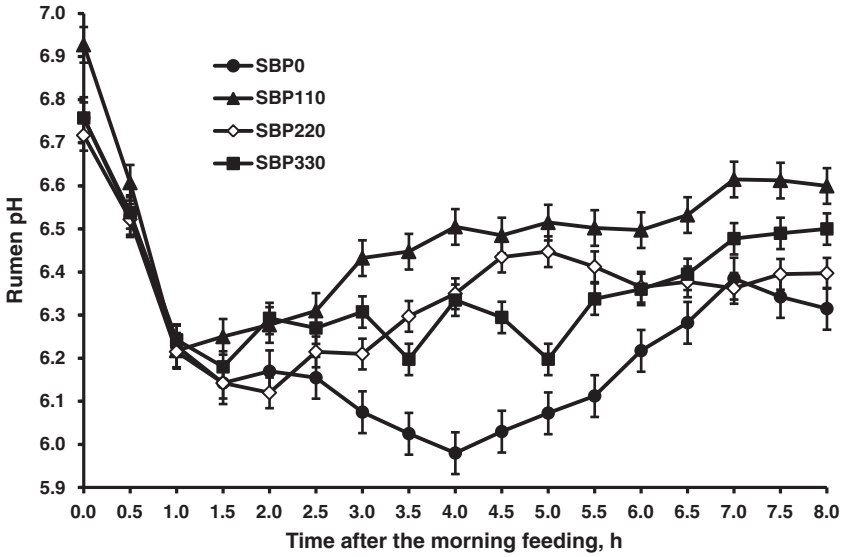

Fig. 1. Ruminal pH of Holstein steers fed low-forage diets in which dried molassed sugar beet pulp was substituted for barley at rates of 0.0 g/kg (SBP0), 110 g/kg (SBP110), 220 g/kg (SBP220) or 330 g/kg (SBP330). (Treatment effect: P<0.01; Time effect: P<0.01; Treatment×Time effect: P=0.975).

3.2. Ruminal fermentation and pH

Ruminal pH over the 8 hours after feeding is shown in Fig. 1. Ruminal pH was significantly influenced by the treatment and sampling time (P<0.01). Inclusion of SBP in the diet resulted in linear (P<0.05) and quadratic (P<0.01) increases in mean ruminal pH. The lowest and the highest mean ruminal pH values were observed in steers fed SBP0 and SBP110, respectively (6.22 *vs.* 6.49), but mean ruminal pH was similar for SBP220 and SBP330 (Table 4). Other pH indices, including minimum and maximum pH, pH range and variance were not significantly affected by dietary SBP content. For all treatments, rumen pH decreased after the morning feeding and then increased. The ruminal pH of steers whose diets included SBP reached its minimum values about 1.5–2 h after the morning feeding, but the ruminal pH of steers fed the basal diet (SBP0) decreased markedly from 2 to 4 h after feeding and reached its minimum values around 4 h after feeding. Previous experiments have shown a variety of responses in ruminal pH to the substitution of SBP for grains. Bodas et al. (2007) reported that partial replacement of barley grain with SBP (12% DM) in the basal concentrate markedly (P<0.01) increased ruminal pH (5.5 *vs.* 6.7) and prevented ruminal acidosis in growing lambs. In another study (Mahjoubi et al., 2009), ruminal pH increased linearly with the substitution of SBP for barley grain in the diet of late lactation cows. In contrast, ruminal pH was not affected in

Table 4
Effects of inclusion of dried molassed sugar beet pulp instead of barley in low-forage diets on ruminal pH and ammonia-N concentration.

Item	Diets[d]					*P*-value[e]		
	SBP0	SBP110	SBP220	SBP330	SEM	Trt	L	Q
Ruminal pH								
Mean	6.22[c]	6.49[a]	6.35[b]	6.36[b]	0.10	<0.01	0.03	<0.01
Minimum	5.91	6.13	5.99	6.00	0.14	0.75	0.83	0.47
Maximum	6.76	6.93	6.76	6.75	0.09	0.52	0.66	0.39
Range	0.85	0.80	0.76	0.76	0.10	0.88	0.47	0.82
Variance	0.053	0.048	0.043	0.041	0.011	0.86	0.43	0.88
Ammonia-N (mg/dl)								
Mean	31.69[a]	26.74[b]	26.42[b]	25.94[b]	0.73	<0.01	<0.01	<0.01
Minimum	22.78[a]	18.41[ab]	16.89[b]	16.78[b]	1.32	0.05	0.03	0.15
Maximum	40.94	35.14	34.78	34.77	1.80	0.12	0.057	0.16
Range	18.16	18.25	18.00	16.38	2.05	0.90	0.80	0.72
Variance	42.92	43.27	43.45	41.71	6.80	0.99	0.91	0.88
Volatile fatty acids (4 h post-feeding)								
Total VFA (mM)	129.7[a]	108.2[b]	114.6[b]	111.7[b]	3.96	0.02	0.03	0.04
Acetate (mol/100 mol)	59.3[b]	61.7[a]	61.9[a]	62.4[a]	0.83	0.03	0.01	0.16
Propionate (mol/100 mol)	26.4[a]	24.1[b]	23.3[b]	21.8[c]	0.62	<0.01	<0.01	0.31
Butyrate (mol/100 mol)	9.8[b]	10.4[b]	10.9[ab]	11.8[a]	0.34	0.02	<0.01	0.67
Acetate/propionate	2.31[b]	2.64[a]	2.73[a]	2.90[a]	0.14	<0.01	<0.01	0.34

[a, b, c] Means with different superscript letters within rows are significantly different.
[d] Barley was replaced with SBP at rates of 0.0 g/kg (SBP0), 110 g/kg (SBP110), 220 g/kg (SBP220) or 330 g/kg (SBP330).
[e] Trt: treatment effect, L: linear effect, Q: quadratic effect.

some studies when SBP replaced barley (Mandebvu and Galbraith, 1999) or corn (O'Mara et al., 1997; Voelker and Allen, 2003c).

Increasing ruminal pH with the inclusion of SBP in the diet can be attributed to greater pectin *vs* starch fermentation and higher ratio of acetate to propionate production in the rumen (Table 4). Marounek et al. (1985) reported that compared to starch fermenters, pectin fermenters produce very little lactate and a higher ratio of acetate to propionate and are inhibited at low pH. Furthermore, increasing ruminal pH with the inclusion of SBP in the diet can be attributed to increases in peNDF and chewing time (Yang and Beauchemin, 2006b) because salivary secretion increases when dairy cows chew during eating and ruminating time.

In other hands, the quadratic increase in ruminal pH with added dietary peNDF (from SBP) in the present study is consistent with previous reports (Mertens, 1997; Zebeli et al., 2006). This might be because the correlation between ruminal pH and chewing activity in the literature is usually poor (Krause et al., 2002), especially for diets containing highly fermentable ingredients such as barley grain (Beauchemin and Yang, 2005; Yang and Beauchemin, 2006a). The relationship between peNDF and rumen pH is inconsistent because the concept of peNDF does not account for differences in ruminal fermentability of feeds, which can have a major impact on ruminal pH (Krause et al., 2002).

Ruminal ammonia concentrations were affected by the treatment and sampling time ($P<0.01$) (Fig. 2). Substitution of SBP for barley caused linear ($P<0.01$) and quadratic ($P<0.01$) decreases in mean ruminal ammonia concentration. The SBP0 treatment produced the highest mean ammonia concentration; however, no significant difference was found among SBP110, SBP220 and SBP330 (Table 4). Moreover, inclusion of SBP in the diet resulted in a linear decrease in minimum and maximum ammonia concentrations ($P=0.03$, $P=0.057$, respectively), but the range and variance of ammonia concentration were similar among treatments. Fluctuations in ruminal ammonia concentration after the morning feeding followed a similar pattern for all treatments (Fig. 2). Ruminal ammonia concentration increased during the first two hours after the morning feeding and then decreased. The highest ruminal ammonia concentration was observed in steers fed SBP0 from 3.5 to 4 h after the morning feeding (Fig. 2). In agreement with our findings, Mandebvu and Galbraith (1999) reported that replacement of barley with SBP resulted in a linear decrease in ruminal ammonia concentration. Our results also confirm those of Voelker and Allen (2003c), who reported that ruminal ammonia concentration was affected quadratically by inclusion of SBP in the diet; the maximum and minimum ammonia concentrations were observed in treatments containing 12% SBP and 24% SBP, respectively (21.4 *vs.* 17.8 mg/dl). However, various other reports have indicated no effect of SBP on ruminal ammonia concentration (Alamouti et al., 2009; Bodas et al., 2007; Mahjoubi et al., 2009). Ruminal ammonia concentration at any given time is a combined result of ammonia production, ammonia absorption and microbial ammonia uptake and utilization. The decrease in ruminal ammonia concentration with dietary SBP content in the present study may be due to either slower release of ammonia from the diet or more rapid uptake by microorganisms in the presence of a rapidly fermentable source of carbohydrates (*i.e.*, molasses). In addition, amylolytic microorganisms are more dependent on amino acids and peptides (Russell et al., 1983), while fibrolytic bacteria are capable of using only ammonia-N (Bryant, 1973). Therefore, substituting high-fiber SBP for high-starch barley might allow more extensive utilization of ammonia-N and thus decrease ruminal ammonia concentration.

As expected, substituting SBP for barley grain linearly increased the molar proportion of acetate ($P=0.01$) and butyrate ($P<0.01$) in total VFA, and linearly decreased the total VFA ($P=0.03$) and molar proportion of propionate ($P<0.01$). Therefore the ratio of acetate to propionate

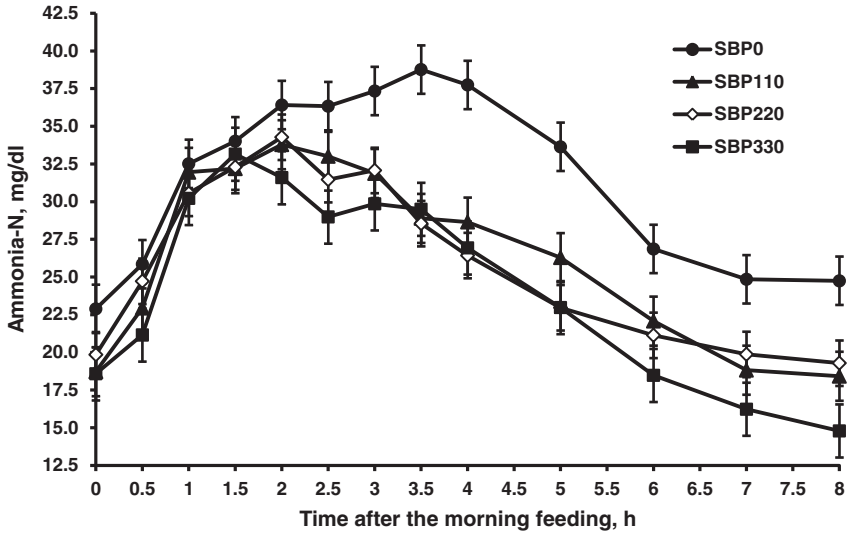

Fig. 2. Ruminal ammonia-N concentrations of Holstein steers fed low-forage diets in which dried molassed sugar beet pulp was substituted for barley at rates of 0.0 g/kg (SBP0), 110 g/kg (SBP110), 220 g/kg (SBP220) or 330 g/kg (SBP330). (Treatment effect: $P<0.01$; Time effect: $P<0.01$; Treatment × Time effect: $P=0.018$).

increased linearly ($P<0.01$) with inclusion of SBP in the diet. Fermentation results in our study were consistent with other *in vivo* experiments, such as Voelker and Allen (2003c) and Mahjoubi et al. (2009), in that by adding a lipogenic at the expense of a glucogenic feed, the levels of acetic acid increased and propionic acid decreased. In addition Marounek et al. (1985) found that the main VFA produced from soluble fiber fermentation is acetic acid. However some studies reported no effect on acetate (Mandebvu and Galbraith, 1999) or butyrate concentrations (Bodas et al., 2007) with SBP replacing barley grain. In other hands decreasing total VFA concentration in the rumen of SBP-fed steers could be partly due to dilution effect as result of higher water intake (data not shown) (because high water-holding capacity of SBP). However, the differences in ruminal VFA concentrations between treatments could be also related to changes in fermentation pattern. The mechanism by which SBP increased butyrate proportion in ruminal fluid is not well understood. However, it indicates a stimulation of butyrate-producing microbiota in the rumen by SBP containing diets (Brossard et al., 2004).

3.3. Total tract nutrient digestibility

Data on the apparent digestibility of DM, CP, NDF and ADF through the total tract are shown in Table 5. Inclusion of SBP at all tested rates resulted in linear ($P<0.01$) and quadratic ($P<0.01$) increases in the digestibility of DM. In addition, the total tract apparent digestibility of NDF increased ($P<0.01$) in a quadratic manner with the inclusion of SBP, but the digestibilities of CP and ADF were similar among treatments. Our results are consistent with those of Alamouti et al. (2009), who reported that cows fed higher proportions of SBP exhibited higher apparent digestibility of DM and NDF. Voelker and Allen (2003b) observed greater DM and NDF digestion when pelleted SBP replaced high-moisture corn in the diet of lactating dairy cows. In contrast, O'Mara et al. (1997) reported that SBP had no effect on the total tract DM digestibility of lactating dairy cows when it was substituted for corn.

Higher ruminal pH is correlated ($r=0.41$, $P<0.05$) with more rapid digestion of potentially digestible NDF (Voelker and Allen, 2003b). In the present experiment, ruminal pH was improved at all levels of SBP inclusion (Fig. 1); therefore, the increase in NDF digestibility might be due to the higher ruminal pH. On the other hand, the quadratic effect on NDF digestibility could be attributed to higher NDF content of SBP-containing diets (325 *vs.* 369 g/kg DM for SBP0 and SBP330, respectively) that might resulted in reduction in the rate of NDF digestibility, whereas the total extent of NDF digestibility have increased. In addition, the fiber in SBP has a shorter lag time and more rapid digestion rate than most other sources of fiber (Bhatti and Firkins, 1995). Therefore, increasing the contribution of SBP fiber to total NDF might increase the overall rate of NDF digestion.

3.4. Blood biochemical parameters

Blood biochemical parameters and venous blood gasses of steers fed the experimental diets are given in Table 6. Plasma glucose concentration was not affected by the treatments ($P>0.05$). A lack of effect of increasing SBP on plasma glucose concentration has also been reported in growing lambs (Mandebvu and Galbraith, 1999) fed a barley based diet. In contrast, plasma glucose concentration decreased in lactating dairy cows when SBP content was increased at the expense of barley (Mahjoubi et al., 2009) or corn (Voelker and Allen, 2003a). This result may be attributed to lower production of propionate in the rumen (Mahjoubi et al., 2009); propionate is the main precursor of glucose in ruminants. On the other hand, variation in animal responses might be explained by different energy balances; Van Knegsel et al. (2005) reported that decreases in plasma glucose and insulin levels were often associated with a negative energy balance.

Plasma urea nitrogen before the morning feeding decreased linearly ($P<0.01$) with SBP inclusion and was highest for SBP0 and lowest for SBP330 (21 *vs.* 16.25 mg/dl). However, there was no treatment effect on PUN at four hours after feeding. These results are partly consistent with those of Mandebvu and Galbraith (1999), who reported that the substitution of SBP for barley linearly reduced PUN concentrations in growing male lambs. This pattern may be partially due to the observed reductions in provision of ruminal ammonia for subsequent hepatic conversion to urea (Mandebvu and Galbraith, 1999). Despite the differences observed in ruminal fermentation parameters, in this study, blood pH and venous blood gasses were not affected by treatments. Mean values of pH, pCO_2, pO_2, O_2Ct, BEecf, BEb and HCO_3^- were within the ranges found for appropriate physiological functions of the blood (Brown et al., 2000). Blood gas analysis is a valuable tool to diagnose acidosis in

Table 5
Effects of inclusion of dried molassed sugar beet pulp instead of barley in low-forage diets on total tract apparent digestibility of nutrients.

Item	Diets[d]					*P*-value[e]		
	SBP0	SBP110	SBP220	SBP330	SEM	Trt	L	Q
Nutrient digestibility (g/kg)								
Dry matter	696.7[c]	717.0[ab]	722.5[a]	709.2[b]	2.5	<0.01	<0.01	<0.01
Crude protein	730.0	740.2	723.0	726.5	11.5	0.75	0.61	0.78
Neutral detergent fiber	611.2[b]	634.2[a]	636.7[a]	620.2[ab]	4.6	0.02	0.20	<0.01
Acid detergent fiber	536.5	529.7	540.7	551.7	10.6	0.52	0.25	0.41

[a, b, c] Means with different superscript letters within rows are significantly different.
[d] Barley was replaced with SBP at rates of 0.0 g/kg (SBP0), 110 g/kg (SBP110), 220 g/kg (SBP220) or 330 g/kg (SBP330).
[e] Trt: treatment effect, L: linear effect, Q: quadratic effect.

Table 6
Effect of inclusion of dried molassed sugar beet pulp instead of barley in low-forage diets on blood biochemical parameters and venous blood gasses of Holstein steers.

Item	Diets[c]					*P*-value[d]		
	SBP0	SBP110	SBP220	SBP330	SEM	Trt	L	Q
Glucose (mg/dl)								
Before feeding	97.5	97.0	96.5	91.5	5.73	0.86	0.50	0.71
4 h post-feeding	93.75	90.75	95.00	95.25	6.05	0.94	0.75	0.80
Plasma urea nitrogen (mg/dl)								
Before feeding	21.00[a]	19.75[ab]	17.5[ab]	16.25[b]	1.25	0.053	0.01	1.00
4 h post-feeding	21.75	19.25	21.5	20.25	1.63	0.69	0.76	0.71
pH	7.334	7.338	7.337	7.333	0.01	0.98	0.97	0.73
pCO_2 (mmHg)	46.97	47.05	47.12	48.00	1.36	0.94	0.62	0.77
pO_2 (mmHg)	29.10	29.22	28.87	29.15	1.50	0.99	0.98	0.96
O_2Ct (ml/dl)	6.70	6.50	6.57	6.35	0.51	0.96	0.69	0.98
BEecf (mmol/L)	0.87	0.55	0.52	0.30	0.31	0.64	0.25	0.87
BEb (mmol/L)	0.35	0.15	0.15	0.40	0.29	0.89	0.91	0.48
HCO_3^- (mmol/L)	25.17	25.45	25.47	25.80	0.28	0.53	0.18	0.93

[a, b] Means with different superscript letters within rows are significantly different.
[c] Barley was replaced with SBP at rates of 0.0 g/kg (SBP0), 110 g/kg (SBP110), 220 g/kg (SBP220) or 330 g/kg (SBP330).
[d] Trt: treatment effect, L: linear effect, Q: quadratic effect.
pCO_2: Partial pressure of carbon dioxide; pO_2: Partial pressure of oxygen; O_2Ct: Oxygen content; BEecf: Base excess of extracellular fluid; BEb: Base excess of blood; HCO_3^-: Bicarbonate ions.

ruminants, especially in dairy cows because it provides good assessment of acidosis while being less invasive than rumen pH analysis. Moreover, blood gas analysis can help us to differentiate respiratory acidosis from metabolic acidosis, especially in a subacute form such as subacute ruminal acidosis (SARA). During SARA, blood pH decreases are an indicator of physiological problems. In other hand, Brown et al. (2000) found that changes in blood acid/base status are small during SARA. The lack of treatment effect on blood gas and biochemical parameters in our study is consistent with the report by Bodas et al. (2007) in which there was no change in blood parameters when barley (12% of concentrate) was replaced with SBP for fattening lamb. The absence of effects on blood parameters in this study could be due to either not enough concentrate in the ration, or short duration of the experimental period, probably insufficient to exhibit ruminal and metabolic acidosis.

4. Conclusions

The results of the present study suggest that SBP might be a source of physically effective fiber that promotes increased chewing time when it is substituted for barley in low-forage diets. Inclusion of SBP, especially at lower inclusion rates, had a significant effect on rumen synchronizing, as observed in the increased rumen pH and decreased ammonia-N concentration. In addition, inclusion of SBP in the experimental diets improved the apparent digestibility of DM and NDF. Overall, we conclude that the substitution of SBP for highly fermentable grain, such as barley, may reduce the risk of rumen abnormality in ruminants fed low-forage diets.

Acknowledgements

The financial support of Ferdowsi University of Mashhad is kindly acknowledged. Special thanks are offered to Mr. Hashemi-Attar for his useful laboratory assistance in this experiment.

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