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Effects of microwaving, and moist and dry heating on ruminal degradability of protein and dry matter in soybean meal

F. Rezaii[1*] and J. Bayat kouhsar[2]

[1]Agriculture Department, Payame Noor University, Tehran, Iran.
[2]Department of Animal Science, Faculty of Agriculture, University of Gonbad, Gonbad, Iran.
*Corresponding author, E-mail address: frezaii@pnu.ac.ir

Abstract Two experiments were conducted to evaluate the effects of microwaving, autoclaving (moist heating), and roasting (dry heating) on gas production parameters and *in situ* rumen degradability of soybean meal (SBM). SBM was treated by roasting at 140℃ for 30 and 60 min, and at 160℃ for 30 and 60 min, autoclaving at 121℃ for 20 min, and microwaving for 2, 4, and 6 min. A gas production trial was performed using rumen fluid collected from fistulated sheep. Cumulative gas production was recorded at 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 h after incubation. The organic matter digestibility (OMD), metabolizable energy (ME), net energy (NE), and short-chain fatty acid (SCFA) in gas production trial were calculated after 24 h incubation. For *in situ* technique, three fistulated sheep of the Dalaq breed (with an average weight of 45 ± 1 kg) were housed in individual cages and fed at the level of maintenance. Samples of the processed and unprocessed SBM were ground to pass through a 3 mm screen, and 5 g of each sample were transferred into nylon bags. The results showed that treatments had a significant effect on gas production potential (P<0.05). With increasing the heating duration, gas production potential increased in roasted treatments. Processing methods significantly reduced gas production (P<0.05). The lowest volume of gas production was recorded for the autoclave treatment. Processing with Micro-6 and moist heating (autoclave) resulted in a significant decrease in organic matter digestibility (OMD) compared to dry heating (roasting). Micro-6 treatment significantly (P<0.05) decreased the concentration of SCFA compared to other treatments. The results of *in situ* experiments showed that processing of SBM significantly reduced the rate of dry matter and protein degradation (P<0.05). Processed SBM had a lower rapid degradable fraction (a) and higher slowly degradable fraction (b) compared to the control treatment. In conclusion, the methods of heating (autoclaving, dry heating, and microwaving) of SBM decreased the gas production and gas production rate and ruminal protein degradability.

Keywords: soybean meal, heat process, microwave, degradability

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Introduction

Protein content is a necessary component of the diet required for growth and reproduction. For maximizing dairy cow productivity and efficiency of protein utilization, supplying proper quantity and pattern of essential amino acids (EAA) in metabolizable protein (MP) is essential (Ipharraguerre and Clark, 2014). Modern protein evaluation systems describe the actual protein requirement that is digested and absorbed from the small intestine (Wei Gao, 2015**)**. Available MP for absorption in the small intestine depends on the flow and digestibility of microbial crude protein (MCP) and dietary ruminal undegradable protein (RUP) (NRC, 2001). The application of MP for production purposes requires the evaluation of ruminal MCP production, the quantity of RUP in feeds, and their intestinal digestibility of MCP and RUP. The approach most frequently considered for improving the quantity and profile of EAA that reach the small intestine of dairy cows is to feed CP supplements high in RUP (NRC, 1985; 2001). The main benefit of RUP is that the original amino acids in the protein meal are absorbed in the small intestine instead of being converted into microbial protein in the rumen, thereby providing a different balance of EAA for better animal nutrition and production (Schroeder, 1997).

SBM is the most commonly used protein source in the animal diet because of its excellent amino acid profile (Min et al., 2009). In addition, it contains dietary fiber, minerals, and essential fatty acids (Esteves et al., 2010). Therefore, the use of high-quality protein sources like SBM may be more efficient if their proteins are mainly protected from ruminal degradation to supply directly amino acids for the host animal (Thanh and Suksombat, 2015). There are several methods to prevent degradation of dietary protein in the rumen (El-Shabrawy, 1996). Of these methods, roasting and extrusion are most commonly used in the feed industry. All methods involve the Maillard reaction. This reaction can be controlled by optimizing the heating process (El-Waziry, 2007). Thorough heating of protein supplement causes denaturation of protein and provides effective protection against microbial fermentation in the rumen (Dakowski et al., 1996). However, overheated protein sources may result in lowered intestinal digestibility in ruminants (Dakowski et al., 1996). Thus, careful control of heating conditions is required to optimize the nutritive value (Ljokjel et al., 2000). The aim of the present study was to evaluate the effect of additional heat treatment in different forms (autoclaving, roasting, and microwaving) of SBM on the RUP content and digestibility of nutrients compared to unprocessed SBM.

Materials and methods

Treatments and chemical analysis

This study was conducted in the Agricultural Faculty of Gonbad University. The treatments included un-processed SBM (control), roasted SBM at 140°C for 30 and 60 min (140-30 and 140-60), 160°C for 30 and 60 min (160-30 and 160-60), autoclaved SBM at 121°C for 20 min (autoclave), and microwaved SBM at 120°C under 500 watt for 2 (Micro-2) 4 (Micro-4), and 6 min (Micro-6).

For the roasting process, SBM was spread out in an aluminum tray and placed in a digital oven set at 140 and 160°C for 30 and 60 min. In the autoclaving process, SBM was prepared as 500-g batches and autoclaved at 121°C and one-atmosphere pressure for 20 min.

After autoclaving, roasting, and microwaving processes, the SBM samples were transferred to a tray to cool down, transferred into plastic bags and refrigerated at 4°C until use.

The chemical composition of processed SBM samples consisting of dry matter, organic matter, ash, crude protein, and crude fiber were determined in accordance with AOAC (2005).

In situ procedure

To measure the ruminal disappearance of SBM dry matter, the nylon bag technique was used (Mohrez and Orskov, 1977). For this purpose, three fistulated sheep of the Dalaq breed (average body weight of 45 ± 1 kg, SD) were used. The sheep were housed in individual cages, fed at the maintenance level (approximately 1.5 kg) with free access to drinking water. The fistulated sheep were fed daily in the morning and the afternoon (0800 and 1600). Synthetic polyester nylon bags (8 × 14 cm with a pore diameter of 45 to 50 µm, and indigestible in the rumen) were used.

The processed SBM samples were ground to pass 3 mm screen. Then, 5 g of each sample was transferred into nylon bags. Four nylon bags per treatment in each of the incubation time were placed in the rumen of fistulated sheep and incubated for 2, 4, 8, 12, 24, 36, 48, 72, and 96 h. To estimate the amount of feedstuff disappearance at time zero (the water-soluble fraction) the bags containing the feed samples were rinsed for 30 min with cold water until water was quite clear. After incubation, the bags containing the undigested feed materials were removed from the rumen, immediately rinsed with cold water for 30 min, oven-dried at 65°C for 48 h, and weighed for calculation of dry matter degradability (Orskov and McDonald, 1979) using the following formula:

$$\text{Dry matter Degradability (\%)} = \frac{\text{dry matter before ruminal incubation- dry matter after ruminal incubation}}{\text{dry matter before ruminal incubation}} \times 100$$

The parameters of dry matter degradation were estimated using the SAS software (SAS, 2003). The following non-linear relationship (Orskov and McDonald, 1979) was used to fit data:

$P = a + b\,(1 - e^{-ct})$

in which,

P: degradability potential

a: the rapidly soluble fraction

b: the potentially degradable fraction which disappears at a constant rate

e: Euler's number

c: a constant rate of degradation

t: time of ruminal incubation

The effective dry matter degradability of processed SBM was estimated using the following equation (Orskov and McDonald, 1979):

$ERD = a + [\frac{b \times c}{c + k}]$

where,

ERD: effective ruminal degradability

a, b, and c: previously described

k: passage rate

Gas production procedure

The volume of gas produced was measured by using the pressure indicator and glass vials containing artificial saliva and filtered ruminal fluid (Menk et al., 1979). Samples of processed SBM were ground using a grinding mill to pass 1-mm screen, oven-dried at 65℃ for 48 h, and 200-mg sub-sample transferred into glass vials. For each sample, four replicates were used as the test while another four vials without sample were used as the control. Ruminal fluid was prepared from three sheep used in the degradation experiment. Flasks containing the filtered rumen fluid were bubbled with carbon dioxide and incubated at 39℃. Artificial saliva and rumen fluid were mixed at a ratio of 2:1 and 30 mL of the mixture were added to glass vials containing 0.2 gr sample or control. Immediately, each vial was bubbled with carbon dioxide for 10 s and sealed using rubber stoppers and aluminum cover. The vials were transferred to a shaking water bath at 39℃ and incubated for 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 h after which the gas pressure was measured using a pressure gauge.

Cumulative gas production was determined according to Orskov and McDonald (1979). The OMD, ME, and NE were determined according to Menk et al. (1979) equations. The amount of SCFA was calculated using the Makar's (2004) equation.

OMD (%) =14.88+ 0.889 GP+ 0.45 CP + 0.0651 XA

ME (MJ/kgDM) = 2.20 + 0.136 GP + 0.057 CP + 0.0029 CF

NE (MJ/kgDM) = -0.36+ 0.114 GP+ 0.0054CP+ 0.0139 EE– 0.0054 XA

SCFA (mmol) = 0.0222 GP- 0.00425

OMD: organic matter digestibility
ME: metabolizable energy
NE: net energy
SCFA: short-chain fatty acids
GP: Net gas production after 24 hours (per 200 mg sample dry matter)
CP: crude protein (%)
XA: ash (%)
CF: crude fiber (%)
EE: ether extract (%)

Gas production parameters were estimated as described by Orskov and McDonald (1979):

$y = b\ (1 - e^{-ct})$

where,
y: the gas produced at the time of incubation
b: gas production from an insoluble fermentable fraction
e: Euler's number
c: gas production rates for b
t: incubation time

Statistical analysis

The data were analyzed in a completely randomized design using the GLM procedure (SAS, 2003) as:

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

where,
Y_{ij}: the dependent variable
μ: the overall mean
T_i: main effect of treatments
e_{ij}: experimental error

Results

Gas production parameters of processed SBM

The effects of heating processing (roasting, microwaving, and autoclaving) on gas production parameters including the predicted ME (MJ/kg DM), NE (MJ/kg DM), and OMD (%) are shown in Table 1. The heating processes had a significant effect on gas production potential ($P<0.05$). Control SBM samples recorded the lowest gas production potential (228.2 mL/g DM) compared to processed treatments (235.5, 262.9, 262.2, 270.2, 226.6, 289.5, 254.4, and 262.2 mL/g DM, respectively, for 140-30, 140-60, 160-30, and 160-60, Micro-2, Micro-4, Micro-6, and autoclave). By increasing the heating time, the gas production potential was increased in roasted treatments. Processing with microwave at 2, 4, and 6 min had a significant effect on the gas production potential so that the highest and the lowest values were found for 4 and 2 min, respectively. There was a significant effect of processing methods on gas production (mL) in various incubation times (Table 2).

Energy contents and OMD

There was a significant difference between thermal processing methods in terms of estimated parameters ($P<0.05$). Processing with Micro-6 and moist heating (autoclave) resulted in a significant decrease in OMD compared to dry heating (Table 1). There was no significant difference between the dry and moist heating methods in terms of ME. However, processing with Micro-6 significantly reduced ME ($P<0.05$). In addition, the moist heating process significantly decreased NE compared to dry heating (at 160℃, Table 1).

Short chain fatty acids (SCFA)

In Micro-6 treatment, the concentration of SCFA was significantly ($P<0.05$) decreased compared to other treatments (Table 1).

Table 1. Gas production parameters and energy contents of processed and unprocessed soybean meal

Treatment[1]	Gas production potential (mL/g DM)	Gas roduction constant rate (mL/g DM)	OMD[2] (%)	ME[3] (MJ/kg)	NE[4] (MJ/ kg)	SCFA[5] (mmol)
Control	228.2±4.74	0.035±0.0018	62.68[abc]	8.99[ab]	3.02[b]	0.66[ab]
140-30	235.5±4.51	0.037±0.0017	61.96[bc]	8.66[ab]	2.73[b]	0.61[ab]
140-60	262.96±6.73	0.037±0.0024	63.53[ab]	9.10[ab]	3.10[ab]	0.68[ab]
160-30	262.2±2.38	0.038±0.0008	66.14[a]	9.22[a]	3.53[a]	0.70[a]
160-60	270.2±5.82	0.035±0.0018	66.07[a]	9.20[a]	3.50[a]	0.70[a]
Micro-2	226.6±5.66	0.0466±0.0034	63.79[ab]	9.04[ab]	3.04[b]	0.67[ab]
Micro-4	289.5±10.42	0.0292±0.0023	64.07[ab]	9.04[ab]	3.06[b]	0.67[ab]
Micro-6	254.4±4.97	0.038±0.0019	59.45[c]	8.55[b]	2.96[b]	0.59[b]
Autoclave	262.2±6.16	0.034±0.0019	60.97[bc]	8.67[ab]	2.74[b]	0.61[ab]
SE			1.137	0.173	0.146	0.028

[1]Soybean meal was treated by roasting at 140℃ for 30 and 60 min (140-30 and 140-60), and at 160℃ for 30 and 60 min, (160-30 and 160-60); microwaving for 2, 4, and 6 min, (Micro-2, Micro-4, and Micro-6) min; and autoclaving at 121℃ for 20 min (Autoclave).
[2]OMD: organic matter digestibility, [3]ME: metabolizable energy, [4]NE: net energy, [5]SCFA: short-chain fatty acids.
[a,b] Within each column, means with common letter(s) are not different (P> 0.05).

Table 2. Gas production of processed and unprocessed soybean meal at different times of incubation

Treatment[1]	Time after incubation (h)									
	2	4	6	8	12	24	36	48	72	96
Control	11.75[bcd]	26.37[bc]	39.75[bcd]	51.37[bc]	90.12[b]	151[ab]	193.75[abc]	216[abc]	237.37[abc]	248.37[abc]
140-30	17.50[abc]	30.75[abc]	48.37[abc]	56.62[bc]	86.37[b]	138.75[ab]	178.37[bc]	196[cd]	219.37[bcd]	229.75[bcd]
140-60	21.25[a]	34.87[ab]	53.87[a]	64.25[ab]	92.87[ab]	155[ab]	198.25[ab]	219.87[abc]	243.50[ab]	255[a]
160-30	18.12[abc]	33.25[ab]	50.50[ab]	62.62[ab]	98.50[ab]	159.25[a]	201.25[ab]	221.87[ab]	244[ab]	253.75[ab]
160-60	19.87[abc]	32.37[ab]	45[abc]	53.87[bc]	96.62[ab]	158.50[a]	199.37[ab]	222[ab]	247.50[a]	258.62[a]
Micro-2	24.5[a]	37.5[a]	49.12[abc]	71.12[a]	107[a]	152.62[ab]	180.25[bc]	197.50[bcd]	218.50[cd]	228.75[cd]
Micro-4	20[ab]	33.87[ab]	49.62[ab]	61.62[ab]	97.12[ab]	152.87[ab]	191.62[abc]	212.50[abcd]	236.25[abc]	248.50[abc]
Micro-6	11[cd]	23.12[d]	37.5[cd]	48[c]	82.87[b]	134.75[b]	208.25[a]	230.87[a]	251.75[a]	263.12[a]
Autoclave	6[d]	16.75[d]	30.87[d]	45.62[c]	84.50[b]	138.87[ab]	172.87[c]	189.36[d]	206.87[d]	217.37[d]
SE	2.8	2.69	3.70	3.98	5.03	6.39	7.15	7.64	7.71	7.75

[1]Soybean meal was treated by roasting at 140℃ for 30 and 60 min (140-30 and 140-60), and at 160℃ for 30 and 60 min, (160-30 and 160-60); microwaving for 2, 4, and 6 min, (Micro-2, Micro-4, and Micro-6) min; and autoclaving at 121℃ for 20 min (Autoclave).
[a,b] Within each column, means with common letter(s) are not different (P> 0.05).

Dry matter degradability of processed SBM

The PD, ED, quickly degradable fraction (a), slowly degradation fraction (b), and dry matter constant rate of degradation (c) are shown in Table 3. Dry heating at 160℃ for 60 min significantly reduced the quickly degradable fraction (a). In general, processing of SBM with different methods (except for autoclave processed SBM) significantly reduced the degradation rate (P<0.05). Different processing methods significantly reduced the amount of effective degradation compared to the control (P<0.05). The effective degradability (ED) was also affected by the processing method (P<0.05). Dry heating process at 160℃ for 60 min resulted in the lowest ED value compared to other treatments (P<0.05). In microwave-heated samples, decrease in rapidly degradable fraction, PD, and ED and increase in slowly degradable fraction were observed with increasing heating time (Table 3). *In situ* degradation rate of DM (Table 4) was significantly affected by the processing method (P<0.05) with the SBM samples processed at 160℃ for 60 min having the lowest degradation rate at all incubation periods.

Protein degradability of processed SBM

The control SBM samples showed the highest, and 160 -60 and autoclave samples the lowest crude protein degradation at all incubation times (Table 6). *In situ* degradation parameters such as PD and ED of SBM crude protein are shown in Table 5. All processed samples (except those processed by microwave for 2 min) had a lower rapidly degradable fraction (a) and higher slowly degradable fraction (b) (P<0.05). Increased duration of dry heating and microwave processing reduced the quickly degradable fraction and increased the slowly degradable fraction. The PD and ED values of soybean CP were decreased by different heat processing (Table 5).

Table 3. Degradability coefficients and effective degradability of processed and unprocessed soybean meal dry matter

Treatment[1]	Degradability Coefficients[2]			PD[3]	Effective degradability[4]		
	a	b	c		3	5	8
Control	23.08±1.94	74.28±3.20	0.067±0.007	97.36	75.55	65.81	57.13
140-30	24.95±2.34	76.26±5.95	0.047±0.090	101.22	71.70	62.12	53.37
140-60	24.02±2.14	79.49±6.87	0.040±0.008	103.51	69.59	59.50	50.65
160-30	20.02±2.08	76.73±4.66	0.050±0.007	96.75	68.25	58.68	49.81
160-60	18.91±1.91	76.73±4.66	0.050±0.007	96.75	61.64	52.27	44.01
Micro-2	24.96±2.10	77.84±4.51	0.052±0.007	102.81	74.50	64.84	55.81
Micro-4	21.27±2.06	80.27±4.34	0.053±0.0007	101.55	72.59	62.65	53.33
Micro-6	21.09±2.10	91.57±4.30	0.058±0.008	91.57	67.69	59.10	50.87
Autoclave	21.13±2.73	70.44±4.66	0.064±0.008	91.58	69.31	60.92	52.68

[1]Soybean meal was treated by roasting at 140℃ for 30 and 60 min (140-30 and 140-60), and at 160℃ for 30 and 60 min, (160-30 and 160-60); microwaving for 2, 4, and 6 min, (Micro-2, Micro-4, and Micro-6) min; and autoclaving at 121℃ for 20 min (Autoclave).
[2]a: quickly degradable fraction, b: slowly degradable fraction, c: constant rate of degradation.
[3]Potential of degradability.
[4]Effective degradability calculated with a passage rate (k) of 0.03, 0.05, and 0.08 per h.

Table 4. *In situ* rumen degradation of processed and unprocessed soybean meal DM (%)

Treatments[1]	Time after incubation (h)							
	0	2	4	6	8	12	24	48
Control	24.40^{b}	27.40^{b}	42.93^{a}	48.40^{a}	54.06^{a}	69.93^{a}	75.46^{a}	97.33^{b}
140-30	22.20^{bc}	27.06^{bc}	42.66^{a}	48.53^{a}	49.40^{b}	62.60^{cd}	66.73^{b}	97.13^{b}
140-60	22.00^{c}	26.26^{bcd}	39.46^{c}	41.60^{d}	47.73^{b}	61.13^{de}	6366^{b}	95.06^{c}
160-30	18.46^{d}	24.13^{e}	37.86^{c}	39.06^{e}	48.83^{b}	60.33^{e}	65.06^{b}	93.26^{d}
160-60	18.33^{d}	19.73^{f}	35.26^{d}	34.53^{f}	40.40^{d}	52.40^{f}	58.80^{c}	84.60^{f}
Micro-2	26.80^{a}	30.66^{a}	38.20^{c}	42.26^{cd}	53.33^{a}	70.26^{a}	73.60^{a}	98.60^{a}
Micro-4	21.80^{c}	24.53^{de}	39.93^{bc}	43.53^{bc}	47.66^{b}	66.33^{b}	71.53^{a}	97.86^{ab}
Micro-6	20.66^{cd}	25.26^{cde}	38.53^{c}	44.40^{b}	44.40^{c}	63.73^{c}	66.13^{b}	90.33^{e}
Autoclave	19.00^{d}	23.93^{e}	42.00^{ab}	48.20^{a}	53.40^{a}	60.46^{e}	65.26^{b}	93.80^{d}
SE	0.751	0.660	0.733	0.608	0.635	0.621	1.372	0.350

[1]Soybean meal was treated by roasting at 140℃ for 30 and 60 min (140-30 and 140-60), and at 160℃ for 30 and 60 min, (160-30 and 160-60); microwaving for 2, 4, and 6 min, (Micro-2, Micro-4, and Micro-6) min; and autoclaving at 121℃ for 20 min (Autoclave).
[a,b] Within each column, means with common letter(s) are not different (P> 0.05).

Table 5. Degradability coefficients and effective degradability of processed and unprocessed soybean meal crude protein

Treatment[1]	Degradability Coefficients[2]			PD[3]	Effective degradability		
	a	b	c		3	5	8
Control	36.88±1.68	63.99±2.58	100.87	0.077±0.008	83.05	75.82	68.41
140-30	34.78±1.89	65.71±3.08	103.50	0.070±0.009	80.96	73.32	65.66
140-60	31.04±2.15	68.66±3.80	99.71	0.064±0.043	77.80	69.60	61.57
160-30	30.74±1.72	70.74±3.50	101.48	0.055±0.007	76.71	68.01	59.76
160-60	29.50±1.56	64.28±2.85	93.79	0.061±0.074	72.81	65.08	57.56
Micro-2	36.33±1.25	63.13±2.11	99.46	0.067±0.006	80.10	72.67	65.29
Micro-4	34.15±1.88	66.34±3.98	100.50	0.053±0.008	76.74	68.54	60.83
Micro-6	28.01±1.59	70.47±3.23	98.48	0.055±0.006	73.88	65.22	57.00
Autoclave	29.94±1.75	69.94±3.18	99.64	0.062±0.007	76.95	68.56	60.41

[1]Soybean meal was treated by roasting at 140℃ for 30 and 60 min (140-30 and 140-60), and at 160℃ for 30 and 60 min, (160-30 and 160-60); microwaving for 2, 4, and 6 min, (Micro-2, Micro-4, and Micro-6) min; and autoclaving at 121℃ for 20 min (Autoclave).
[2]a: quickly degradable fraction, b: slowly degradable fraction, c: constant rate of degradation.
[3]Potential of degradability.

Table 6. *In situ* rumen degradation of processed and unprocessed soybean meal CP (%) at different times of incubation

Treatments[1]	Time after incubation (h)					
	0	2	6	12	24	48
Control	38.73[a]	45.51[a]	55.51[a]	80.95[a]	89.87[a]	99.06[a]
140-30	36.86[a]	42.12[b]	53.12[b]	78.09[b]	86.33[ab]	98.56[a]
140-60	31.76[b]	38.93[c]	48.93[c]	75.21[c]	79.64[c]	98.16[ab]
160-30	30.26[bc]	38.55[c]	48.55[c]	71.11[d]	77.68[c]	98.23[ab]
160-60	30.80[b]	36.11[d]	46.11[d]	68.33[e]	76.43[c]	91.16[d]
Micro-2	37.03[a]	44.59[a]	53.93[ab]	75.32[c]	85.48[b]	97.32[ab]
Micro-4	36.53[a]	38.60[c]	48.60[c]	72.16[d]	78.86[c]	96.21[bc]
Micro-6	28.16[c]	35.45[d]	45.45[d]	67.75[e]	76.09[c]	94.88[c]
Autoclave	29.36[bc]	38.93[c]	48.93[c]	72.24[d]	79.02[c]	97.82[ab]

[1]Soybean meal was treated by roasting at 140℃ for 30 and 60 min (140-30 and 140-60), and at 160℃ for 30 and 60 min, (160-30 and 160-60); microwaving for 2, 4, and 6 min, (Micro-2, Micro-4, and Micro-6) min; and autoclaving at 121℃ for 20 min (Autoclave).
[a,b]Within each column, means with common letter(s) are not different (P> 0.05).

Discussion

Gas production

The lowest amount of gas production at all incubation times was for autoclave treatment. Increased duration of treatment with microwave (2, 4, and 6 min) reduced gas production in the first 12 h of incubation. However, after 36 h incubation, gas production was increased by increasing the processing duration. In agreement with these results, other papers showed that gas production from the insoluble fractions (b) was significantly decreased when SBM was autoclaved (El-Waziry et al., 2007) or roasted (El-Waziry et al., 2005).

Energy contents and OMD

The decrease in OMD due to Micro-6 and moist heating (autoclave) was in line with the findings of El-Waziry et al. (2007). The highest and lowest OMD percentages were found untreated and autoclaved SBM, respectively.

In agreement with our findings, a decrease in the predicted ME and NE of SBM was reported when SBM was treated by 30 min autoclaving (El-Waziry et al., 2007) or roasting at 146℃ for 30 min **(**El-Waziry et al., 2005). Samadi and Yu (2011) reported that moist and dry heating did not alter the energy values (ME, NE) of soybean seeds. Decrease in ME and NE have been attributed to increased Maillard products, which decreases ruminal degradability (El-Waziry et al., 2007).

SCFA concentration

The result of this work supported the findings that showed that SCFA concentrations were significantly ($P<0.05$) decreased when SBM was treated by dry heating (El-Waziry et al., 2005) and autoclaving (El-Waziry et al., 2007). The rate of total SCFA production depends on the substrate-soluble carbohydrate (starches and sugars> pectin> cellulose) (Stevens and Hume, 2004), thereby decreased production of SCFA can be attributed to the decrease in the soluble fractions and the degradation rate (Table 3).

Dry matter degradability of processed SBM

In agreement with our findings, a significant decrease in the soluble fractions (a) of SBM was reported when SBM was treated by autoclaving (El-Waziry et al., 2007) or roasting (El-Waziry et al., 2005) processes. Similar results were reported by Ljokjel et al. (2000), who evaluated autoclaved SBM at 120 and 130℃ for 30 min using the *in situ* technique. El-Waziry et al. (2005 and 2007) observed that the rate constant of gas production for the insoluble fraction (c) of SBM was significantly reduced when SBM was treated by autoclaving or roasting processes. Some papers also reported that heat treatment significantly reduced the rapidly soluble fraction in SBM (Konishi et al., 1999; El-Waziry, 2005).

Protein degradability of processed SBM

Our findings are in agreement with those of Konishi et al. (1999) who reported that heat treatment decreased the rapidly soluble fraction (a) of crude protein when roasted at 133℃, 143℃, or 153℃ for 3 h. Consistent with these findings, a significant decreases in the soluble protein of flaxseeds (51.88 to 18.82%, CP) (Doiron et al., 2009), camelina seeds (Peng et al., 2014) (52.73 to 20.41% CP), and soybeans (43.38 to 11.35% CP) (Samadi and Yu, 2011) by moist heating (at 120℃ for 1 h) were reported compared to raw seeds. In this experiment, there was no significant difference in the amount of soluble protein between the dry heat treatment (higher temperature and longer time; 160℃ for 60 min) and moist heat treatment (lower temperature and shorter time; 120℃ for 20 min). The result of the present study confirmed that moist heating process had a greater effect on reducing the soluble protein compared to dry-heat treatment of SBM.

Heating SBM decreased the slowly degradable fraction (b) compared to the control. This finding is consistent with the suggestion that feed protein is more gradually degraded in the rumen after moist heating (Doiron et al., 2009; Samadi and Yu, 2011; Peng et al., 2014). Moist heating significantly decreased CP degradability compared to camelina (75.4 to 57.9%) (Peng et al., 2014) and soybean raw seeds (86.2 to 66.4%) (Samadi and Yu, 2011).

Autoclaving (Ljokjel et al., 2000), expelling (Titgemeyer and Shirley, 1997), and roasting (Faldet et al., 1991) reduced the effective rumen degradability of proteins (EPD) in SBM. Konishi et al. (1999) also found that ED of crude protein in SBM and rapeseed meal was significantly reduced by increasing the heating temperature (when roasted at 133, 143 or 153℃ for 3 h) at all ruminal outflow rates. Samadi and Yu (2011) showed that moist heating had a much greater effect on the chemical and nutrient profiles of SBM crude protein than dry heating. Cleale et al. (1987) noted that moisture levels higher than 20% were needed to effectively protect proteins from degradation in the rumen. However, in the present study, dry heating had an equal or greater effect than moist heating; because in dry heating treatments (140 and 160℃ for 30 and 60 min) SBM was exposed to a higher temperature for a longer time compared to the moist heating treatment (121℃ for 20 minutes).

Heating treatment was reported to reduce SBM protein degradation in the rumen resulting from a lower solubility of the protein in the rumen (Konishi et al., 1999; El-Waziry, 2005; Samadi and Yu, 2011; Peng et al., 2014). SBM contains 0.5±1% reducing sugars and almost 13% non-reducing oligosaccharides (Bach Knudsen, 1997) that might be broken down to reducing monosaccharides during heating (Ljokjel et al., 2000). The cross-linkages between these reducing monosaccharides and peptide chains form complexes between free amino and aldehyde groups which reduce the protein solubility. Furthermore, heat processing reduces protein degradation in the rumen by changing the protein molecular structure (Peng et al., 2014). It was shown that even if feed tissues contain the same protein content, their nutritive value may be different if their α-helix-to-β-sheet ratios in the protein secondary structures are different (Samadi and Yu, 2011). The change in the ratio of α-helix to β-sheet by moist heating was reported by Doiron et al. (2009). The results of numerous pieces of research showed that moist heating (120℃ for 60 min) significantly decreased the ratio of α-helix to β-sheet compared to dry heating process (120℃ for 60 min). These changes in protein intrinsic molecular structures were associated with decreased CP solubility and protein degradation in the rumen, as well as increased intestinal digestibility of RUP in soybean seeds (Samadi and Yu, 2011) and camelina seeds (Peng et al, 2014). These findings indicated that changes in protein subfractions and protein digestibility in the rumen were related to the changes occurring due to heating of the protein molecular structures.

Conclusions

Results of the present study suggested that dry and moist heating and microwaving of SBM decreased the gas production, and gas production rate and ruminal protein degradability. Although heat production is a common phenomenon during SBM processing, the results of the present study showed that extra heat could significantly affect the protein and DM degradability in the rumen. Generally, the methods of heating (autoclaving, dry heating, and microwaving) affected protein degradability. The results of the present study showed that microwaving of SBM might result in greater gas production compared whit the moist heating.

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تأثیر روش گرمادهی بر ویژگی‌های تجزیه‌پذیری شکمبه‌ای پروتئین و ماده خشک کنجاله سویا

ف. رضایی[1]* و ج. بیات کوهسار[2]

[1]**بخش کشاورزی، دانشگاه پیام نور، تهران، ایران.**

[2]**گروه علوم دامی، دانشکده کشاورزی، دانشگاه گنبد، گنبد، ایران.**

***نویسنده مسئول، پست الکترونیک:** frezaii@pnu.ac.ir

چکیده در پژوهش کنونی اثر گرمادهی با ماکروویو، اتوکلاو (گرمای مرطوب) و برشته کردن (گرمای خشک) بر فراسنجه‌های تولید گاز و تجزیه‌پذیری شکمبه‌ای کنجاله سویا بررسی شد. کنجاله سویا در دمای ۱۴۰ و ۱۶۰ درجه سانتی‌گراد به مدت ۳۰ و ۶۰ دقیقه برشته، در دمای ۱۲۱ درجه سانتی‌گراد به مدت ۲۰ دقیقه اتوکلاو، و در زمان‌های ۲، ۴، و ۶ دقیقه در ماکروویو گرمادهی شد. آزمایش تولید گاز با مایع شکمبه گوسفند فیستوله شده انجام و تولید گاز پس از ۲، ۴، ۶، ۸، ۱۲، ۲۴، ۳۶، ۴۸، ۷۲ و ۹۶ ساعت انکوباسیون ثبت شد. گوارش‌پذیری ماده آلی، انرژی قابل متابولیسم، انرژی خالص و اسیدهای چرب کوتاه زنجیر پس از ۲۴ ساعت محاسبه شد. آزمایش ***in situ*** در سه بره فیستوله شده از نژاد دالاق (با میانگین وزن ۱±۴۵ کیلوگرم) انجام شد. حیوانات در قفس‌های انفرادی نگهداری و در سطح نگهداری تغذیه شدند. نمونه‌های فرآوری شده و فرآوری نشده کنجاله سویا آسیا شدند تا از الک ۳ میلی‌متری رد شوند و ۵ گرم از هر نمونه در کیسه‌های نایلونی ریخته شد. درصد تجزیه‌پذیری شکمبه‌ای ماده خشک کنجاله سویای فرآوری شده در زمان‌های مختلف انکوباسیون با رابطه غیرخطی تعیین شد. تیمارها اثر معنی‌داری بر پتانسیل تولید گاز داشتند ($P<0.05$). در روش برشته کردن، با افزایش زمان گرمادهی پتانسیل تولید گاز افزایش یافت. روش‌های فرآوری اثر معنی‌داری بر کاهش تولید گاز داشتند ($P<0.05$). کمترین مقدار تولید گاز در روش گرمادهی با اتوکلاو به دست آمد. روش‌های گرمادهی با ماکروویو به مدت ۶ دقیقه و گرمای مرطوب (اتوکلاو) در مقایسه با گرمادهی خشک (برشته کردن) گوارش‌پذیری ماده آلی را به طور معنی‌داری کاهش داد. گرمادهی با ماکروویو به مدت ۶ دقیقه در مقایسه با بقیه تیمارها به طور معنی‌داری ($P<0.05$) غلظت اسیدهای چرب کوتاه زنجیر را کاهش داد. گرمادهی با ماکروویو به مدت ۶ دقیقه غلظت اسیدهای چرب کوتاه زنجیر را در مقایسه با سایر تیمارها کاهش داد ($P<0.05$). یافته‌های ***in situ*** نشان داد که فرآوری کنجاله سویا نرخ تجزیه ماده خشک و پروتئین را کاهش داد ($P<0.05$). کنجاله سویای فرآوری شده در مقایسه با تیمار کنترل بخش سریع‌التجزیه (a) کمتر و کند تجزیه (b) بیشتر داشت. در مجموع روش‌های گرمادهی (اتوکلاو، گرمای خشک و ماکروویو) سبب کاهش تولید گاز و نرخ آن و کاهش تجزیه‌پذیری پروتئین در مقایسه با کنترل شدند.