Feeding processed soybean to mid-lactation Holstein cows: ingestive behaviour and rumen fermentation characteristics

Imaneh Sadr-Arhamia, Gholam Reza Ghorbania, Shahryar Kargarb, Ali Sadeghi-Sefidmazgia, Morteza Hosseini Ghaffaric, † and Mariangela Caroprese
aDepartment of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran; bDepartment of Animal Science, School of Agriculture, Shiraz University, Shiraz 71441-65186, Iran; cDepartment of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton T6G 2P5, Canada; dDipartimento di Scienze Agrarie, degli Alimenti e dell’Ambiente, Università di Foggia, Via Napoli, 25, Foggia, Italia

ABSTRACT
The objective of this study was to evaluate the effects of roasted soybean (RSB), extruded soybean (ESB) and their equal blend (RSB + ESB) compared with soybean meal (SBM) on nutrient intake, feed preference, meal and rumination patterns, feeding and chewing behaviour and rumen fermentation characteristics of mid-lactation dairy cows. Eight Holstein dairy cows were used in a replicated 4 × 4 Latin square design with four 28-d periods. Cows received one of the four following experimental diets: (1) 13.88% of diet dry matter (DM) as SBM; (2) 15.22% of diet DM as RSB, (3) 15.55% of diet DM as ESB and (4) 7.69% RSB plus 7.69% ESB (RSB + ESB). Each experimental period consisted of a 21-d diet adaptation period and a 7-d data collection period. Meal patterns, including meal size and inter-meal interval, were not affected by the experimental diets and thereby DM intake was not different among diets. Sorting index was not different across dietary treatments but intake of particles retained on 1.18-mm sieve and on pan increased and decreased in both SBM and ESB as compared with RSB and RSB + ESM, respectively. Total time spent eating and ruminating and rumen volatile fatty acid concentrations were unaffected by dietary treatments. Feeding processed soybean instead of SBM had minimal effects on sorting behaviour and meal patterns and thereby no changes in feed intake occurred. Finally, RSB and ESB and their equal blend had a similar effect on feed intake and chewing behaviour of mid-lactation Holstein cows.

HIGHLIGHTS
- Feeding processed soybean products had the minor effect on feed intake and can be considered as alternative feedstuffs when the price is competitive.

Introduction
Feed intake is a function of both meal size and inter-meal interval, determined by satiety and hunger, respectively (Kargar et al. 2013, 2014). Heat treated soybean (SB) vs. SB meal (SBM) contain a greater amount of oil (NRC 2001), which may provide additional energy for high-producing dairy cows, assuming it does not negatively affect ruminal fermentation and fibre digestibility and thereby feed intake. However, heating process may rupture the fat micelles within the SB, which can allow a more rapid release of oil into the rumen (Reddy et al. 1993) and potentially decreases DM intake (DMI) by lowering both fibre digestibility (Sutton et al. 1983) and the degradable part of dietary protein in the rumen (Giallongo et al. 2015) or by increasing postprandial plasma concentrations of the gut peptides (e.g. cholecystokinin and glucagon-like-peptide-1) through increased flow of unsaturated fatty acids to the duodenum (Allen 2000). Heat treated SB (fed at more than 10% of dietary DM) has been variable effect on DMI in previous studies. Some studies reported positive (Anderson et al. 1984; Giallongo et al. 2015) or negative effects (Akbarian et al. 2014), whereas other investigators showed no effects on feed intake (Guillaume et al. 1991; Bailoni et al. 2004; Amanlou et al. 2012). It remains unclear from above mentioned studies that how meal pattern (including meal size and inter-meal interval) is
influenced when heat treated SB products are fed to dairy cows. Therefore, the need to evaluate the use of different heated products of SB or their blend vs. SBM at higher rate of inclusion seemed appropriate when dietary energy is balanced in SBM supplemented diets using an inert source of fat in the rumen.

Higher secretion of gut peptides may induce reduced gut motility (Allen 2000) which can cause a reduction in DMI by decreasing meal size and a decrease in time spent rumination by decreasing rumination duration (Harvatine and Allen 2006). Although the inconsistent effect of feeding SBM (as rumen degradable source of protein with diminished oil content) vs. roasted SB (RSB) or extruded SB (ESB); both as rumen undegradable source of protein containing higher amounts of unsaturated oil) on DMI has been reported in some research studies (Amanlou et al. 2012; Akbarian et al. 2014; Giallongo et al. 2015), there is no available published data, to the best of our knowledge, to report feeding and chewing behaviour of cows fed RSB, ESB and/or their blends. Therefore, investigation of feeding behaviour when cows are fed diets containing heat processed SB products is required to clarify regulation mechanism(s) for feed intake. We hypothesised that feeding ESB or RSB would have comparable feed intake and rumen fermentation characteristics to that of SBM. We also hypothesised that feeding blended SB products would perform equally to those of ESB or RSB if not affect meal size or inter-meal interval; therefore, RSB and ESB or their blend can be used interchangeably in dairy cow diets, allowing dairy producers more choices of feed ingredients. The objectives of this study were to investigate the effects of RSB and ESB alone, and/or their equal blend on nutrient intake, feed preference, meal and rumination patterns, feeding and chewing behaviour and ruminal fermentation characteristics of mid-lactation Holstein cows.

Materials and methods

Cows, experimental design and diets

The experiment was conducted at the Dairy Research Facilities of the Lavark Research Station from Isfahan University of Technology, Isfahan, Iran. Guidelines for the care and use of animals were approved by the Animal Care Committee of the University. Eight Holstein dairy cows (BW = 534 ± 52 and DIM = 104 ± 5; mean ± SD) were used in a replicated 4 × 4 Latin square design with four 28-d periods. Each experimental period consisted of a 21-d diet adaptation period and a 7-d collection period. Cows were assigned to one of the four experimental diets: (1) 13.88% of diet DM as SBM, (2) 15.22% of diet DM as RSB, (3) 15.55% of diet DM as ESB, and (4) equal blending of RSB and ESB at 7.69% of diet DM (RSB + ESB). The level of SB products was chosen at the amount that to meet crude protein (CP) requirement mainly from these products as for SBM which rely on soybean meal. Basal diet contained 20.04% alfalfa hay, 20.04% corn silage, 17.55% barley grain, 21.10% corn grain, 13.88% SBM, 2.29% corn gluten meal, 2.04% fat supplement, 1.02% vitamin-mineral mix, 1.02% sodium bicarbonate, 0.41% calcium carbonate, 0.41% di-calcium phosphate and 0.20% white salt. Across diets, the DM, CP, non-fibrous carbohydrate (NFC), neutral detergent fibre (NDF), ether-extract (EE) and net energy for lactation (NEL) concentrations were 55.1% (DM basis), 15.4%, 37.6%, 33.7%, 5.4% and 1.67 Mcal/kg of DM, respectively. Cows were housed individually in box stalls (4 m × 4 m) located in a roofed area with open sides. Each box stall was equipped with a concrete feed bunk and automatic water troughs. Clean wood shavings and sand were used for bedding and refreshed twice daily. Cows were allowed to exercise in an outdoor lot daily from 1700 to 1800 h. Dietary forage to concentrate ratio was 40:60 and the forage component of the experimental diet was a mixture of corn silage and chopped alfalfa hay. All diets were formulated according to the Cornell Net Carbohydrate and Protein System (version 5.0) nutrient allowance for a lactating dairy cow weighing 534 kg and producing 37 kg/d of milk with 3.11% milk true protein and 3.40% fat and consuming 22 kg of DM. Diets were formulated to be iso-caloric and iso-nitrogenous. Dietary ingredients were mixed for ~8 min in a total mixed ration (TMR) mixer wagon (Fan Avaran Keshavarzi Arya Co., Isfahan, Iran). The feed was provided twice daily at 0700 and 1400 h ad libitum and was adjusted daily to allow at least 10% refusals. Soybean meal and ESB were used as intact forms and not further processed, but RSB and the grains were ground using a hammer mill with 3 mm screen size (model 5543 GEN, Isfahan Dasht, Isfahan, Iran).

Feed sampling and analyses

The TMR amounts offered and refused were measured daily for each cow with DMI determined daily for each cow. Refusals from individual cows were used for calculation of nutrient intake. To determine DM and nutrient composition, representative samples of forages (pooled within the period), treatment TMR
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was determined by oven drying at 60°C for 48 h. The physical effectiveness factor (p,ef) was determined as the DM proportion of particles retained on two sieves (p,ef>8; Lammers et al. 1996) and on three sieves (p,ef>1.18; Kononoff et al. 2003) of the PSPS, respectively. The physically effective NDF of two (pNDF>8) and three sieves (pNDF>1.18) were calculated by multiplying the NDF concentration of the feed by the fraction on pef>8 and pef>1.18, respectively.

Sorting behaviour and chewing activities

The sorting index was calculated as the ratio of actual intake to expected intake of particles retained on each sieve of the PSPS (Kargar et al. 2013, 2014). The predicted intake of an individual fraction was calculated as the product of the DMI of the total diet multiplied by the DM percentage of that fraction in the fed TMR. A sorting index of 100, >100 and <100 indicated no sorting, sorting for and sorting against, respectively.

On the third day of each collection period, eating and ruminating activities were monitored visually (by six trained people) for each cow over a 24-h period. A total of six individuals in two-person teams rotated throughout the 24-h period; two individuals observed the cows simultaneously for 2 h with a 4-h rest period before recording again. All activities including eating and ruminating were noted every 5 min, and each activity was assumed to persist for the entire 5-min interval between observations (Kargar et al. 2010, 2013, 2014). To calculate the time spent eating or ruminating per kilogram of DM, NDF, and forage NDF, pNDF>8 and pNDF>1.18 intakes, average intake within the experimental period was used. A period of rumination was defined as at least 5 min of ruminating activity followed by at least 5 min without ruminating activity. Total chewing time was calculated as the sum of eating and ruminating time.

Rumen fluid sampling and volatile fatty acid analyses

Rumen fluid sampling and analysing of volatile fatty acids (VFA) were done according to Kargar et al. (2012). Briefly, at the end of each experimental period, rumen fluid from the ventral sac was sampled using the rumenocentesis technique. Rumen samples were obtained at 4 h after the morning feeding. Rumen fluid was obtained at least three times from each cow at the same time to collect three repeatable pH values (and averaged to have one pH value per cow per period) and then rumen pH was determined using a mobile pH metre (HI 8314 membrane pH metre, Hanna Instruments, Villafranca, Italy). For stabilising, each millilitre of rumen fluid was acidified with 20 μL of 50% H2SO4 and stored at −20°C for subsequent VFA analyses by gas chromatography (model CP-9002, Chrompack, Middelburg, The Netherlands) with a 50 m (0.32 mm ID) silica-fused column (CP-Wax Chrompack Capillary Column, Varian, Palo Alto, CA). Helium was used as the carrier gas and oven initial and final temperatures were 55 and 195°C, respectively. The detector and injector temperatures were set at 250°C. Crotonic acid (1:7, v/v) was used as the internal standard.

Statistical analyses

Data were composited within period and subjected to MIXED MODEL procedure of SAS (SAS Institute 2003) according to the following model:

\[ Y_{ijklm} = \mu + S_i + P_j + T_k + C_l + e_{ijklm} \]

where \( Y_{ijklm} \) is the variable of interest, \( \mu \) is the overall mean, \( S_i \) is the fixed effect of square (\( i = 1–2 \)), \( P_j \) is the fixed effect of period (\( j = 1–4 \)), \( T_k \) is the fixed effect of treatment (\( k = 1–4 \)), \( C_l \) is the random effect of cow (\( l = 1–8 \)) and \( e_{ijklm} \) is the residual error. The REML method was used to estimate least squares means, and the Kenward-Roger method was used to calculate denominator degrees of freedom. Normality of distribution and homogeneity of variance for residuals were tested using PROC UNIVARIATE (SAS Institute 2003). In case of non-normality, parameters were normalised by
The similar results were reported by Kargar et al. (2013, 2014), where the percentage of particles retained on the third sieve (1.18–8 mm) increased \( p = .04 \) for SBM and ESB than for RSB and RSB + ESB diets, resulting in greater \( \text{pef}_{>1.18} \) \( p = .03 \) and \( \text{peNDF}_{>1.18} \) \( p = .03 \). As reported by Kargar et al. (2013, 2014), the feed materials retained on the bottom pan (<1.18 mm) decreased for SBM and ESB compared with RSB. The proportion of SBM and ESB retained on the 1.18-mm sieve was more than that of RSB and a blend of RSB and ESB. However, the geometric mean particle length was not changed across experimental diets and averaged 3.84 mm.

**Results and discussion**

**Diet characteristics and particle size distribution**

Across diets, the DM, CP, NFC, NDF and EE concentrations (mean ± SE) were 55.1 ± 0.4, 15.4 ± 0.2, 37.6 ± 0.6, 33.7 ± 0.5 and 5.4 ± 0.3% (DM basis), respectively. Data on particle size distribution of forages (including corn silage and alfalfa hay) and TMR are presented in Table 1. The proportion of the particles retained on the top (>19 mm) and middle sieves (8–19 mm) of the PSPS did not differ among diets indicating no difference in both \( \text{pef}_{>8} \) and \( \text{peNDF}_{>8} \) across experimental diets. The similar results were reported by Kargar et al. (2013, 2014), where the feed materials retained on the 1.18-mm sieve was more than that of RSB and a blend of RSB and ESB. However, the geometric mean particle length was not changed across experimental diets and averaged 3.84 mm.

**Nutrient intake**

Intake of nutrients (other than CP, EE and \( \text{peNDF}_{>1.18} \)) was not different across experimental diets (Table 2). The

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**Table 1. Physical characteristics of forage and experimental diets measured using the Penn State Particle Separator.**

| Item | Forage | Diet LSMa |
|------|--------|-----------|
| % of DM retained on sieve | | |
| 19.0 mm | | 28.80 | 5.60 | 7.30 | 7.60 | 6.90 | 7.62 | 0.87 | .48 |
| 8.0 mm | | 56.70 | 28.80 | 23.30 | 23.90 | 23.30 | 24.20 | 0.94 | .83 |
| 1.18 mm | | 13.00 | 43.00 | 45.60 | 39.20 | 44.10 | 40.90 | 1.39 | .04 |
| Pan | | 1.50 | 22.70 | 23.90 | 29.30 | 30.50 | 28.30 | 2.77 | .03 |
| \( \text{pef}_{>8} \) | | 0.86 | 0.34 | 0.31 | 0.32 | 0.30 | 0.31 | 0.02 | .88 |
| \( \text{pef}_{1.18} \) | | 0.99 | 0.77 | 0.76 | 0.71 | 0.74 | 0.72 | 0.03 | .04 |
| \( \text{peNDF}_{>8} \), % of DM | | 51.32 | 16.69 | 10.22 | 9.82 | 9.95 | 9.49 | 0.56 | .64 |
| \( \text{peNDF}_{1.18}, \% \) of DM | | 59.11 | 37.58 | 25.46 | 22.05 | 24.46 | 22.08 | 0.91 | .03 |
| GMPS,d mm | | 13.04 | 4.16 | 4.11 | 3.72 | 3.81 | 3.71 | 0.24 | .10 |
| SDgm,e mm | | 2.06 | 2.93 | 2.95 | 3.16 | 2.97 | 3.08 | 0.05 | .01 |

LSM: Least Square Means.
aParticle length variables were measured using the Penn State Particle Separator (The Pennsylvania State University, University Park; Kononoff et al. 2003).
bSBM: soybean meal at 13.9% of dietary DM; RSB: roasted soybean at 15.2% of dietary DM; ESB: extruded soybean at 15.5% of dietary DM; and RSB + ESB: roasted soybean and extruded soybean at equal blend of 7.7% of dietary DM.
c\( \text{pef}_{>8} \) and \( \text{pef}_{>1.18} \): physical effectiveness factor determined as the DM proportion of particles retained on 2 sieves (19 and 8 mm) or 3 sieves (19, 8, and 1.18 mm), respectively, of the Penn State Particle Separator (Lammers et al. 1996; Kononoff et al. 2003); \( \text{peNDF}_{>8} \) and \( \text{peNDF}_{1.18} \): physically effective NDF calculated as dietary NDF content (% DM) multiplied by \( \text{pef}_{>8} \) and \( \text{pef}_{1.18} \), respectively.
dGeometric mean particle size, calculated according to the method of the American Society of Agricultural Engineers (ASAE, 1995; method S424.1).
eGeometric SD of particle size, calculated according to the method of the American Society of Agricultural Engineers (ASAE, 1995; method S424.1).
fMeans within a row with different superscripts are significantly different \( (p < .05) \).

**Table 2. Nutrient intake as influenced by feeding processed soybean to mid-lactation dairy cows.**

| Item | SBM | RSB | ESB | RSB + ESB | SEM | p value |
|------|-----|-----|-----|-----------|-----|---------|
| Intake, kg/d | | | | | | |
| Organic matter | 21.00 | 20.00 | 20.40 | 20.00 | 0.42 | .42 |
| Crude protein | 3.59d | 3.29d | 3.38d | 3.28d | 0.07 | .02 |
| NFC | 8.62 | 8.18 | 8.53 | 7.75 | 0.31 | .22 |
| Neutral detergent fibre (NDF) | 7.69 | 7.18 | 7.35 | 7.24 | 0.19 | .26 |
| Forage NDF | 4.94 | 4.68 | 4.81 | 4.71 | 0.09 | .11 |
| Ether extract | 1.02d | 1.23c | 1.26c | 1.27d | 0.02 | <.01 |
| \( \text{PeNDF}_{>8} \) | 2.35 | 2.29 | 2.24 | 2.23 | 0.09 | .78 |
| \( \text{PeNDF}_{>1.18} \) | 5.85c | 5.09d | 5.50d | 5.21d | 0.18 | .01 |

LSM: Least Square Means.
aSBM = soybean meal at 13.9% of dietary DM; RSB = roasted soybean at 15.2% of dietary DM; ESB = extruded soybean at 15.5% of dietary DM; and RSB + ESB = roasted soybean and extruded soybean at equal blend of 7.7% of dietary DM.
b\( \text{peNDF}_{>8} \) and \( \text{peNDF}_{1.18} \) = calculated by multiplying \( \text{pef}_{>8} \) (DM retained on 19- and 8-mm sieves) and \( \text{pef}_{1.18} \) (DM retained on 19-, 8-, and 1.18-mm sieves) by the NDF content of the diet (DM basis), respectively.
cdMeans within a row with different superscripts are significantly different \( (p < .05) \).
lack of effect on feed intake, which is a function of meal size and inter-meal interval (Kargar et al. 2013, 2014), was attributed to the similar meal size or inter-meal interval indicating that all diets had the same animal acceptance in this study. Feeding heat processed SB products (RSB or ESB) vs. SBM resulted in varied DMI in different research studies. Several studies reported increase (Anderson et al. 1984; Giallongo et al. 2015), decrease (Akbarian et al. 2014) and/or no changes (Guillaume et al. 1991; Bailoni et al. 2004; Amanlou et al. 2012) in DMI for cows fed heat processed SB products vs. SBM. Inconsistencies between the results of this study and above-mentioned studies is difficult to explain, although forage to concentrate ratio, forage source, forage particle size and the relative proportion of forage source did vary among studies. However, our results are in line with other investigators (Mohamed et al. 1988) who observed no differences in DMI for cows fed heat processed SB products compared with SBM. In the previous studies (Guillaume et al. 1991; Bailoni et al. 2004; Amanlou et al. 2012), feeding RSB to dairy cows had no effect on DMI when compared to ESB. Greater intake of CP (p = .02) and also less EE intake (p < .01) in SBM compared with other experimental diets is a reflection of numerical differences in those component contents of the original diets. Overall, in the present experiment, average DMI was 22.0 kg/d, or ~4.1% of mean BW, and this is within the anticipated range for dairy cows producing >35 kg/d of milk (NRC 2001).

### Sorting behaviour and meal and rumination patterns

Experimental diets did not affect sorting activity, which is expressed as the sorting index (Table 3). Regardless of the type of experimental diets fed, cows sorted, to a similar extent, against long particles (>19 mm). This result is not unexpected, as most investigations of feed sorting have conveyed that cattle sort widely against long particles, regardless of the substrate, making up the bulk of the long-particle fraction (Kargar et al. 2013, 2014).

Experimental diets did not affect the intake of particles retained on the 19-mm and 8-mm sieves. However, intake of particles retained on the 19-mm sieve tended (p = .07) to decrease in RSB and ESB diets as compared with SBM diet. Corresponding to differences in the profile of particles of diets (Table 1), intakes of particles retained on the 1.18-mm sieve and feed materials in the pan, increased (p < .01) and decreased (p < .01) in SBM and ESB diets as compared with RSB and RSB + ESB diets, respectively.

Eating patterns were not affected by experimental diets (Table 3). The number of eating bouts per day, meal length, inter-meal interval, eating rate and meal size were not different among experimental diets (p > .05) and correspond with no difference in DMI across diets (data not shown). Rumination patterns were not different among experimental diets. The number of ruminating bouts per day, bout length and meal size were not different among experimental diets.

### Table 3. Sorting index, particle size intake and meal and rumination patterns as influenced by feeding processed soybean to mid-lactation dairy cows.

| Item                          | SBM     | RSB     | ESB     | RSB + ESB |
|-------------------------------|---------|---------|---------|-----------|
| Sorting index,a,b%            |         |         |         |           |
| 19                            | 91.57   | 88.04   | 80.96   | 83.97     |
| 8                             | 98.15   | 98.18   | 97.16   | 98.35     |
| 1.18                          | 101.20  | 101.3   | 101.5   | 101.3     |
| Pan                           | 99.70   | 100.7   | 101.5   | 101.3     |
| DMI, kg/d                     |         |         |         |           |
| 19                            | 1.52    | 1.47    | 1.27    | 1.19      |
| 8                             | 5.18    | 5.03    | 5.03    | 5.14      |
| 1.18                          | 10.54   | 8.61c   | 10.08b  | 9.09b     |
| Pan                           | 5.47d   | 6.40d   | 5.72d   | 6.20b     |
| Meals                         |         |         |         |           |
| Bouts/d                       | 15      | 15      | 16      | 17        |
| Length, min/meal              | 19      | 20      | 16      | 16        |
| Interval, min                 | 74      | 79      | 77      | 72        |
| Eating rate, kg of DM/min     | 0.083   | 0.074   | 0.084   | 0.079     |
| Meal size, kg of DM           | 1.53    | 1.49    | 1.40    | 1.29      |
| Rumination                    |         |         |         |           |
| Bouts/d                       | 17      | 18      | 18      | 17        |
| Bout length, min/bout         | 29      | 27      | 28      | 28        |
| Bout interval, min            | 56      | 51      | 52      | 57        |

LSM: Least Square Means.

aSBM: soybean meal at 13.9% of dietary DM; RSB: roasted soybean at 15.2% of dietary DM; ESB: extruded soybean at 15.5% of dietary DM; and RSB + ESB: roasted soybean and extruded soybean at equal blend of 7.7% of dietary DM.
bSorting index was calculated as the percentage of actual intake relative to the predicted intake of each particle fraction. Values equal to 100% indicate no sorting, values <100% indicate selective refusals (sorting against) and values >100% indicate preferential consumption (sorting for) Kargar et al. (2013, 2014).
c,dMeans within a row with different superscripts are significantly different (p < .05).
interval between bouts were not affected by experimental diets ($p > .05$; Table 3) and correspond with no difference in rumination time across diets (Table 4). To the best of our knowledge, no similar published data are available for comparison. However, the lack of treatment effect on meal and rumination patterns may imply that heat-processed SB products (RSB and ESB) did not interfere with ruminal fermentation or post-prandial concentrations of gut peptides (although we did not measure those variables) (Allen 2000); therefore, no changes in time spent eating and rumination were observed in this study.

**Chewing activity**

Time spent eating was not different across experimental diets ($p = .28$). Characteristics of eating behaviour are impacted mainly by physical factors that affect ease of ingestion and mastication (Kargar et al. 2013, 2014). Thus, despite observed differences in PSPS fractions all diets were apparently consumed with similar ease as forage-to-concentrate ratio, forage NDF concentration and geometric mean particle size were not different among diets. This may imply that the observed difference in materials fractioning caused by SBM and heat-processed SB products has a minimal effect on feeding behaviour of dairy cows. However, eating time when expressed as minutes per kg of DMI was tended ($p = .08$) to be greater in cows fed SBM diets than that of cows fed other diets. As expected, eating time when expressed as minutes per unit of peNDF $> 1.18$ intake was greater in RSB diet compared with SBM and ESB diets ($p = .02$). Because daily eating time was not different among diets, an increase in daily eating time per unit of peNDF $> 1.18$ in RSB diet compared with SBM and ESB diets can be attributed to less peNDF $> 1.18$ intake.

**Table 4. Chewing activities as influenced by feeding processed soybean to mid-lactation dairy cows.**

| Item                          | SBM      | RSB      | ESB      | RSB + ESB | SEM   | $p$ value |
|-------------------------------|----------|----------|----------|-----------|-------|-----------|
| **Eating time**               |          |          |          |           |       |           |
| min/d                         | 281      | 298      | 274      | 279       | 8.53  | .28       |
| min/bout                      | 19       | 20       | 17       | 17        | 1.18  | .12       |
| min/kg of DMI                 | 12.5     | 14.1     | 12.8     | 12.9      | 0.41  | .08       |
| min/kg of NDF intake          | 37.1     | 42.3     | 38.8     | 38.7      | 1.49  | .13       |
| min/kg of forage NDF intake   | 57.6     | 64.7     | 58.6     | 59.4      | 4.79  | .73       |
| min/kg of peNDF $> 1.18$      | 123.2    | 133.7    | 128.6    | 125.7     | 5.20  | .54       |
| min/kg of peNDF $> 1.18$      | 48.9     | 59.4     | 52.0     | 54.0      | 2.09  | .02       |
| **Ruminating time**           |          |          |          |           |       |           |
| min/d                         | 481      | 478      | 483      | 464       | 13.93 | .77       |
| min/bout                      | 29       | 28       | 28       | 28        | 1.74  | .86       |
| min/kg of DMI                 | 21.3     | 22.5     | 22.1     | 21.6      | 0.82  | .72       |
| min/kg of NDF intake          | 64.6     | 67.5     | 67.0     | 64.4      | 2.35  | .71       |
| min/kg of forage NDF intake   | 97.8     | 103.2    | 101.7    | 99.0      | 5.62  | .90       |
| min/kg of peNDF $> 1.18$      | 223.7    | 237.5    | 253.8    | 227.2     | 16.96 | .62       |
| min/kg of peNDF $> 1.18$      | 83.2     | 94.9     | 90.2     | 89.5      | 3.71  | .22       |
| **Total chewing time**         |          |          |          |           |       |           |
| min/d                         | 762      | 775      | 758      | 743       | 13.52 | .44       |
| min/bout                      | 24       | 24       | 23       | 22        | 0.97  | .53       |
| min/kg of DMI                 | 33.8     | 36.6     | 34.9     | 34.5      | 0.92  | .23       |
| min/kg of NDF intake          | 100.1    | 109.8    | 105.8    | 103.1     | 3.02  | .54       |
| min/kg of forage NDF intake   | 155.4    | 168.0    | 160.3    | 158.4     | 9.24  | .90       |
| min/kg of peNDF $> 1.18$      | 351.7    | 347.1    | 353.7    | 334.4     | 13.49 | .63       |
| min/kg of peNDF $> 1.18$      | 132.2    | 154.2    | 142.2    | 143.5     | 4.63  | .03       |
| Resting, min/d                | 678      | 665      | 683      | 697       | 13.52 | .44       |

LSM: Least Square Means.

$^a$SBM: soybean meal at 13.9% of dietary DM; RSB: roasted soybean at 15.2% of dietary DM; ESB: extruded soybean at 15.5% of dietary DM; and RSB + ESB: roasted soybean and extruded soybean at equal blend of 7.7% of dietary DM.

$^b$peNDF: physically effective NDF of 2 (peNDF $> 8$) and 3 sieves (peNDF $> 1.18$), respectively.

$^c,d$Means within a row with different superscripts are significantly different ($p < .05$).
Ruminal fermentation characteristics

Ruminal fluid pH values ($p = .80$) and total VFA concentrations ($p = .24$) were not different across experimental diets (Table 5); however, the molar proportion of propionate tended ($p = .07$) to be increased in SB containing diets compared with SBM diet. This finding is thought to be due to a modification of the ruminal population (a reduction in cellulolytic and methanogenic bacteria), as observed with most fat sources (including SB) containing C18 fatty acids. Furthermore, the increase in propionate can be due to the competition for metabolic hydrogen between methane and propionate production pathways (Doreau and Chilliard 1997). The pH values of all diets were in an acceptable range to maintain a healthy rumen environment and also milk fat percentage (NRC 2001). These data agree with others (Mielke and Schingoethe 1981; Mohamed et al. 1988; Giallongo et al. 2015) that showed heat-processed SB had no effect on ruminal pH. In agreement with other investigators (Mielke and Schingoethe 1981; Giallongo et al. 2015), heat-processed SB had no effect on total VFA concentration in the rumen. Overall, the lack of diet effect on ruminal pH, and VFA concentrations lends credence to us that cellulolytic bacterial populations were not affected (Kargar et al. 2012).

Conclusions

At the same dietary forage to concentrate ratio and geometric mean particle size, feeding processed soybean instead of SBM had minimal effects on sorting behaviour and meal patterns and thereby no changes in feed intake occurred. Finally, RSB and ESB and their equal blend had a similar effect on feed intake and feeding and chewing behaviour of mid-lactation Holstein cows and can be considered as alternative feedstuffs when the price is competitive.

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Ethics statement

All animal procedures were approved by the Animal Care Committee of Isfahan University of Technology as recommended by the Iranian Council of Animal Care (1995).

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