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ORIGINAL ARTICLE

Effect of particle size and partial replacement of alfalfa hay by soya bean hulls on nutrient intake, total tract digestibility and rumen degradability of diets by Holstein steers

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Summary

This study examined the effect of alfalfa hay (AH) particle size and the replacement of soya bean hull (SH) for AH within the diet of restricted fed Holstein steers on dry matter intake (DMI), total tract digestion, ruminal digestion, ruminal pH and ammonia nitrogen content, and faecal pH. Four rumen-cannulated Holstein steers averaging 353 ± 9.6 kg of BW were assigned to a 4 × 4 Latin square experiment with four periods and a 2 × 2 factorial arrangement of treatments. Factor A was AH particle size (fine vs. coarse) and factor B was diet SH content (0% vs. 10%; substituted for AH). Steers were fed at 1% of body weight of TMR containing 400 g/kg forage and 600 g/kg concentrate. Chopping of AH to fine particles decreased ($p = 0.01$) amount of dietary materials retained on the medium sieve (8 mm). The inclusion of SH significantly increased ($p = 0.01$) materials retained on the 1.18-mm sieve and tended to decrease ($p = 0.07$) materials on 19-mm sieves. The inclusion of SH increased ($p = 0.01$) ether extract (EE) intake and increased ($p = 0.07$) DMI. Inclusion of SH increased ($p = 0.01$) EE digestion and decreased ($p < 0.01$) faecal pH. Neither AH particle size nor SH inclusion in diets affected ($p > 0.10$) the *in situ* ruminal degradability coefficients of DM (‘*a*’, ‘*b*’, ‘*c*’ or ‘*a*+*b*’). No interaction of AH×SH was seen on nutrient intake, digestibility and *in situ* ruminal degradability of Holstein steers.

Keywords Holstein steer, soya bean hull, particle size, degradability, faecal pH

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Introduction

Soya bean hulls (SH) have been used as a replacement for forages in diet of dairy cows and beef cattle because of their high energy and fibre content. However, in contrast to forage fibre, the SH fibre is highly digestible making it a poor source of effective fibre (Behgar et al., 2011). Hsu et al. (1987) showed that the digestion of SH was higher *in situ* than *in vivo,* which was attributed to the short retention time of soya bean hulls in the rumen. Similarly, Nakamura and Owen (1989) observed that the passage rate of ytterbium-labelled SH was two times greater than labelled alfalfa hay (AH). Rapid ruminal passage rate of SH has been mainly related to their small particle size and high specific gravity in the rumen (Titgemeyer, 2000). Increased dry matter intake (DMI) has been also suggested to have a role in the high passage rate of SH from rumen (Mulligan et al., 2001); however, increasing the level of DMI of steers did not completely explain high passage rate of SH and its effects on ruminal fibre digestibility (Woods et al., 1999).

It has been well established that replacement of a portion of hay of diet with SH decreased the mean particle size of diet (Weidner and Grant, 1994b; Slater et al., 2000) and also resulted in reduced rumination time (Weidner and Grant, 1994b) and ruminal mat consistency (Weidner and Grant, 1994a).

Addition of long hay to diets containing SH has been shown to interact with SH in the rumen, resulting in decreased passage rate of SH, increased rumination time and increased extent of ruminal digestion (Weidner and Grant, 1994a,b). Behgar et al. (2011) found that increasing diet forage particle size can prevent milk fat depression in dairy cows fed SH-based diets. However, in this study, faecal pH decreased in cows fed coarse diets containing SH compared to those fed fine diets, suggesting that larger particle size could shift the digestion of SH from rumen to the lower gut.

Although a large number of studies have studied SH as a replacement for forage or grain in cattle diets, there is very limited information on the effect of particle size of diets on the utilization of SH by cattle. Therefore, the objective of the present study was to evaluate the impact of AH particle size in SH-based diets on total tract digestion, *in situ* degradation of diets and rumen condition in Holstein steers fed at restricted level of intake.

Material and methods

Steers, diets and treatments

Four rumen-cannulated Holstein steers with the initial average body weight of 353 ± 9.6 kg were housed in individual stalls and were maintained according to the ethics committee for the University of Ferdowsi Mashhad. Steers were fed a TMR daily at 0800 restricted at 1% of body weight. The experimental design was a 4 × 4 Latin square with four periods and a 2 × 2 factorial arrangement of treatments. Factor A was AH particle size (fine vs. coarse) and factor B was diet SH content (0% vs. 10%; substituted for AH). The diet was formulated according to NRC (1996) recommendations (Table 1). The basal diet contained 60% barley-based concentrate and 40% forage (alfalfa hay–corn silage). Differences in average particle size of the diets were created by changing of AH cutting length and SH substitution with AH. AH was chopped by using a small chopper (Moharek Machine, Iran) fitted with 5-mm and 20-mm sieves to obtain hay with fine and coarse particle size respectively. Each experimental period consisted of 14 days for adaptation and 5 days for data collection including measurement of feed and nutrient intake, nutrient digestibility, rumen pH, rumen $N\text{-}NH_3$, faecal pH and rumen degradability.

Feed and nutrient intake

Feed intake was recorded daily during the last 5 days of each period. Feeds and orts were sampled daily, immediately stored at −20 °C. The samples were pooled on a weekly basis, dried at 55 °C for 48 h (UN 30, Memmert, Germany) and then ground through 2-mm screen (Retsch Cutting Mill, Retschmule, Germany) for subsequent analyses. The samples were analysed in duplicate for crude protein (CP; Kjeltec Auto 1300, Foss, Denmark), ether extract (EE; Soxtec System HT 1043, Tecator, Foss) and organic matter (OM; AOAC, 1998). Neutral detergent fibre (NDF) was determined according to Van Soest et al. (1991) without amylase addition.

Table 1 Ingredients and chemical composition of the total mixed diets (DM basis)

Items	Diets AH Fine	Diets AH Coarse	Diets AH+SH Fine	Diets AH+SH Coarse
Ingredients, %				
Alfalfa hay	20.0	20.0	10.0	10.0
Soya bean hull	–	–	10.0	10.0
Corn silage	20.0	20.0	20.0	20.0
Barley grain	30.0	30.0	30.0	30.0
Soya bean meal	4.00	4.00	2.50	2.50
Wheat bran	25.1	25.1	26.5	26.5
Calcium carbonate	0.50	0.50	0.50	0.50
Salt	0.29	0.20	0.20	0.20
Vitamin and mineral premix	0.30	0.30	0.30	0.30
Chemical, % of DM				
Dry matter	55.8	56.6	57.9	57.8
Organic matter	92.7	91.5	92.6	92.9
Crude protein	12.9	12.9	13.1	13.1
Neutral detergent fibre	37.3	37.3	38.2	38.2
Ether extract	2.70	2.70	3.20	3.20

AH, alfalfa hay; SH, soya bean meal.

Particle size distribution

The Penn State Particle Separator (PSPS) was used to measure particle size distribution of individual diet ingredients (i.e. SH, AH and corn silage) and total mixed rations as described by Kononoff and Heinrichs (2003). Percentage of DM retained on each sieve, geometric mean (Xgm) and standard deviation was calculated as outlined by the American Society of Association Executives (2001).

Rumen sampling and total tract digestibility

Samples of rumen fluid (250 ml) were obtained pre- and post-feeding (1-h intervals up to 7 h post-feeding) via rumen cannula. The samples were squeezed through layers of cheesecloth for pH determination (Metrohm 691, Switzerland) and stored at −20 °C for subsequent ammonia nitrogen ($N\text{-}NH_3$) analysis (Preston, 1986). Faecal samples (250 g; grab samples) were collected on day 15 and day 17 before feeding and dried at 55 °C. Dried faecal samples composited by steer and period and were analysed in duplicate for DM, OM, CP, EE and NDF using the same methods to feed samples. Apparent total digestibility of nutrients was estimated by the marker ratio technique using acid insoluble ash (AIA) as an internal marker (Van Keulen and Young, 1977).

Faecal pH

The pH of faecal grab samples was determined on days 15 and 17 of each period before morning feeding. Fifty grams of fresh faecal samples was mixed with 50 ml of deionized water, and pH was recorded immediately (Slater et al., 2000).

Nylon bag procedure

Nylon bags (18.5 × 9 cm^2 with a pore size of 48 μm) containing 5 g ground (2 mm) of feed samples (3 bags/steer) were incubated in the rumen of rumen-cannulated steers for 2, 4, 8, 16, 24, 48, 72 and 96 h. Steers were offered the same diet as were in the bags in each period of experiment. Bags were inserted into rumen in reverse order relative to incubation time and removed simultaneously. The bags were then washed under tap water until the rinse water was clear. Zero time disappearance was obtained by washing unincubated bags under tap water. The percentage of DM disappearance at each time was calculated from the proportion remaining feed in the bag after incubation time relative to original feed weight (DM basis).

Statistical analyses

Data were analysed as 4 × 4 Latin square. The statistical model was $Y_{ij(kl)} = \mu + Raw_i + Col_j + A_{(K)} + B_{(l)} + (AB)_{(kl)} + \varepsilon_{ij(kl)}$; where $Y_{ij(kl)}$ = observation $_{ij(kl)}$, μ= overall mean, Raw_i = effect of period I, Col_j = effect of steer *j*, $A_{(k)}$ = effect of level *k* of particle size, $B_{(l)}$ = effect of level l of SH, $(AB)_{kl}$ = effect of interaction of level *k* of particle size with level l of SH and $\varepsilon_{ij(kl)}$ = random error with mean 0 and variance σ^2. Data were analysed using the GLM procedure of SAS (V 9.0).

Dry matter disappearance was fitted using the NLIN option of SAS (SAS, 2002) using the following model: P = a+b(1-ect): where P = potential disappearance at time t, '*a*' = the fraction of soluble material, '*b*' = the fraction that is potentially degradable in the rumen and '*c*' = constant rate of degradation of fraction '*b*' (/h). Effective degradability (ED) was calculated according to the equation by Ørskov and McDonald (1979): ED = a + (b × c)/(c + 0.05), assuming an outflow rate (c) of 0.05/h (AFRC, 1993). Duncan's multiple range tests were used to determine the differences among treatments. Differences were declared significant at $p \leq 0.05$ and tendencies declared at $0.05 > p \leq 0.10$.

Results and discussion

Particle size distributions of diets

Particle size distributions of diets are shown in Table 2. Chopping of AH to fine particles decreased ($p = 0.01$) dietary DM retained on the medium sieve (8 mm). The inclusion of SH increased ($p = 0.01$) the amount of material (DM basis) retained on 1.18-mm sieve and tended to decrease ($p = 0.07$) the amount of material on 19-mm sieve.

Intake and nutrient digestibility

Intake of nutrients is shown in Table 3. Despite of restricted feeding, orts were present and steers fed SH tended ($p = 0.07$) to increase their DM intake compared to those fed on AH. This finding is in agreement with Trater et al. (2001) who observed an increase in DM intake with increase in dietary SH levels in Holstein steers fed at 1.75% of BW. In contrast, Loest et al. (1999) reported no change

Table 2 Particle size distribution of dietary treatments

Items	Diets				SE	p Value		
	AH		AH+SH					
	Fine	Coarse	Fine	Coarse		PS	SH	PS×SH
19 mm	7.85	6.97	5.35	5.22	0.85	0.58	0.07	0.68
8 mm	17.0	18.2	15.3	19.1	0.59	0.01	0.58	0.09
1.18 mm	39.3	39.1	43.1	41.4	0.69	0.23	0.01	0.34
Pan	35.9	35.8	36.2	34.3	0.67	0.19	0.44	0.23

AH, alfalfa hay; SH, soya bean meal.

in DM intake of steers fed SH-based diet at 1.5% of BW.

Intake of NDF and CP was not affected ($p > 0.1$) by particle size of AH or by SH inclusion in diets. In contrast, *ad libitum* consumption of steers with higher level of SH has been shown to increase intake of DM, OM, and fibre and their digestibility (Grigsby et al., 1992; Galloway et al., 1993). Inclusion of SH in diets increased EE intake ($p < 0.01$) in steers fed SH-containing diets. This effect was expected because of the higher EE level in SH than AH. Inclusion of SH in diets increased EE content of diets from 2.70% to 3.20% of DM.

Apparent total tract digestibilities of diets are shown in Table 3. The change in AH particle size did not affect ($p > 0.10$) the digestibility of DM, OM, CP, EE or NDF among dietary treatments. In contrast, studies with dairy cows (Kononoff and Heinrichs, 2003; Yang and Beauchemin, 2005; Behgar et al., 2011) showed increase in the digestibility of DM, OM and NDF as AH particle size of diets increased. The lack of effect of AH particle size on nutrient digestibility in the present

Table 3 Effects of particle size of alfalfa hay and soya bean hull inclusion in diet on nutrient intake and total tract digestion

	Diets					p Value		
	AH		AH+SH					
Items	Fine	Coarse	Fine	Coarse	SE	PS	SH	PS×SH
Intake (kg/days)								
Dry matter	3.80	3.81	3.94	3.93	0.06	0.97	0.07	0.85
Neutral detergent fibre	1.45	1.46	1.47	1.47	0.02	0.98	0.52	0.85
Crude protein	0.50	0.50	0.51	0.51	<0.01	0.98	0.26	0.85
Ether extract	0.10	0.10	0.13	0.13	<0.01	0.99	<0.01	0.86
Digestibility (% of intake)								
Dry matter	71.4	70.9	69.3	70.6	0.98	0.71	0.32	0.43
Organic matter	73.2	75.0	72.6	73.1	1.25	0.43	0.29	0.66
Crude protein	54.9	59.7	58.7	63.9	3.03	0.15	0.23	0.95
Ether extract	73.0	71.0	84.4	81.4	2.13	0.34	<0.01	0.94
Neutral detergent fibre	60.0	57.8	56.1	56.8	1.23	0.51	0.11	0.27

AH, alfalfa hay; SH, soya bean meal.

Table 4 Effects of particle size of alfalfa hay and soya bean hull inclusion in diet on rumen pH, rumen N-NH_3 and faecal pH

	Diets					p Value		
	AH		AH+SH					
Items	Fine	Coarse	Fine	Coarse	SE	PS	SH	PS×SH
Rumen pH (h post-feeding)								
0*	7.09	7.01	6.99	6.96	0.06	0.41	0.32	0.72
1	6.62	6.64	6.59	6.64	0.06	0.58	0.84	0.81
2	6.42	6.44	6.35	6.39	0.03	0.39	0.07	0.64
3	6.45	6.37	6.33	6.33	0.04	0.22	0.14	0.51
4	6.43	6.31	6.26	6.38	0.05	0.98	0.43	0.07
5	6.46	6.40	6.28	6.33	0.05	0.92	0.03	0.29
6	6.51	6.44	6.35	6.40	0.06	0.87	0.15	0.35
7	6.55	6.42	6.41	6.43	0.06	0.39	0.39	0.28
Rumen NH_3-N (mg/dl)								
2	16.0	21.8	24.4	19.2	5.79	0.96	0.67	0.42
4	17.7	20.8	22.0	16.8	1.72	0.58	0.97	0.07
Faecal pH	7.46	7.34	7.22	7.20	0.05	0.24	0.01	0.41

AH, alfalfa hay; SH, soya bean meal.
*Before morning feeding.

study can be explained by the fact that the steers were under restricted feeding strategy. Le Liboux and Peyraud (1998) showed interaction of feeding level and diet particle size on cell wall digestibility and rumen fermentation pattern. There was a shift of starch digestion from the rumen to the intestine when forage particle size was increased, although total digestion of starch was not changed.

Inclusion of SH increased digestibility of EE ($p < 0.01$) which are likely due to the greater EE intake (Palmquist, 1991) or substituting of SH oil for waxes and other indigestible EE components in AH. Digestibility of NDF was tended to decrease ($p = 0.10$) by SH inclusion. This is in agreement with Behgar et al. (2011) who substituted SH for AH in the diets of dairy cows at 10% of dietary DM. Substituting higher amounts of SH (i.e. 70–100% of diet DM) for AH in steers fed at 1.75% of BW increased digestibility of NDF (Trater et al., 2001).

Ruminal environment

Ruminal pH and N-NH_3 concentration, and faecal pH are shown in Table 4. Inclusion of SH in diets decreased ruminal pH at 2 h and 5 h post-feeding ($p = 0.07$ and $p = 0.03$ respectively). The slightly lower ruminal pH with SH inclusion could be related to the enhanced concentrations of volatile fatty acids (VFA) in the rumen (Weidner and Grant, 1994a). However, concentrations of ruminal VFA were not measured in the present study. In contrast to present study, SH inclusion did not affect ruminal pH in dairy cows up to 4 h post-feeding (Behgar et al., 2011). There was no effect of dietary treatments on rumen N-NH_3 content which was consistent with lack of difference in CP content among experimental diets.

Faecal pH was decreased with the inclusion of SH ($p = 0.01$), particularly in steers received coarse AH. These results are consistent with previous findings in dairy cows fed SH (Behgar et al., 2011) and was related to the increase in rumination and chewing activity of animals fed coarse diets resulting in increase in rumen fluid passage rate and subsequent escape of small SH particles to lower tract. In contrast, Slater et al. (2000) did not observe any change in faecal pH in dairy cows fed increasing levels of SH (3.5% to 23% of diet DM). The reduction of faecal pH with SH inclusion in diets is likely due to the bypass of SH particles from rumen which are rich in readily digestible NDF and starch. Yang et al. (2002) showed a shift of starch digestion from the rumen to the intestine

Table 6 Effects of particle size of alfalfa hay and soya bean hull inclusion in diet on in situ disappearance of DM, *a*, *b*, *c*, a+b* and ED† values

	Diets							
	AH		AH+SH			p Value		
Items	Fine	Coarse	Fine	Coarse	SE	PS	SH	PS×SH
a	8.9	49.0	48.0	48.9	1.77	0.80	0.80	0.84
b	38.8	39.0	41.5	42.3	3.06	0.89	0.38	0.93
c (/h)	0.04	0.05	0.03	0.04	0.01	0.87	0.51	0.97
*a+b**	87.7	88.0	89.5	91.2	3.71	0.81	0.54	0.86
ED†	66.9	67.5	64.1	64.8	1.52	0.69	0.14	0.95

DM, dry matter; AH, alfalfa hay; SH, soya bean meal.
*Total DM degraded in the rumen. †Effective rumen degradability at out flow rate of 0.05/h.

Table 5 Rumen dry matter degradability (%) of experimental diets in individual time of incubation

	Diets							
	AH		AH+SH			p Value		
Times	Finze	Coarse	Fine	Coarse	SE	PS	SH	PS×SH
0	44.3	46.0	45.0	45.5	1.14	0.96	0.51	0.71
2	53.7	51.0	58.0	54.5	1.00	0.02	0.01	0.66
4	55.1	57.0	59.2	55.2	0.60	0.15	0.11	<0.01
8	60.5	59.2	62.3	56.3	1.70	0.10	0.76	0.25
12	65.0	61.0	64.5	59.0	1.50	0.04	0.47	0.65
16	68.5	67.6	66.4	61.1	0.90	0.03	0.01	0.08
24	74.7	73.8	73.0	66.4	0.87	0.02	<0.01	0.04
48	83.1	82.8	84.0	77.9	0.62	<0.01	0.04	0.01
72	85.3	85.0	87.0	82.0	0.90	0.03	1.00	0.08
96	86.5	84.4	86.7	89.2	0.98	0.07	1.00	0.12

AH, alfalfa hay; SH, soya bean meal.

when AH particle size was increased in diets of dairy cows.

Rumen degradability of DM

Rumen DM degradability of experimental diets is shown in Table 5. In most incubation times (up to 72 h), feeding steers with diet containing fine AH increased rumen degradability of diets ($p < 0.05$). Similar results were observed for diets containing SH in early periods of incubation (up to 4 h), but rumen degradation of SH-containing diets was decreased ($p < 0.05$) with increasing incubation time. This effect could in part be explained by the effect of SH on the rumen pH and escape of SH particles from the rumen to lower gut that may decrease available nutrients for microbial growth and fermentation.

The effect of dietary treatments on rumen DM degradable fraction (*a+b*), degradation coefficients (*a*, *b* and *c*) and effective degradability (ED) of experimental diets is shown in Table 6. There was no significant effect of AH, SH and AH×SH interaction on '*a+b*', '*a*', '*b*' and '*c*' values or ED of DM. This result is in agreement with other studies (Le Liboux and Peyraud, 1998; Yang et al., 2002). Yang et al. (2002) replaced chopped AH with ground AH in the diet of dairy cow and showed no effect on ruminal OM digestion. Similarly, no effect of AH particle size was observed on ruminal OM and fibre digestion by Le Liboux and Peyraud (1998).

Conclusion

The results of this experiment showed that the inclusion of SH decreased diet particle size and ruminal pH at 5 h post-feeding as well as faecal pH. No interaction of AH particle size and SH inclusion in diets was seen on nutrient intake and digestibility of restricted fed Holstein steers.

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