PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 8 (5): 695-703, 2009
ISSN 1680-5194

Rumen Fermentation Characteristics and Lactation Performance in Dairy Cows Fed Different Rumen Protected Soybean Meal Products

M.A. Soltan
Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Alexandria University, Egypt

Abstract: One hundred lactating Holstein dairy cows were cows were assigned to investigate the effect of untreated Soybean Meal (SBM) by different treated SBM products; heat+ soy hulls addition (HS), extrusion treatment (EP), addition of tannin plant extract and essential oil (PA) or addition of tannin plant + pelleting (HPA) on rumen fermentation, milk production and composition of dairy cows from 17th-25th after calving. Basal experimental diet was formulated (containing untreated SBM) and used as control, SBM was replaced by four treated SBM products and fed to the five groups (20 cows per each). Solvent extracted untreated SBM exhibited greater effective degradability of CP and AA when compared with treated SBM products (HS, EP, PA or HPA). This was due to a greater fraction of soluble protein. Moreover, treated SBM products (HS, EP, PA and HPA) contained relatively low concentrations of lysine, arginine, histidine, alanine, praline, serine, aspartic acid and glutamic acid in different levels compared with SE product which suggesting binding and cross linking reactions involving these AA as a result of the treatment methods. Treated SBM feeding instead of untreated one had no effect ($p>0.05$) on dry matter intake while, improved milk production and milk-to-feed ratio across the whole experimental periods by about (2.2, 1.9, 3.2 and 4.4%) and by (2.5, 1.9, 3.8 and 4.4%) respectively. Moreover, treated SBM reduced ($p<0.05$) the concentrations of ruminal NH_3-N by about 8.5, 7.8, 13.2 and 13.2% respectively, while had no effect ($p>0.05$) on total VFA, acetate, butyrate concentrations and slightly decreased propionate in the rumen when compared with cows fed on untreated SBM containing diet. Regarding blood serum units treated SBM had no effect ($p>0.05$) on blood serum glucose concentration, however cows fed on diets containing EP, PA and HPA treated SBM instead of untreated SBM showed a reduction ($p<0.05$) in blood urea N by about 4.9, 6 and 7.7% respectively, on the other hand HS treated SBM leading to non significant ($p>0.05$) reduction in blood urea N by about 3.8%. Treated SBM products increased milk fat percentage, fat yield and protein yield and had no effect on milk lactose percentage and the present study suggested that HS and EP treatment methods of SBM is less effective and the cow performance lesser respond than PA or HPA methods which depend on tanninferous plant species that protect protein from degradation in the rumen due to presence of small amounts of condensed tannin in the plant species and may be more available and digestible in the intestine more than the previous processing.

Key words: Soybean meal, SBM heat or plant extracts (tannin) treatment, milk production and composition, rumen fermentation

INTRODUCTION

Controlling the rate and extent of degradation of dietary protein to balance the protein supply from microbial synthesis is then of great interest to ruminant nutritionists, because inefficient utilization necessitates over feeding of protein and the most costly ingredient of the ration (Broderick *et al*., 1991). Improvement of ruminant protein is a matter of practical concern that the amounts of protein and amino acids delivered to the intestine commonly limit productivity of these animals is shown by their responses to post ruminal supplementation (Broderick *et al*., 1991; Ipharraguerre *et al*., 2005). Selection of the proper source of supplemental crude protein for feeding offers an excellent opportunity for influencing the supply of amino acids to dairy cows. This is because the crude protein source modulates the intestinal supply of amino acids by affecting the passage of Ruminal Undegredable Protein (RUP) and microbial nitrogen to the lower intestinal tract (Clark *et al*., 1992). In addition, the contribution of RUP to the ruminal outflow of total protein and its amino acids composition impact the pattern of amino acids available for absorption in the small intestine (Rulquin and Verite, 1993; NRC, 2001).

Although soy proteins are a good source of digestible lysine and histidine (Santos *et al*., 1998; NRC, 2001), they are low in methionine (1.44-1.47% of crude protein) and can be extensively degraded (= 57.4-69.6%) by ruminal microbes (NRC, 2001), which may compromise their value for contributing quantitatively and/or qualitatively to supply of essential amino acids in metabolizable protein. This limitation motivated the development of several methods for decreasing the ruminal degradability of proteins in soybean meal (SBM). Nowadays, there are several commercial sources of treated SBM are available for use in the diets for dairy

cattle. The heat-generating extrusion process which decreased SBM protein degradability in the rumen (Aldrich and Merchen, 1995). The use of heat and soy hulls is yet another industrial method for protecting SBM protein from ruminal degradation (Heitritter *et al*., 1998). This method also involves non enzymatic browning, but it be more environmentally acceptable than the chemical treatment methods because it uses a natural ingredients (Soy hulls). Plant secondary compounds (herbs extract containing tannin, terpenenoid, volatile essential oils) with some mineral and vitamins supplementation have potential to be used as protein protecting in the rumen (El-Waziry *et al*., 2005; Tandon *et al*., 2008), however the published information on the heat treatment and soy hulls or plant secondary compounds methods are lacking and scientific studies are needed to evaluate its efficacy.

According to Waltz and Stern (1989), treatment of SBM can increase the supply of amino acids to the duodenum of ruminants by 40-70% and Bateman *et al*. (2005) and Ipharraguerre *et al*. (2005) have concluded that an increase in RUP can increase milk yield. Based on the processing method of SBM, the intestinal availability of amino acids greatly differed, as SBM heat treatment leading to reduction of amino acids intestinal digestibility (Ceresnakova *et al*., 2002) and while little information about the other procedure and the effect of different processing on ruminal fermentation characteristics (El-Waziry *et al*., 2007). Therefore, the objective of the current study was to evaluate the effect of multiple methods of treating SBM on ruminal fermentation characteristics, milk production and composition as well as some blood parameters of dairy cows.

MATERIALS AND METHODS

This experiment was carried out to evaluate the effect of five types of SBM processing on ruminal fermentation characteristics, milk production and composition as well as some blood parameters of dairy cows.

Cows, Experimental design and Diets: One hundred lactating Holstein dairy cows were allotted into five groups (20 cows per each). The first group (20 cows) was assigned to the control fed for 9 weeks on the basal diet containing normal SBM without treatment and the other four cow groups the SBM replaced by other treated commercial source as outlined in Table 1. The cows averaged 112±15 DIM at the start of the experiment, with an average Body Weight (BW) of 575 kg. They were housed in bedded pack housing system with outside feeding. The housing system provides 0.45 cm head lock for feeding and about 30 m^2 yard per cow. The barns have open ridges to facilitate cleaning and manure removing. About 30% of the whole area is sloppy shaded at highet 9 m in the center with fan but no available cooling system in the cow's yard. Waterers were available in suitable size all the day time. Cows were fed ad libitum on a Total Mixed Ration (TMR) to meet the predicted requirements for energy, protein, minerals and vitamins according to NRC (2001). Ingredient and chemical compositions of the used diet are presented in Table 2.

Soy bypass protein sources: Five types of SBM supplements were investigated: solvent-extracted of normal commercial SBM (SE), Extrusion Processing (EP, Super soy; Arasco KSA), heat treated SBM with soy hulls (HS; Aminoplus; Ag Processing Inc., Omaha, NE), Treated SBM with plant secondary compounds (PA, Soy bypass, NF UAE) and heat treated SBM with plant secondary compounds (HPA; ACD100, FEEDCO, KSA with Centraly's Co. "France" supervision). The EP treatment by using SBM are fed into an extruder barrel, where a central revolving shaft forces the meal through the extruder, the SBM are treated by the heat generated through friction (130°C) and steam which is frequently injected during the process. The HS treatment involved mixing SBM with soy hulls (10:1 wt ratio), adding water to achieve 30-50% moisture and then further cooking at 95°C to obtain final moisture of 12-13%. The PA treatment with 1.25% of a commercial product (AM-3-Pro) production by Centraly's Co. "France" containing essential oil and plant extract plus vitamin B6 (600 mg/kg), niacin (20000mg/kg), iron (42000 mg/kg), zinc (180000mg/kg), manganese (31000mg/kg) and cobalt (50 mg/kg). The HPA treatment produced same as PA plus heat treatment (75-80°C) during pelleting process.

Feed intake: Diets were offered in equal amounts twice daily (08:00 and 15:00). Feed consumption was recorded daily by weighing feeds offered to and refused by the cows. Samples of TMR and feed ingredients were collected daily and kept frozen. Samples were composite by period (each 3 weeks), dried at 55°C for 48 h, ground through a 1-mm screen (Wiley mill) and analyzed for DM, total nitrogen, NDF, ADF, EE composition.

Milk production and milk composition: Cows were milked twice daily in the milking pallor (05:00 and 17:00), and milk yield was recorded at each milking. During the last week of each 21-day period, milk samples were taken from each cow at each milking, pooled on a yield basis, and stored at +4°C with a preservative (bronopol-B2) until analyzed for fat, protein and lactose.

Ruminal fermentation characteristics: Ruminal fluids were collected at 0, 4 and 8 h after feeding from 5 cows from each group on 21 of each period of the experiment. Ruminal fluids collected through a speculum were inserted into the cow mouth and a lubricated rubber tube

Table 1: Outline of the experimental design

Groups No.	% of processing methods of used SBM: Solvent extraction (Control)	Heat with soy hulls treatment	Extrusion treatment	Plant secondary compounds (Tannin herbs extract) addition	Plant secondary compounds (Tannin herbs extract) addition + pelleting
1	100	0	0	0	0
2	0	100	0	0	0
3	0	0	100	0	0
4	0	0	0	100	0
5	0	0	0	0	100

Table 2: Ingredient composition (% as fed) and chemical composition (on dry matter basis) of the basal used diet

Ingredient composition		Chemical composition	
Ingredient		Items	%
Berseem hay	50.00	Moisture%	11.4
Yellow Corn	20.50	Crude protein%	16.2
Barley grain	15.35	Ether extract%	3.25
Soybean meal	6.50	Crude Fiber%	15.8
Sugar beet pulp	1.90	NDF%	27.5
NaCl (Iodinized)	0.10	ADF%	20.7
Dicalcium phosphate	0.50	Ash%	8.8
Lime stone	1.00	NE_L (MJ/kg DM)***	1.58
Sodium Bicarbonate	0.30	Calcium %	1.05
Rumen protected fat*	1.25	Phosphorus%	0.40
Mineral and vitamin premix**	0.10		
Molasses	2.50		

*Super Sp-202, produced by Allgreen Co. (Malaysia) a special form of a hydrogenated triglyceride which has min. 99% fat as palm oil, acid value, 220 max, melting point, 55°C min. **Cattle premix produced by Centraly's Co. (France) contains the following elements per kg. (10000000 IU vitamin A, 1000000, IU vitamin D_3, 10000 mg vitamin E, 100000 mg magnesium, 50000 mg manganese, 45000 mg zinc, 50000 mg iron, 6000 mg copper, 800 mg iodine, 100 mg selenium. ***Calculated according to NRC (2001).

was inserted through the speculum into the rumen via the esophagus. Ruminal contents (250 ml) were removed using an electric pump. Samples were monitored visually to ensure they were not contaminated with saliva. The pH was measured immediately using pH meter (Orion research model 201). The whole contents were squeezed through 4 layers of cheesecloth. The samples were acidified to pH 2 with 50% H_2SO_4 and frozen at -20°C for later determination of Volatile Fatty Acids (VFA) and ammonia (NH_3-N) concentrations.

Chemical analysis: Analytical DM contents of TMR and feed ingredients were determined by oven-drying at 105°C for 48 h (AOAC, 1990; method 930.15). Ash contents of TMR and feed ingredients were determined by incineration at 550°C overnight. Crude protein in TMR and feed ingredient were determined by using Kjeldahl method according to Randhir and Pradhan (1981) and ether extract was determined according to Bligh and Dyer (1959) technique as modified by Hanson and Olly (1963). NDF and ADF in TMR and feed ingredients were determined according to (AOAC, 1990; method 973.18). Protein, fat and lactose concentrations in milk samples were analyzed (AOAC, 1990) by infrared spectrophotometer (System 6000 MilkoScan; Foss Electric). Concentrations of NH3-N and VFA in ruminal fluid were analyzed by colorimetry (Weatherburn, 1967) and by GLC (Varian 3700; Varian Specialties Ltd, Brockville, Ontario, Canada), respectively. Nitrogen fractions, defined according to the Cornell Net Carbohydrate and Protein System, were determined on SBM products using the methods of Licitra *et al.* (1996). To analyze AA, samples were ground to pass a 0.5- mm screen; SBM products as well as bag residues were acid-hydrolyzed with 6 *N* phenol-HCl for 24 h at 110°C (AOAC, 2000) and AA concentrations of the hydrolysates were determined by the isotope dilution method (Calder *et al.*, 1999). Briefly, 2 mL of the hydrolysate was diluted with 3 mL of ultrapure water and 1 mL of this solution was then combined with 200 _L of a mixture of labeled AA (13C and 15N AA isotope standards; CDN Isotopes, Pointe-Claire, Quebec; Cambridge Isotope Laboratories Inc., Andover, MA), which served as an internal standard. The solution was eluted through a poly-prep chromatography column (resin 100-200 mesh H; Bio-Rad, Hercules, CA), then derivatized with *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide and dimethylformamide 1:1 (394882, 27.0547; Sigma-Aldrich) according to the method of Calder and Smith (1988). Amino acids were quantified using GC-MS (Hewlett-Packard Model GC6890-MS5973; Agilent Technologies, Wilmington, DE) and a mass selective detector (Hewlett-Packard, Palo Alto, CA). The AA Met, Cys, Arg and Pro were analyzed separately by subjecting the samples to performic acid oxidation, followed by HCl hydrolysis (AOAC, 2000); these 4 AA were analyzed with a Biochrom 20 AA analyser (Amersham Pharmacia Biotech, Piscataway, NJ).

Blood sampling: Five cows from each treatment group were randomly selected for blood sampling. Blood samples were collected at the end of the experimental period. Blood samples were obtained by vein puncture

during or immediately after evening milking. Blood was collected into a 20 ml tube and allowed to clot at ambient temperature. Blood samples were centrifuged at 3000 rpm for 15 min. Only clear non-hemolyzed sera were obtained and kept frozen until further analysis. Samples were analyzed for glucose according to Trinder (1969). Serum samples were analyzed to determine urea nitrogen concentration according to Coulomb and Faverau (1963).

Statistical analysis: The analysis of variance for the obtained data was performed using Statistical Analysis System (SAS, 1996) to assess significant differences.

RESULTS AND DISCUSSION

Chemical analysis of the used SBM products: The chemical compositions of SBM used in the present study are presented in Table 3. The crude protein content of the SBM products showed little deviation from 50%. The concentration of soluble protein was numerically lower in the treated SBM products than in SE. The HS, EP and HPA products were all exposed to higher temperatures during the respective processing methods and heat denaturizing feed proteins is known to reduce their solubility (Liu, 1999) on the other hand PA SBM product not exposed to heat which reflected on the higher soluble protein compared with other SBM heat treated products. Compared with the NRC (2001) model, NDF, ADF, Neutral Detergent Insoluble Crude Protein (NDICP) and Acid Detergent Insoluble Crude Protein (ADICP) were higher for HS, EP, PA and HPA SBM products. The increases in NDICP and ADICP for the treated SBM products were also the result of exposure to heat and chemical reactions during the processing (Demjanec *et al*., 1995; McKinnon *et al*., 1995; Borucki *et al*., 2007). An increase in NDICP reflects an increase in the feed protein fraction that is slowly degraded in the rumen (Mustafa *et al*., 2000), whereas an increase in ADICP by about 180.9, 238.1, 28.6 and 85.7% were observed for HS, EP, PA and HPA products respectively when compared with SE and that values are an indication of heat-damaged protein, which leads to reduced protein digestibility (Faldet *et al*., 1992; Can and Yilmaz, 2002). The contents of NDF and ADF were also higher in the treated products than in SE, these increases are likely to be artifacts of the increases in NDICP and ADICP (Van Soest and Mason, 1991; Borucki *et al*., 2007).

Results of the Amino Acids (AA) analysis of the different SBM products (Table 3) revealed that the AA composition of SE was comparable to that reported by Degussa (Degussa Feed Additives, 1996); however, values for HS, EP, PA and HPA were lower than those reported by the NRC (2001). The analytical data indicated that PA and HPA treated products contained slightly higher methionine concentration compared with other treated SBM products and that may be related to the addition of synthetic methionine during processing. Moreover, treated SBM products (HS, EP, PA and HPA) contained relatively low concentrations of lysine, arginine, histidine, alanine, praline, serine, aspartic acid and glutamic acid in different levels compared with SE product which suggesting binding and cross linking reactions involving these AA as a result of the treatment methods (Adrian, 1974; Gerrard, 2002; Borucki *et al*., 2007). These reactions would reduce the AA release upon acid hydrolysis (Ford and Shorrock, 1971). Only one previous published data (Borucki *et al*., 2007) indicated the AA composition of some treated SBM products (heat treated with hulls, expeller heat treatment and lignosulfate treated) but no available information has been published on the AA composition of PA and HPA products, the data provided here would quite useful in formulating diets for dairy cattle.

Dry Matter Intake (DMI): Approximately similar DMI was observed by cows fed on treated SBM (HS, EP, PA and HPA) products (Table 4) during the first three weeks of the experiment when compared with cows group fed of SE SBM. However, during the next periods of the experiment DMI slightly decreased ($p>0.05$) by cows fed on PA treated SBM, while there was an increase in DMI of the other treated SBM (HS, EP and HPA) products across the experimental period by about 0.3, 0.1 and 0.2% respectively when compared with untreated product. The approximately similar DMI was in agreement with that obtained by Mabjeesh *et al*. (2000) they concluded that DMI was similar for cows fed on roasted whole cotton seed and those fed on the whole cotton seed without treatment.

Milk production performance and milk-to-feed ratio: Improvement ($p<0.05$) in milk production at the first three weeks of production (Table 5) by cows fed on diets containing HS, PA or HPA teated SBM instead of untreated SBM product by about 1.9, 2.6 and 3.5% respectively while using EP treated product non significantly improved milk production by about 1.0% when compared with those fed on untreated SBM. Moreover, more improvement in milk production of the next experimental periods while, the improvement across the whole experimental periods by different SBM products feeding was observed by about 2.2, 1.9, 3.2 and 4.4% respectively when compared with the control. The highest improvement was observed when SE untreated SBM was replaced by HPA treated SBM followed by PA and followed by HS treated SBM, while the lowest response by cows fed on EP treated SBM when compared with the control. Milk production improvement was in harmony with those obtained by (Socha, 1991) summarized that lactating cows

Table 3: Effect of different rumen protected soybean meal products on milk composition of dairy cows

Items	Soybean meal product treatments				
	SE (Control)	HS treatment	EP treatment	PA treatment	HPA treatment
CP % of DM	52.7	51.3	50.9	48.9	49.1
Soluble P (% of CP)	28.6	15.5	16.2	19.9	15.3
NDICP (% of CP)[1]	4.9	30.3	28.3	33.3	37.7
ADICP (% of CP)[2]	2.1	5.9	7.1	2.7	3.9
Ash%	7.4	7.6	7.8	8.2	8.6
Ether extract%	1.2	1.8	14	1.9	2.2
NDF%	8.9	15.8	14.9	10.3	11.5
ADF%	5.2	9.9	7.9	6.5	7.3
Essential Amino Acids (EAA) (% of DM)					
Methionine	0.67	0.65	0.73	0.78	0.79
Lysine	3.41	2.95	3.21	3.23	3.11
Arginine	4.11	3.95	3.79	3.80	3.79
Histidine	1.51	1.31	1.39	1.36	1.39
Isoleucine	2.41	2.56	2.27	2.22	2.33
Leucine	4.16	4.34	3.78	3.74	3.83
Phenylalanine	2.77	2.31	2.6	2.55	2.63
Threonine	2.1	1.96	1.91	1.89	1.91
Valine	2.56	2.26	2.24	2.31	2.46
Non Essential Amino Acids (NEAA) (% of DM)					
Glycine	2.31	2.21	2.09	2.07	2.1
Cystine	0.85	0.73	0.78	0.80	0.79
Alanine	2.45	2.29	2.14	2.12	2.17
Proline	2.9	2.65	2.62	2.65	2.72
Serine	2.85	2.68	2.52	2.51	2.51
Aspartic acid	6.27	5.33	5.76	5.64	5.8
Glutamic acid	10.11	9.61	9.2	9.10	9.25

1 = Neutral Detergent Insoluble Crude Protein (NDICP), 2 = Acid Detergent Insoluble Crude Protein (ADICP)

Table 4: Effect of different rumen protected soybean meal products on dry matter intake (DMI kg/cow/day) of dairy cows

Stage of lactation	Soybean meal product treatments				
	SE (Control)	HS treatment	EP treatment	PA treatment	HPA treatment
	(N = 21)				
17-19 weeks	19.87±0.04	20.06±0.1	20.07±0.07	19.93±0.06	19.91±0.05
20-22 weeks	20.06±0.08	20.21±0.1	19.94±0.07	20.00±0.05	20.16±0.04
23-25 weeks	19.94±0.08	19.80±0.06	19.89±0.05	19.87±0.05	19.88±0.05
Average (17 - 25 weeks)	19.96±0.03	20.02±0.03	19.97±0.02	19.93±0.02	19.99±0.02
Relative to control	100	+ 0.3	+ 0.1	- 0.2	+ 0.2

Values are means ± standard error. No significant differences (P>0.05). N = No. of observation.

Table 5: Effect of different rumen protected soybean meal products on milk production performance (kg/cow/day) of dairy cows

Stage of lactation	Soybean meal product treatments				
	SE (Control)	HS treatment	EP treatment	PA treatment	HPA treatment
	(N = 21)				
17-19 weeks	31.3±0.07^{c}	31.9±0.13^{bd}	31.6±0.10^{bc}	32.1±0.12^{ad}	32.4±0.07^{a}
20-22 weeks	31.8±0.07^{c}	32.6±0.14^{b}	32.6±0.14^{b}	32.7±0.10^{b}	33.2±0.11^{a}
23-25 weeks	31.8±0.07^{c}	32.3±0.08^{b}	32.3±0.08^{b}	33.0±0.10^{a}	33.4±0.13^{a}
Average (17 - 25 weeks)	31.6±0.03^{d}	32.3±0.05^{c}	32.2±0.05^{c}	32.6±0.05^{b}	33.0±0.04^{a}
Relative to control	100	+ 2.2	+ 1.9	+ 3.2	+ 4.4

Values are means ± standard error. Mean values with different letters at the same raw differ significantly at ($p \leq 0.05$). N = No. of observation.

increased milk production when heat treated SBM was used as a protein supplement compared to untreated SBM. However, milk response to heat treated SBM have been inconsistent. Hoffiman *et al*. (1991) reported that substitution of expeller SBM for untreated SBM in diets fed to dairy cows not affected the milk production.

Table 6: Effect of different rumen protected soybean meal products on milk-to-feed ratio of dairy cows

	Soybean meal product treatments				
Stage of lactation	SE (Control)	HS treatment	EP treatment	PA treatment	HPA treatment
	(N = 21)				
17-19 weeks	1.58±0.01[b]	1.59±0.01[b]	1.58±0.01[b]	1.61±0.01[ab]	1.63±0.01[a]
20-22 weeks	1.59±0.01[b]	1.62±0.01[ab]	1.63±0.01[a]	1.63±0.01[a]	1.65±0.01[a]
23-25 weeks	1.57±0.01[b]	1.61±0.01[ab]	1.59±0.01[b]	1.62±0.01[a]	1.63±0.01[a]
Average (17 - 25 weeks)	1.59±0.01[c]	1.62±0.01[b]	1.61±0.01[b]	1.64±0.01[a]	1.65±0.01[a]
Relative to control	100	+ 2.5	+ 1.9	+ 3.8	+ 4.4

Values are means ± standard error. Mean values with different letters at the same raw differ significantly at ($p \leq 0.05$). N = No. of observation.

Table 7: Effect of different rumen protected soybean meal products on rumen fermentation characteristics of dairy cows

	Soybean meal product treatments				
Items	SE (Control)	HS treatment	EP treatment	PA treatment	HPA treatment
	(N = 15)				
Rumen pH	6.30±0.03[a]	6.23±0.02[b]	6.22±0.01[b]	6.22±0.01[b]	6.20±0.03[b]
NH_3-N (mg/100mL)	12.9±0.48[a]	11.8±0.23[b]	11.9±0.30[b]	11.2±0.65[b]	11.2±0.35[b]
Total VFA (mM)	86.7±0.63[a]	85.2±1.18[a]	86.3±0.38[a]	85.8±0.30[a]	85.4±0.48[a]
Acetate (A) mol/100mL	61.8±0.38[a]	62.7±0.38[a]	62.9±0.55[a]	62.4±0.35[a]	62.7±0.35[a]
Propionate (P) mol/100mL	24.0±0.28[a]	22.7±0.50[b]	22.5±0.40[b]	22.7±0.23[b]	23.2±0.45[ab]
Butyrate mol/100mL	14.2±0.28[a]	14.6±0.92[a]	14.6±0.98[a]	14.9±0.48[a]	14.1±0.65[a]
A:P ratio	2.58±0.04[b]	2.77±0.05[a]	2.80±0.04[a]	2.75±0.03[a]	2.71±0.05[a]

Values are means ± standard error. Mean values with different letters at the same raw differ significantly at ($p \leq 0.05$). N = No. of observation.

The milk production response variation between different SBM products of the present study may be caused by decreased microbial synthesis, poor essential AA profiles or low digestibility of the HS or EP treated SBM compared with the other sources used in the present study. These explanations are in agreement with those reviewed by Santos and Huber (1996).

Improvement ($p>0.05$) in milk-to-feed ratio was observed (Table 6) during the first three week of the experimental period with replacement of untreated SBM by HS, PE or PA treated products by about 0.6, 0.0 and 1.9% respectively while HPA treated product significantly improved milk-to-feed ratio by about 3.2% when compared with the control and more improvement was observed in the next periods. Substitution of untreated SBM by different treated SBM products (HS, PE, PA and HPA) improved ($p<0.05$) milk-to-feed ratio across the experimental period by 2.5, 1.9, 3.8 and 4.4% respectively. The highest improvement was observed when untreated SBM was replaced by HPA treated product, followed by PA, then HS and the lowest response by cows fed on EP treated product instead of untreated one. The possible explanation for milk-to-feed ratio may be related to the higher milk production without increasing of dry matter intake.

Rumen fermentation characteristics: Ruminal pH reduction ($p<0.05$) was observed with inclusion of different treated HS, EP, PA or HPA) SBM products instead of SE SBM (Table 7) by about 1.1, 1.3, 1.3 and 1.6% respectively when compared with control. Moreover, the concentrations of NH_3-N were significantly ($p<0.05$) reduced when SBM replaced by treated SBM (HS, EP, PA or HPA) by about 8.5, 7.8, 13.2 and 13.2% respectively. A decrease in rumen NH_3-N concentrations was observed by previous studies (Mabjeesh *et al*., 2000; El-Waziry *et al*., 2005; El-Waziry *et al*., 2007), but the present results are in contrast with those obtained by (Stern *et al*., 1985; Tice *et al*., 1993) they reported that no decline in ruminal NH_3-N of cows fed diets containing heat treated soybeans compared to cows fed whole raw soybeans. A decrease in rumial NH_3-N may indicate that treated SBM lowered proteolysis, degradation of peptides and deamination of amino acids in the rumen (Newbold *et al*., 1990), the mentioned explanations clarify the PA and HPA processing methods are more effective compared with HS or EP methods.

The VFA concentrations (Table 7) were reduced ($p>0.05$) when treated SBM (HS, EP, PA and HPA) replaced the untreated SBM l dairy cow diets by about 1.7, 0.5, 1 and 1.5% respectively. The molar proportions of individual VFA were slightly differ between groups as treated SBM fed cows showed an increase ($p>0.05$) of acetate concentration, similar in butyrate while exhibited reduction in propionate concentrations and consequently the acetate to propionate ratio significantly increased by about 7.4, 8.5, 6.6 and 5% respectively compared with cow fed on diet containing untreated

Table 8: Effect of different rumen protected soybean meal products on milk composition of dairy cows

Items	Soybean meal product treatments				
	SE (Control)	HS treatment	EP treatment	PA treatment	HPA treatment
	(N = 21)				
Milk fat %	3.15±0.03^{b}	3.25±0.02^{a}	3.22±0.02^{a}	3.20±0.02^{a}	3.20±0.02^{a}
Milk fat yield (g/cow/day)	997.0±15.3^{b}	1049.8±8.0^{a}	1036.0±7.3^{a}	1041.5±5.8^{a}	1052.5±15.5^{a}
Milk protein %	3.16±0.03^{c}	3.21±0.03^{cb}	3.17±0.03^{c}	3.25±0.02^{ab}	3.28±0.04^{a}
Milk protein yield (g/cow/day)	1001.0±14.3^{d}	1036.8±9.5^{c}	1021.8±12.0^{c}	1059.5±9.8^{b}	1082.5±22.0^{a}
Milk lactose %	4.69±0.02^{a}	4.68±0.02^{a}	4.70±0.01^{a}	4.71±0.04^{a}	4.72±0.02^{a}
Milk lactose yield (g/cow/day)	1487.5±12.5^{c}	1512.5±6.5^{bc}	1513.3±7.5^{b}	1534.0±17.0^{ab}	1556.8±21.9^{a}

Values are means ± standard error. Mean values with different letters at the same raw differ significantly at ($p \leq 0.05$). N = No. of observation.

Table 9: Effect of different rumen protected soybean meal products on some blood serum parameters of dairy cows

Items	Soybean meal product treatments				
	SE (Control)	HS treatment	EP treatment	PA treatment	HPA treatment
	(N = 5)				
Glucose, mg/dl	57.6±0.48^{a}	58.1±0.55^{a}	57.7±0.55^{a}	58.7±0.38^{a}	58.6±0.50^{a}
Urea N, mg/dl	18.2±0.32^{a}	17.5±0.23^{ab}	17.3±0.20^{b}	17.1±0.35^{b}	16.8±0.45^{b}

Values are means ± standard error. Mean values with different letters at the same raw differ significantly at ($p \leq 0.05$). N = No. of observation.

SBM. The present data are in agreement with (Mabjeesh *et al*., 2000) stated that total VFA concentration was similar for roasted and untreated whole cotton seeds containing diets, however, the results are in contrast with (El-Waziry *et al*., 2007) they observed that the VFA concentrations were significantly ($p<0.05$) decreased when SBM treated by heat or tannins addition.

Milk composition: Higher ($p<0.05$) milk fat% and milk fat yield g/cow/day (Table 8) by about (3.2, 2.2, 1.6 and 1.6%) and (5.3, 3.9, 4.5 and 5.6%) when cows fed on diets containing treated SBM (HS, EP, PA or HPA) respectively when compared with cows group fed on untreated SBM. Replacement of untreated SBM by HS or EP treated SBM increased ($p>0.05$) milk protein % by about 1.6 and 0.3% respectively, while using PA or HPA treated SBM increased ($p<0.05$) milk protein% by about 2.8 and 3.8% respectively, on the other hand all products of treated SBM increased ($p<0.05$) milk protein yield by about 3.6, 2.1, 5.8 and 8.1% respectively when compared with cows group fed on untreated SBM. Moreover, treated SBM had no effect ($p>0.05$) on milk lactose percentage, while increased milk lactose yield compared to cows fed on untreated SBM. The higher milk fat percentages observed in this study with the treated SBM products were results of the additional postruminal digested energy (Mabjeesh *et al*., 2000). The lack of response in milk protein response in cows fed on HS or EP treated SBM instead of untreated SBM may be explained the moderate increase in rumen undegradable supply by those treated SBM sources or due to heating creates crosslinkages between peptide chains and the presences of carbohydrate, forms complexes between amino free aldehyde groups through Maillard reactions and some of these complexes undigested, while treated SBM by plant extract and essential oils are one of two distinct types of polymers of flavonoid phenols and as chemical additives for decreasing ruminal degradation of feed proteins were used to protect rapidly protein sources such as SBM (Makkar, 2003). The value of that processing method to ruminant protein nutrition lies in the sensitivity of the bonding to pH over normal pH range in the rumen, protein remain bound to the tannin, but at the low pH normally occurring in the abomasums, the protein released and become available for digestion (Broderick *et al*., 1991).

Blood serum units: The concentration of blood serum glucose and urea N are presented in Table 9. Substitution of SE SBM by HS, EP, PA or HPA treated SBM products had no effect ($p>0.05$) on blood serum glucose concentration when compared with the control. However cows fed on diets containing EP, PA and HPA treated SBM instead of SE SBM showed a reduction ($p<0.05$) in blood urea N by about 4.9, 6 and 7.7% respectively when compared with control, while substitution of SE by HS treated SBM exhibited non significant ($p>0.05$) reduction in blood urea N by about 3.8%. The lower blood urea N concentrations reflect improvement in metabolism of nitrogenous components in the diet (Mabjeesh *et al*., 2000). The urea N concentrations in various animal groups indicated that the HS treatment of SBM was the lowest response followed by EP treatment, while SBM treatment by plant extract with essential oil without or with heat treatment more effective.

Conclusion: Solvent extracted SBM exhibited greater effective degradability of CP and AA when compared with treated SBM products (HS, EP, PA or HPA). This was due to a greater fraction of soluble protein. The inclusion of treated SBM products in dairy cow diets improved milk production and milk-to-feed ratio while, decreased the concentration of ruminal NH_3 and blood urea N and increased CP utilization for milk protein secretion. Moreover, treated SBM products increased milk fat percentage and yield. However, the present study suggested that HS and EP treatment methods of SBM is less effective and the cow performance lesser respond than PA or HPA methods which depend on tanninferous plant species that protect protein from degradation in the rumen due to presence of small amounts of condensed tannin in the plant species and may be more available and digestible in the intestine more than the previous processing.

REFERENCES

Adrian, J., 1974. Nutritional and physiological consequence of the Maillard reaction. World Rev. Nutr. Diet., 19: 71-122.

Aldrich, C.G. and N.R. Merchen, 1995. Heat treatment of whole soybeans: influence on protein digestion by rumiants. J. Anim. Sci., 73 (suppl.): 95.

AOAC, 2000. Official Methods of Analysis. 17th Ed. Association of Official Analytical Chemists International, Arlington, VA.

AOAC., 1990. Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemists International, Arlington, VA.

Bateman, H.G., J.H. Clark and M.R. Murphy, 2005. Development of a system to predict feed protein flow to the small intestine of cattle. J. Dairy Sci., 88: 282-295.

Bligh, E.G. and W.J. Dyer, 1959. Fat extraction (Cited by Pearson's chemical analysis of foods). 8th Ed. (1963).

Broderick, G.A., R.J. Wallace and E.R. Orskov, 1991. Control of rate and extent of protein degradation. In physiological aspects of digestion and metabolism in ruminant (T. Tsuda, Y. Sasaki and R. Kawashima, Eds), pp: 541-592. Academic Press, Tokyo, Japan.

Borucki S.I.C., L.E. Phillip, H. Lapierre, P.W. Jardon and R. Berthiaume, 2007. Ruminal Degradability and Intestinal Digestibility of Protein and Amino Acids in Treated Soybean Meal Products, J. Dairy Sci., 90: 810-822

Calder, A.G. and A. Smith, 1988. Stable isotop ratio analysis of leucine and ketoisocaproic acid in blood plasma by gas chromatography mass spectrometry. Use of tertiary butyldimethylsilyl derivatives. Mass Spectrum, 2: 14-16.

Calder, A.G., K.E. Garden, S.E. Anderson and G.E. Lobley, 1999. Quantification of blood and plasma amino acids using isotope dilution electron impact gas chromatography mass spectrometry with U-^{13}C amino acids as internal standard. Rapid Commun. Mass Spectrum, 13: 2080-2083.

Can, A. and A. Yilmaz, 2002. Usage of xylose or glucose as non-enzymatic browining agent for reducing ruminal protein degradation of soubean meal. Small Rumin. Res., 46: 173-178.

Ceresnakova, A., A. Sommer, M. Chrenkova and P. Dolesova, 2002. Aminoacid profile of escaped feed protein after rumen inclusion and their intestinal digestibility. Arch. Anim. Nutr., 56: 409-418.

Clark, J.H., T.H. Klusmeyer and M.R. Cameron, 1992. Microbial protein synthesis and flows of nitrogen fractions to te duodenum of adiry cows. J. Dairy Sci., 75: 2304-2310.

Coulomb, J.J. and L. Faverau, 1963. A simple semi-micro method for colorimetric determination of urea. Chem., 9: 102-108.

Demjanec, B., N.R. Merchen, J.D. Cermin, Kr., C.G. Aldrich and L.L. Berger, 1995. Effect of roasting on site and extent of digestion of soybean meal by sheep. I. Digestion and nitrogen and amino acids. J. Anim. Sci., 73: 824-834.

Degussa Feed Additives, 1996. The Amino Acid Composition of Feedstuffs. 4th ed. Degussa, Ridgefield Park, NJ.

El-Waziry, A.M., M.E.A. Nasser and S.M.A. Sallam, 2005. processing methods of soybean meal. 1- Effect of roasting and tannic acid treated soybean meal on gas production and rumen fermentation *in vitro*. J. Appl. Sci., 1: 313-320.

El-Waziry, A.M., M.E.A. Nasser, S.M.A. Sallam, A.L. Abdallah and I.C.S. Bueno, 2007. Processing methods of soybean meal, 2. Effect of autoclaving and Qucbraho tannin treated soybean meal on gas production and rumen fermentation *in vitro*. J. Appl. Sci., 3: 17-24.

Faldet, M.A., L.D. Satter and G.A. Broderick, 1992. Determining optimal heat treatment of soybean by measuring available lysine chemically and biologically with rats to maximize protein utilization by ruminants. J. Nutr., 122: 151-160.

Ford, J.E. and C. Shorrock, 1971. Metabolism of heat damaged protein in the rat. Influence of heat damage on the excretion of amino acids and peptides in the urine. Br. J. Nutr., 26: 311-319.

Gerrard, J.A., 2002. Protein-protein crosslinking in food: Methods consequences, applications, trends. Food Sci., 49: 1282-1288.

Hanson, S.W.F. and J. Olly, 1963. Fat extraction. (Cited by Pearson's chemical analysis of foods). 8th Ed. (1963).

Heitritter, G.V., J.B. Yeates and P.L. Huffman, 1998. Method for manufacturing protein protected ruminant feed. US Pat. No. 5,82,355. http://www.uspto.gov/ Accessed Aprill 10, 2006.

Hoffiman, P.C., R.R. Grummer, R.D. Shaver, G.A. Broderick and T.R. Brendel, 1991. Feeding supplemental fat and undegraded intake protein to early lactating dairy cows. J. Dairy Sci., 74: 3468.

Ipharraguerre, I.R., J.H. Clark and D.E. Freeman, 2005. Rumen fermentation and intestinal supply of nutrients in dairy cows fed Rumen-Protected Soy Products. J. Dairy Sci., 88: 2879-2887.

Licitra, G., T.M. Hernandez and P.J. Van Soest, 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol., 57: 347-358.

Liu, K., 1999. Chemistry and nutritional value of soybean components. Page 25-114 in Soybeans: Chemistry, Technology and Utilization Monsanto, Ed. Aspen Publishers, Gaithersburg, MD.

Mabjeesh, S.J., J. Galindez, O. Kroll and A. Arieli, 2000. The effect of roasting nonlinted whole cottonseed on milk production by dairy cows. J. Dairy Sci., 83: 2557-2563.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannin rich feeds. Small Ruminant Res., 49: 241.

McKinnon, J.J., J.A. Olubobpkun, A. Mustafa, R.D.H. Cohen and D.A. Christensen, 1995. Influence of dry heat treatment of canola meal on site and extent of nutrient disappearance in ruminants. Anim. Feed Sci. Technol., 56: 243-252.

Mustafa, A.F., D.A. Christensen, J.J. McKinnon and R. Newkirk, 2000. Effects of stage of processing of canola seed on chemical composition and in vitro protein degradability of canola meal and intermediate products. Can. J. Anim. Sci., 80: 211-214.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. 7th Rev. Ed. Nat. Acad. Press, Washungton, DC.

Newbold, C.J., R.J. Wallace and N. Mckain, 1990. Effects of the ionophore tetronasin on nitrogen metabolism by ruminal microorganisms *in vitro*. J. Anim. Sci., 68: 1103-1111.

Randhir, S. and K. Pradhan, 1981. Forage evaluation. First published, 1981 printox, New Delhi, Dhawan Printing works.

Rulquin, H. and R. Verite, 1993. Amino acid nutrition in dairy cows: Production effects and animal requirements. Pages 55-77. In Recent Advances in Animal Nutrition. P.C. Garansworthy and D.J.A. Cole, ed. Nottingham Unversity Press, Nottingham, UK.

Santos, F.A.P., F.E.P. Santos, C.B. Theurer and J.T. Huber, 1998. Effects of rumen undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci., 81: 3182-3193.

Santos, F.P. and J.T. Huber, 1996. Quality of bypass proteins fed to high producing cows is important. Feedstuffs, 68: 34 pp, 12.

SAS, 1996. SAS Procedure Guide "version 6.12 Ed". SAS Institute Inc., Cary, NC, USA.

Socha, M., 1991. Effect of feeding heat processed whole soybeans on milk production, milk composition and milk fatty acid profile. M.S. Thesis, Univ. Wisconsin, Madison, WI.

Stern, M.D., K.A. Santos and L.D. Satter, 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat treated whole soybeans. J. Dairy Sci., 68: 45-52.

Tandon, M., R.A. Siddique and T. Ambwani, 2008. Role of bypass proteins in ruminant production. Dairy Planner., 4: 11-14.

Tice, E.M., M.L. Eastridge and J.L. Firkins, 1993. Raw soybeans and roasted soybeans of different particle sizes 1. Digestibility and utilization by lactating cows. J. Dairy Sci., 76: 224-235.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidizes with an alternative oxygen acceptor. Amm. Clin. Biochem., 6: 24.

Van Soest, P.J. and V.C. Mason, 1991. The influence of the Maillard reaction upon the nutritive value of fibrous feeds. Anim. Feed Sci. Technol., 32: 45-53.

Waltz, D.M. and M.D. Stern, 1989. Evaluation of various methods for predicting soy-bean protein from degradation by rumen bacteria. Anim. Feed Sci. Technol., 25: 111-122.

Weatherburn, M.W., 1967: Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem., 39: 971-974.