

## ***In vitro* Evaluation of Different Substitution Levels of Soybean Meal by Guar Meal in a Fattening Diet for Lambs**

### **Research Article**

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Received on: 7 Jul 2014

Revised on: 17 Sep 2014

Accepted on: 15 Oct 2014

Online Published on: Sep 2015

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### **ABSTRACT**

The aim of the current study was to evaluate the replacement effect of soybean meal (SBM) at different levels (0 as D1, 33 as D2, 67 as D3 and 100% as D4 based on crude protein (CP) content) by guar meal (GM) on ruminal gas production kinetics, ruminal digestibility and fermentation characteristics of a fattening diet for lambs. Three experiments were conducted. The first experiment of 144-h incubations was aimed to determine comparatively the gas production kinetics of SBM and GM. The gas production kinetics and the ruminal digestibility and fermentation of the fattening diet, containing different proportions of SBM and GM, were also studied by the incubations of 144-h and 24-h in the second and last experiments, respectively. The results of the first kinetic experiment indicated a higher asymptote of gas production (a), half time to asymptote of gas production ( $T_{1/2}$ ) and metabolizable energy (ME) and a lower fractional rate of gas production ( $\mu$ ) for SBM than GM ( $P < 0.001$ ). Replacing SBM with GM had no effect on gas production kinetics of the fattening diet at any substitution levels in the second kinetic experiment ( $P > 0.05$ ). However, the gas produced after 24-h of incubation ( $GP_{24}$ ), *in vitro* true dry matter degradability (IVTDM), *in vitro* true organic matter degradability (IVTOMD), partitioning factor (PF), microbial biomass production (MBP) and total volatile fatty acids (TVFA) concentration increased with D2 compared to D1 and D3 in the last experiment. The ammonia concentration decreased with D2 and D3 compared to D4 ( $P < 0.05$ ), nevertheless, the ruminal volatile fatty acids (VFA) pattern was not affected by the treatments ( $P > 0.05$ ). These results demonstrated that the protein from SBM might be replaced by that from GM in fattening diets for lambs at the levels up to 67%, but the 33% substitution is recommended because of its beneficial effects on ruminal digestibility and fermentation.

**KEY WORDS** soybean meal, guar meal, gas production.

### **INTRODUCTION**

Guar is a subtropical annual legume cultivated mainly in arid regions and used as animal feed, green manure or for extraction of gum, which the latter is of higher commercial importance (Sharma and Gummagolmath, 2012). The crude protein (CP) content of guar meal (GM) varies depending on the proportion of the hull and germ, as well as the pro-

portion of the gum remaining in the meal after gum extraction (Nagpal *et al.* 1971; Conner, 2002; Sharma and Gummagolmath, 2012). The nutritive value of GM is comparable to that of soybean meal (SBM), though its methionine and lysine contents are lower than those found in SBM (Verma and McNab, 1984) yet, it has some advantages such as lower cost and lower content of trypsin inhibitor compared to SBM (Verma and McNab, 1982; Conner, 2002).

Thus, its substitution to SBM in the diets of ruminants has been studied in some experiments in recent years (Ahmed *et al.* 2000; Vatandoust *et al.* 2010; Turki *et al.* 2011). A higher performance has been reported for animals fed the diets containing GM, especially at lower inclusion levels (Mahdavi *et al.* 2010; Vatandoust *et al.* 2010; Salehpour and Qazvinian, 2011). Part of higher performance in animals fed the diets containing GM in these experiments may be originated from an improved ruminal digestion and fermentation of these diets, particularly regarding a higher ruminal degradability of GM protein compared to that of SBM (Habib *et al.* 2013b; Marghazani *et al.* 2013). However, there are few data related to the impact of GM on ruminal digestibility and fermentation when included in the diets of ruminants. This concerns both *in vivo* and *in vitro* experiments, however, because of higher cost and inconvenience of *in vivo* experiment, *in vitro* experiments, particularly those based on gas production technique can provide valuable information on the effect of supplements on rumen digestibility and fermentation. Hence, the objective of the present study was to assess the effects of the replacing SBM with GM on rumen gas production kinetics and ruminal digestibility and fermentation in a fattening diet for lambs.

## MATERIALS AND METHODS

### Experiments

The current study was composed of three *in vitro* experiments, of which two were completed with the incubations of 144-h (kinetic experiments) and the last with the incubations of 24-h of the samples. The first kinetic experiment was for comparative determination of gas production kinetic features of SBM and GM. The second kinetic experiment was made to assess the effects of the replacing SBM with GM on ruminal gas production kinetics of a fattening diet for lambs at the substitution levels of 33, 67 and 100% (CP basis, i.e. the protein content in the SBM was replaced at the above mentioned levels by that of GM).

The last experiment of 24-h incubation was conducted to evaluate the effects of the replacing SBM with GM at the same levels mentioned above on ruminal digestibility and fermentation of the fattening diet.

### Guar meal and experimental diets

Guar meal was purchased from Ariya Shirin Noush Co. (Ariya Shirin Noush, Shiraz). A diet was formulated according to NRC (1985) to meet the nutrient requirements of growing lambs. Soybean meal in the basal diet (D1) was replaced by GM at the above-mentioned levels in D2-D4 (Table 1). The metabolizable energy and CP contents of the diets were the same. These experimental diets were used as

the fermentation substrates (treatments) in the incubation media.

**Table 1** Chemical composition of guar meal (GM) and soybean meal (SBM)

Supplement	Chemical composition (% of DM)					
	DM	OM	CP	EE	Ash	NDF
SBM	95.77	93.58	44.52	7.22	6.42	9.28
GM	97.40	93.33	62.33	7.19	6.92	18.93

DM: dry matter (%); OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; GM: guar meal; SBM: soybean meal.

### Animals and rumen fluid

Rumen fluids were collected before the morning feeding from three ruminally fistulated matures Mehraban rams (50±4 kg of body weight). To meet nutrient requirements, the rams were fed *ad libitum* a mixed diet composed of (per kg DM) 700 g alfalfa hay and 300 g barley plus minerals and vitamins (NRC, 1985), providing 9.58 MJ ME and 137.4 g CP. Rumen fluids were then pooled and strained through four layer cheese clothes into a pre-warmed, insulated flask and immediately transported to the laboratory.

### *In vitro* gas production

*In vitro* gas production procedure was conducted as described by Menke and Steingass (1988). While incubation substrates in the first kinetic experiment were GM and SBM, the substrates in the other experiments were the experimental diets with different proportions of SBM and GM. In the incubations of 144-h, a representative air-dried sample was ground to pass a 1 mm sieve and sub-samples of 200 mg (DM basis) were weighed into 100 mL glass syringes. Incubation of the samples was conducted in triplicate with 30 mL of buffered rumen fluid under continuous flow of CO<sub>2</sub>. Three syringes containing 30 ml of buffered rumen fluid without substrate were considered as blanks. The syringes were then placed in a water-bath at 39 °C and gas volume was recorded at 2, 4, 6, 8, 12, 24, 48, 72, 96, 120 and 144 h of incubation. In the experiment of 24-h, in order to reduce the inherent error with gravimetric determination of substrate digestibility in the incubations, a higher amount (500 mg) of the substrates were incubated in 100 mL glass syringes with 40 mL of buffered rumen fluid according to the method illustrated by Makkar *et al.* (1995). After 24-h of incubation, contents of the syringes were transferred into centrifuge tubes and were immediately placed in cold water at 4 °C to stop the fermentation. Tube contents were then centrifuged at 15000 × g for 20 min at 4 °C, aliquots of 4 ml of the supernatants were mixed with 1 ml of 25% metaphosphoric acid and frozen at -20 °C until subsequent analysis for volatile fatty acids (VFA) and ammonia content. Remaining residues in the tubes were boiled with neutral detergent solution at 100 °C for 1 h to estimate

the *in vitro* true dry matter degradability (IVTDMD) after being oven-dried at 60 °C for 48 h. The recovered residues were subsequently incinerated in sintered glass crucibles at 600 °C to quantify the *in vitro* true organic matter degradability (IVTOMD).

### Chemical analyses

Dry matter, total ash, ether extract (EE) and CP were measured according to the standard methods described by AOAC (2000). The NDF was determined as described by Van Soest *et al.* (1991) and are expressed exclusive of residual ash. Ammonia concentration in the supernatant was determined as illustrated by Broderick and Kang (1980). VFA concentrations of the samples were quantified according to Ottenstein and Bartley (1971) using a gas chromatograph (GC-FID, PU4410-PHILIPS, England) equipped with a flame ionization detector and a 10PEG column.

### Calculations and statistical analysis

Data of the gas produced at different times during 144-h of incubation were fitted to the model proposed by France *et al.* (1993) as shown in the below, by NLIN procedure of SAS (SAS, 2002).

$$GP = \alpha \{1 - e^{-[b(t-L) + c(\sqrt{t-L})]}\}$$

Where:

GP (mL): is the gas produced at the time.

t and a (mL): are the asymptote of gas production.

b and c: are constants.

L (h) is the lag time.

$T_{1/2}$  (h, time to half asymptote of gas production) and  $\mu$  (/h, fractional rate of gas production at  $T_{1/2}$ ) were calculated using the following equations (France *et al.* 1993):

$$T_{1/2} = [(-c/2 + \sqrt{\{c^2/4 + b[bL + c\sqrt{L} - \ln(0.5)]\}}) / b]^2$$

$$\mu = b + c/(\sqrt{2}T_{1/2})$$

Organic matter digestibility (OMD, %), short chain fatty acids (SCFA, mmol/200 mg DM) and metabolizable energy (ME, MJ/kg DM) for SBM and GM was estimated according to Menke and Steingass (1988), using the following equations:

$$OMD = 14.88 + 0.889 GP + 0.45 CP + 0.0651 Ash$$

$$SCFA = 0.0222 GP - 0.00425$$

$$ME = 1.06 + 0.157 GP + 0.084 CP + 0.22 EE - 0.081 Ash$$

Where:

GP: is 24-h net gas production (mL/200 mg DM).

CP, EE and OM are crude protein, ether extract and organic matter (% of DM), respectively.

The ratio of organic matter truly degraded (mg) to the gas produced (mL) after 24-h of incubation was used as PF (Blummel *et al.* 1997). The microbial biomass production (MBP) was estimated using the equation (shown below) proposed by Blummel (2000).

$$MBP = TSD - (\text{gas volume} \times SF)$$

Where:

TSD: is the true substrate (organic matter) degraded at the end of incubation (24-h).

SF: is stoichiometric factor equivalent to 2.2, and the gas volume refers to the gas produced at 24-h of incubation.

The estimated kinetic parameters and data (measured variables) of 24-h experiment were subjected to analysis of variance by GLM procedure of SAS (SAS, 2002) using the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

$Y_{ij}$ : is the observation.

$\mu$ : is the overall mean for each parameter.

$T_i$ : is the effect of treatments (SBM vs. GM in the first experiment and type of the diet, containing different proportions of SBM and GM, in the other experiments).

$e_{ij}$ : is the residual error.

In the case of statistical significance, declared at  $P < 0.05$ , the means for each parameter or variable were compared between the treatments using Duncan's multiple range test. The means of estimated parameters in the first experiment were compared using the t-test.

## RESULTS AND DISCUSSION

### Chemical composition of SBM and GM

Except the CP and NDF contents, which were higher in GM than SBM, a comparable chemical composition was observed for these two supplements (Table 2). The chemical composition of GM in the current study was also comparable to heat processed GM which was used in the study of Danesh Mesgharan *et al.* (2010).

### Gas production kinetics of SBM and GM

In the first kinetic experiment, the parameters of 'a' and  $T_{1/2}$  were higher for SBM (Table 3) compared to those for GM ( $P < 0.001$ ). A very short lag phase (L) was observed which was almost similar for both supplements, however, the fractional rate of gas production ( $\mu$ ) was higher for GM when

compared to that of SBM ( $P<0.001$ ). Moreover, a higher amount of DOMD, SCFA and ME were determined for SBM than for GM ( $P<0.001$ ).

**Table 2** Ingredients and chemical composition of the fattening diets, containing guar meal (GM) replaced to soybean meal (SBM) at different levels (0 as D1, 33% as D2, 67% as D3 and 100% as D4, based on CP content)

Ingredient (% DM basis)	Diets			
	D1	D2	D3	D4
Alfalfa hay	32.7	31	28.9	27.1
Wheat straw	5.2	5.9	6.9	7.7
Barely	50.1	52.1	54.1	56.1
Soybean meal	10	6.6	3.3	0
Guar meal	0	2.4	4.8	7.1
Limestone	1	1	1	1
Minerals and vitamins	1	1	1	1
Chemical composition (% of DM)				
OM	93.0	93.0	93.0	93.1
CP	15.4	15.4	15.4	15.4
EE	2.2	2.1	2.1	2.0
NDF	28.7	29.1	29.5	29.9
ME (MJ/ Kg DM)	10.9	10.9	10.9	10.9

OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ME: metabolizable energy.

**Table 3** Estimated parameters of gas production kinetic for guar meal (GM) and soybean meal (SBM)

Parameters	Supplements	
	GM	SBM
a (mL)	223.9±9.03 <sup>b</sup>	338.7±6.72 <sup>a</sup>
L (h)	0.032±0.0163	0.027±0.0036
T <sub>1/2</sub> (h)	6.22±0.448 <sup>b</sup>	8.06±0.402 <sup>a</sup>
μ (/h)	0.100±0.0060 <sup>a</sup>	0.080±0.0012 <sup>b</sup>
OMD (%)	78.4±1.100 <sup>b</sup>	92.7±1.03 <sup>a</sup>
DOMD (%)	73.1±1.03 <sup>b</sup>	86.7±0.96 <sup>a</sup>
SCFA (mmol/200 mg DM)	0.81±0.029 <sup>b</sup>	1.19±0.028 <sup>a</sup>
ME (MJ/kg)	13.03±0.195 <sup>b</sup>	14.08±0.181 <sup>a</sup>

<sup>a</sup> a: asymptote of gas production (mL per 200 mg DM); L: lag time; T<sub>1/2</sub>: half time to asymptote; μ: fractional rate of gas production; OMD: organic matter digestibility; DOMD: digestible organic matter in dry matter; SCFA: short chain fatty acids; ME: metabolizable energy; GM: guar meal; SBM: soybean meal. The values with different letters in the same row are significantly different at  $P<0.05$ .

The principal objective of the present study was to assess the replacement effect of SBM by GM in a fattening diet for lambs on its ruminal digestibility and fermentation. This required more information about the nutritional value of these supplements, however, the majority of data in the literatures about these supplements, especially on GM, have focused on their protein content and characteristics (Mondal *et al.* 2008; Jahani-Azizabadi *et al.* 2010; Habib *et al.* 2013b; Marghazani *et al.* 2013). Thus, the parameters of gas production kinetic obtained in the first experiment provided more information, especially on energetic values of SBM and GM, which made it possible to have a reliable iso-energetic and iso-nitrogenous diets (substrates) in the second and third experiments.

The kinetic parameters revealed a greater potential for SBM than for GM to produce the gas during ruminal incubation, resulting in higher values of OMD, SCFA and ME for SBM.

This is due to lower CP content of SBM comparing to GM, because the metabolites originated from the initially degradation of proteins have less chance than those of carbohydrates to be fermented and participated in gas production (Wolin, 1960). Therefore, despite the similar OM content, a higher ME was estimated for SBM than for GM. Nevertheless, a higher fractional rate of gas production, which obtained at shorter T<sub>1/2</sub> for GM, may be due to a higher proportion of soluble or quickly degradable fraction of the OM involved in the gas produced from GM compared to that of SBM.

This hypothesis is supported by the results of some *in sacco* experiments, which reported a higher 'a' fraction for GM than SBM (Habib *et al.* 2013a; Marghazani *et al.* 2013). Additionally, in an *in sacco* experiment in our laboratory (unpublished data), the 'a' fraction of GM dry matter was estimated equivalent to 37.1%, which is much higher than those reported for SBM (Mandal *et al.* 1999; Habib *et al.* 2013a).

#### Ruminal gas production kinetics of the diets containing different levels of GM substituted to SBM

Substitution of SBM by GM had no significant effect on any estimated parameters of gas production ( $P>0.05$ ). However, a numerical increase was observed with 33% replacement (D2) on the parameters 'a' and μ (Table 4). A higher asymptote of gas production in D2 appears to be due to the higher rate of gas production, resulting probably from an appropriate availability of nitrogen and amino acid for rumen microorganisms (Shingfield *et al.* 2003).

#### Ruminal digestibility and fermentation of the diets containing different levels of GM substituted to SBM

Replacement of SBM by GM modified ruminal digestibility of the diets and their fermentation (Table 5), as the highest GP<sub>24</sub> was observed with D2, which was significantly higher than that with D1 and D4 ( $P<0.05$ ).

**Table 4** Ruminal gas production kinetics of a fattening diet for lambs containing different levels of guar meal (GM) substituted to soybean meal (SBM)

Parameters <sup>1</sup>	Diets <sup>2</sup>				SEM	P
	D1	D2	D3	D4		
a	358.4	379.5	341.2	347.9	16.80	0.39
L	0.44	0.63	0.47	0.60	0.150	0.75
T <sub>1/2</sub>	12.3	11.6	11.9	12.5	0.380	0.39
μ	0.060	0.066	0.061	0.061	0.0041	0.62

<sup>1</sup> a: asymptote of gas production (mL per 200 mg DM); L: lag time (h); T<sub>1/2</sub>: half time to asymptote (h); μ: fractional rate of gas production (/h).

<sup>2</sup> The fattening diets containing different levels of GM (0 as D1, 33 as D2, 67 as D3 and 100% as D4, based on CP content) replaced to SBM. SEM: standard error of mean.



The same variation was observed for the IVTDMD and IVTOMD, the highest values for these variables were obtained at 33% inclusion of GM (CP basis) in the diet ( $P < 0.001$ ). The PF, as the index of organic matter partitioning between two pathways, including microbial biomass and VFA production, was also higher with D2 compared to D1 and D4 ( $P < 0.001$ ). The variation of MBP among the diets was highly correlated with that of partitioning factor (PF), as it increased with D2 and D3 compared to D1, but decreased dramatically at the highest level of GM in the diet (D4).

The modification in total volatile fatty acids (TVFA) concentration also followed closely that of  $GP_{24}$ , as its highest value was observed with D2, which tended to be different significantly from those measured with the other diets ( $P = 0.059$ ).

In contrast to MBP, which was higher with D2 and D3 compared to the other diets, the  $NH_3$  concentration was reduced with D2 and D3 compared to D1, but increased considerably with D4. The  $GP_{24}$  variation was coherent with that of TVFA. It was expected, because there is a close linkage between the gas, as waste product, and VFA, as useful product of the fermentation, and both are the products of the same pathway in the rumen fermentation (Blummel *et al.* 1997).

Moreover, there was a high correlation between the fermentation end products ( $GP_{24}$  and TVFA) and ruminal DM and OM digestions, as the highest value for all of these variables were obtained with D2. A huge increase in MBP with D2 and D3 was highly related to a higher DM and OM digested in the same diets. There are no comparable *in vitro* data in the literature; however, some results obtained *in vivo* are coherent with those obtained in the current study. In this regard, Vatandoust *et al.* (2010) reported a higher-milk yield and milk fat percent with 4% replacing GM to SBM in the diet of early lactating Holstein cows. Likewise, inclusion of GM instead of cottonseed meal has been resulted in a higher milk fat and protein percent and yield at the level of 3.48% compared to 0, 5.86, and 11.6% in the diet of Holstein dairy cows (Salehpour and Qazvinian, 2011).

A higher feed intake and weight gain has also been reported during the first month of the finishing period for the lambs fed the diet in which GM substituted to SBM (Mahdavi *et al.* 2010). In other experiment conducted by Turki *et al.* (2011), the sheep fed a diet containing 15% GM had higher weight gain and lower feed conversion ratio compared to those fed diets with 0, 25 and 32% GM. In all of these experiments, lower inclusion levels of GM have resulted in the best performance. It might partly been related to some modifications caused by GM in ruminal fermentation, as it was observed in the present study. Positive

effect of GM on rumen fermentation at lower inclusion levels is of high probability due to its protein characteristics, which may result in a higher synchronization of energy and nitrogen for rumen microorganisms. However, its depressive effect at higher levels may be of different origins. Its anti-nutrients compounds, such as anti-trypsin (Lee *et al.* 2003), phenolic compounds and galactomannans (Van Nevel *et al.* 2005) can negatively affect rumen fermentation, though the amount of these compounds in processed GMs is very low.

The results from the current research demonstrated that the inclusion of SBM and GM together has more beneficial effect on ruminal digestion and fermentation than when each used alone, especially with 33% replacement of SBM by GM. This may be due to a better supply of nutrients to rumen microorganisms, resulting in a higher OM digestion and, thereby, producing a higher  $GP_{24}$  and TVFA with D2.

In data comparing directly the ruminal degradability of protein from GM and SBM, a higher quickly degradable fraction (a) have often been reported for GM: 18.4 vs. 16.8%, (Habib *et al.* 2013b); 22.7 vs. 16.5% (Marghazani *et al.* 2013) for GM and SBM, respectively. Furthermore, a higher ruminal degradability of protein has been reported for GM compared to SBM in most of the experiments (Olaisen *et al.* 2003; Mondal *et al.* 2008; Marghazani *et al.* 2013).

Additionally, Mondal *et al.* (2008), reported that the (A+B<sub>1</sub>) and B<sub>2</sub> fractions of protein were higher in GM compared to SBM (46.4%, 20.8% vs. 41.6%, 15.9% for GM and SBM, respectively). Regarding these results, it can be concluded that the higher solubility or the higher proportion of 'a' fraction of protein in GM than in SBM may explains part of the improved rumen fermentation in D2 in the current study. nitrogen to the rumen has resulted in a better synchronization of energy and nitrogen for rumen microorganisms in D2, giving rise, as it can be deduced. Indeed, it appears that early supply of higher amount of from MBP, to their higher growth in D2 and D3.

This suggestion is supported regarding a high proportion of barley in the diets, as a quickly fermentable substrate in the rumen, which provides a high amount of fermentable energy for rumen microorganism in early times of incubation.

However, decreasing all variables of rumen digestibility and fermentation with D4, may be caused by a poor balance of amino acids, especially by a lower methionine and lysine contents in GM than SBM (Verma and McNab, 1984). The increase in TVFA with D2 was coherent with that of IVTOMD in this diet, however, the increment in IVTDMD and IVTDMD were more pronounced. It seems that the inclusion of GM in D2 and somewhat D3 has not only stimulated the ruminal fermentation, but also caused a redirection of digested OM from VFA and gas production

**Table 5** Ruminal digestibility and fermentation of a fattening diet for lambs containing different levels of guar meal (GM) substituted to soybean meal (SBM)

Parameters <sup>1</sup>	Diets <sup>2</sup>				SEM	P
	D1	D2	D3	D4		
GP <sub>24</sub>	141.1 <sup>b</sup>	145.8 <sup>a</sup>	142.8 <sup>ab</sup>	138.1 <sup>b</sup>	1.32	0.024
IVTDMD	72.5 <sup>c</sup>	94.4 <sup>a</sup>	88.9 <sup>b</sup>	63.3 <sup>d</sup>	0.44	0.0001
IVTOMD	73.2	94.4 <sup>a</sup>	89.4 <sup>c</sup>	66.6 <sup>d</sup>	0.36	0.0001
PF	2.46 <sup>b</sup>	3.06 <sup>a</sup>	2.97 <sup>a</sup>	2.28 <sup>c</sup>	0.029	0.0001
MBP	35.6 <sup>c</sup>	125.5 <sup>a</sup>	109.7 <sup>b</sup>	14.3 <sup>d</sup>	3.74	0.0001
TVFA	86.0 <sup>b</sup>	102.84 <sup>a</sup>	88.79 <sup>b</sup>	86.07 <sup>b</sup>	3.44	0.059
NH <sub>3</sub>	17.24 <sup>ab</sup>	9.56 <sup>b</sup>	13.38 <sup>b</sup>	28.11 <sup>a</sup>	3.48	0.029

<sup>1</sup> GP<sub>24</sub>: gas produced after 24-h of incubation (mL per 500 mg DM); IVTDMD: *in vitro* true dry matter degradability (%); IVTOMD: *in vitro* true organic matter degradability (%); PF: partitioning factor (g organic matter degraded to mL gas produced), MBP: microbial biomass production (mg); TVFA: total volatile fatty acids (mmol/L); NH<sub>3</sub> (mmol/L).

<sup>2</sup> The fattening diets containing different levels of GM (0 as D1, 33 as D2, 67 as D3 and 100% as D4, based on CP content) replaced to SBM.

**Table 6** Ruminal VFAs pattern of a fattening diet for lambs containing different levels of guar meal (GM) substituted to soybean meal (SBM).

Variables <sup>1</sup>	Diets <sup>2</sup>				SEM	P
	D1	D2	D3	D4		
TVFA (mmol/L)	86.1 <sup>b</sup>	102.8 <sup>a</sup>	88.8 <sup>b</sup>	86.1 <sup>b</sup>	3.44	0.059
VFA molar proportion (mmol /100 mmol)						
Acetate (A)	64.5	63.0	61.9	61.5	1.12	0.35
Propionate (P)+ isobutyrate	23.8	22.4	24.5	25.0	1.02	0.4
Butyrate	9.2	11.0	10.7	9.9	0.92	0.55
Isovalerate	0.9	1.9	1.1	1.9	0.58	0.56
Valerate	1.7	1.7	1.9	1.8	0.11	0.49
P/A	0.37	0.36	0.4	0.41	0.021	0.389

<sup>1</sup> TVFA: Total volatile fatty acid; P/A: propionate to acetate ratio.

<sup>2</sup> The fattening diets containing different levels of GM (0 as D1, 33 as D2, 67 as D3 and 100% as D4, based on CP content) replaced to SBM.  
SEM: standard error of the means.

pathway to that of MBP. This may be explanatory for a huge increase in MBP and PF with D2 and D3. This suggestion is supported by a decrease in ruminal NH<sub>3</sub> concentration with D2 and D3. Indeed, the ruminal concentration of NH<sub>3</sub> at any given time reflects the balance between its production from proteins degraded in the rumen and its loss due to NH<sub>3</sub> consumption by the rumen bacteria especially by the cellulolytics (Nolan and Dobos, 2005). Thus, despite the increase in ruminal OM digestion and VFA production with D2 and D3, the decrease in ruminal NH<sub>3</sub> could be indicative of its consumption effectively by the rumen bacteria in these diets.

#### Ruminal VFAs pattern of the diets containing different levels of GM substituted to SBM

Despite the increase in TVFA with D2 (Table 6), the substitution of SBM by GM had no effect on VFA pattern in the diets ( $P>0.05$ ). The ratio of propionate to acetate (P/A) also remained unchanged with inclusion of GM in replace to SBM. These results reveal that replacing SBM by GM in fattening diets has no effect on the rumen proportions of principal microorganism populations.

#### CONCLUSION

The results of this study indicated that replacement of SBM by GM has a stimulatory effect on ruminal digestibility and

fermentation of fattening diets at 33% replacement level (CP content basis). However, this beneficial effect on the rumen microbial ecosystem seems to be directed mainly toward microbial biomass synthesis. Regarding the higher CP content of GM compared to SBM, GM, as a cheaper protein supplement, can be replaced to SBM at the levels of up to 67% (CP content basis) in fattening diets for lambs.

#### ACKNOWLEDGEMENT

This research was supported by the Faculty of Agriculture, Bu-Ali Sina University. The authors would like to thank Arya Shirin Noush Com. for providing the guar meal. We also thank Dr. Ghaziani (Tehran University, Karaj), for her collaboration in analysis of VFA.

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