

IN VITRO RUMINAL AND POST-RUMINAL PROTEIN DEGRADATION OF UNTREATED AND TREATED SOYBEAN MEAL

Marwa F.A. Attia, M.E. A. Nasser, K.A. El-Shazly and S. M.A. Sallam *

²Department of Animal and Fish and Production, Faculty of Agriculture, University of Alexandria, Egypt

*Corresponding author: Prof. Dr. Sobhy M.A. Sallam, Department of Animal and Fish Production, Faculty of Agriculture, University of Alexandria, El-Shatby, Alexandria, Egypt Tel., +203-5921863 - Fax: +203-5922780 - E mail: (s_sallam@yahoo.com)

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SUMMARY

This study was designed to determine the characteristics of rumen fermentation, and post-ruminal protein digestion of untreated and treated soybean meal (SBM) using gas production (GP) technique and three step *in situ/in vitro* procedures. Soybean meal (SBM) was treated by autoclaving, roasting, with ethanol and mixed by various proportions of *Acacia saligna* (AS) leaves (1:3, 1:1, 3:1) ratios. The results showed that the highest cumulative GP was observed in untreated SBM followed by treated SBM with ethanol, roasted SBM and SBM+AS (3:1). The lowest mean values of GP were obtained in SBM+AS (1:3), autoclaved SBM and SBM+AS (1:1). The predicted metabolizable energy (ME, MJ/kg DM) and net energy (NE, MJ/kg DM) varied widely in substrates and was particularly high in untreated SBM, while SBM+AS (1:3) had significantly the lowest values of ME and NE. The highest mean values of NH₃-N concentration were obtained with untreated SBM, while the lowest was obtained with autoclaved SBM and followed by SBM+AS (1:3), ethanol-treated SBM and roasted SBM. Rumen degradable protein (RDP, %) varied from 33.6% in autoclaving SBM to 62.4% in SBM+AS (3:1). Intestinal protein digestion (IPD, %) content was highest in autoclaving-SBM, roasting SBM and untreated SBM and lowest mean value of IPD was recorded in SBM+AS (1:1). The highest mean values for VFA concentrations were obtained with SBM+AS (3:1) and SBM treated with ethanol followed by SBM plus *A. saligna* leaves (1:3). The lowest mean value of VFA concentration was obtained by autoclaving SBM or roasting SBM. It is concluded that autoclaving SBM, ethanol- treated SBM and mixing SBM with *Acacia* leaves (1:3) could be used advantageously to reduce RDP and increase the bypass protein but the autoclaving SBM showed the best treatment on improving the intestinal protein digestion of SBM.

Key words: Intestinal digestion, heating, gas production, *in vitro*

INTRODUCTION

The value of any protein to ruminant animals depends on the efficiency with which both the fraction that is degraded in the rumen and built into microbial protein, and that which is undegraded, are dealt with by the intestinal tract subsequent to leaving the rumen, and in particular how the protein is digested in the small intestine of ruminant. In addition, the benefit of any feed protein to ruminants depends on how the protein is digested in the small intestine (Calsamiglia and Stern, 1995; McNiven *et al.*, 2002). There was a wide variation in ruminal and post-ruminal protein disappearance of the feeds evaluated by different *insitu* and *invitro* digestibility methods. Grains, by-products and protein sources are more susceptible for digestion in the ruminant gastrointestinal tract than forages and halophytes (Danesh Mesgaran and Stern, 2005).

Proteins of soybean meal (SBM), which is a commonly used protein supplement for ruminants, are extensively degraded in the rumen. It is very palatable and has a good amino acid balance and high availability. It is estimated that only 34% SBM protein escape rumen fermentation. Therefore, improvement in ruminal escape characteristics of SBM is of major importance to both beef and dairy producers and the soybean industry. Some attempts to decrease the extent of ruminal degradation of SBM proteins have used treatment with dry or moisture heat (Nakamura *et al.*, 1994), ethanol (Corley *et a.*,

1999), formaldehyde (Mir *et al.*, 1984), tannic acid and quebracho (El-Waziry *et al.*, 2005, 2007), acetic acid (Robinson *et al.*, 1994), and xylose (Harstad and PrestlØkken, 2000).

Accurate estimation of ruminal and intestinal protein disappearance of feed is also essential for ruminant diet formulation. The mobile bag technique, using duodenally cannulated cows, provides an estimation of total tract protein disappearance and correlates well with true intestinal nitrogen digestibility *in vivo* (Hvelplundet *et al.*, 1994). However, the cost and unpredictability of surgically modifying a number of steers or cows, as well as animal welfare issues, have led the nutritionist to investigate laboratory methods to estimate ruminal and post-ruminal protein disappearance (Calsamiglia and Stern, 1995; McNiven *et al.*, 2002; Tejido *et al.*, 2002). Numerous laboratory methods are currently used for estimating ruminal and intestinal disappearance of feed protein using various enzymatic procedures (Calsamiglia and Stern, 1995; McNiven *et al.*, 2002). These methods are simple to conduct and reasonable in cost, compared with the *in vivo* method. In addition, they yield values that are estimates of true digestibility of feed protein. *In vitro* gas production method has been shown to be well correlated with *in vivo* results (Cone *et al.*, 2006), and it is sensitive to the presence of compounds that reduce intestinal protein utilization (Calsamiglia and Stern, 1995). Voluntary intake of a feed depends essentially on the rate of ruminal and post-ruminal digestion of digestible nutrients (Ørskov *et al.*, 1988); especially CP as an index for feed digestion potential. Therefore, in order to define an optimum ruminant diet in a tropical area, it is necessary to choose feedstuffs according to the quantity and quality of available CP. Therefore, the aim of this experiment was to study the effect of some treatments (roasting, autoclaving or ethanol) of SBM and mixing SBM with *Acacia saligna* leaves with different proportions of tannins content gas production, kinetics of gas production, ruminal fermentation, dry and organic matter degradation, ruminal and intestinal protein digestion using gas production technique and three-step *in situ/in vitro* procedures.

MATERIALS AND METHODS

The present study was carried out at the Rumen Microbiology Laboratory, Department of Animal Production, Faculty of Agriculture, Alexandria University, Egypt.

Animals and diet

Three rumen cannulated sheep were used as source of rumen inoculum and for three step *in situ/in vitro* technique. The animals were fed on commercial concentrate mixture and rice straw throughout the experiment period.

Experimental feeds and treatments

Feed samples included untreated SBM in comparison with treated SBM and associative effect of mixing SBM with *A. saligna* leaves in various proportions. The treatments were as follow:

- 1) Untreated SBM.
- 2) Treated SBM with 70% Ethanol (Sadeghi and Shawrang, 2007).
- 3) Roasting SBM at 155°C for 30 min (Nowak *et al.*, 2005).
- 4) Autoclaving SBM at 121°C for 30 min (Broderick and Craig, 1980).
- 5) SBM + *Acacia saligna* leaves (3:1).
- 6) SBM + *Acacia saligna* leaves (1:1).
- 7) SBM + *Acacia saligna* leaves (1:3).

Chemical analyses

All feed samples were dried using a forced-air oven at 50°C for 48 h. The feed samples were ground to pass through a 1-mm screen and analyzed for DM; dry matter-ID number 930.15; OM: organic matter-ID number 942.05; CP: crude protein – as 6.25 x N- ID number 954.01 (AOAC, 1995) and ADF; acid detergent fibre – ID number (973.18) and NDF: neutral-detergent fibre, (Mertens, 2002). Untreated and treated SBM were also analyzed for extractable total phenols (TP), tannins (TT) and condensed tannins (CT). Dried plant material (200 mg) was extracted with acetone: water (10 ml, 70:30 v/v) in an ultrasonic bath for 20 minutes. The contents were centrifuged (4°C, 10 min, 3000 g) and the supernatant was kept on ice until analysis. Total phenols were determined with the Folin-Ciocalteu reagent (Makkaret *et al.*, 1993, Makkar, 2003). Extractable tannins were determined as the difference in total phenols (measured by Folin-Ciocalteu reagent) before and after treatment with insoluble polyvinyl polypyrrolidone (PVPP), as this polymer binds strongly to tannins (Makkaret *et al.*, 1995). TP and TT were expressed as tannic acid

equivalents. Condensed tannins were measured by the HCl-butanol method and the results were expressed as leucocyanidin equivalent (Makkar, 2003).

In vitro gas production technique

In vitro gas production was undertaken according to the procedure described by Menke and Steingass (1988). Samples (100 mg) of the air-dry feedstuffs were accurately weighted into 50 ml calibrated glass syringe fitted with plungers. The buffer solution used for *in vitro* gas production was defined as MB9 (Onodera and Handerson, 1980). Then the buffer pH was adjusted at 6.8 and CO₂ flushed for 30 min. Rumen contents (50% solid: 50% liquid) were collected from three rumen cannulated sheep which was fed with rice straw ad lib and commercial concentrate mixture. The rumen contents were collected before the morning feeding of the animals. Liquids and solids were placed in pre-warmed (39°C) insulated flasks and transported under anaerobic conditions to the laboratory. The rumen contents were squeezed through four layers of cheese-cloth and kept in a water bath at 39°C with CO₂ saturation until inoculation took place. The buffer and inoculum (2:1 v/v) were mixed and kept in a water bath at 39°C with CO₂ saturation (Solivaet *al.*, 2005).

Buffered rumen fluid (15 ml) was pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39°C. Syringes were incubated *in vitro* in water bath for 96 h and gently shaken every 2h. Half of the syringes were terminated after 24 h incubation for determination of the ammonia-N (NH₃-N), volatile fatty acids (VFA) and truly degraded dry and organic matter. The rest of the syringes were continuously incubated up to 96 h and gas production was recorded at 3, 6, 9, 12, 24, 72 and 96 h of incubation *in vitro*.

Three runs were performed for each experiment consisting of four syringes containing only buffered rumen fluid considered as the blank, four syringes for each sample and four syringes for Berseem hay as a high quality roughage and internal standard to correct the variation between runs. The gas values were expressed as ml per 200 mg of DM (remember only 100mg was incubated). The cumulative gas production (Y) at time (t) was fitted to the exponential model of (Ørskov and McDonald, 1979). Gas (t) = a+b*(1-exp-ct), a = the gas production from the soluble fraction (ml), b = the gas production from the insoluble fraction (ml), c = the gas production rate (ml/h), t = incubation time (h).

Rumen fermentation and degradation

The content of the syringes at 24 h of incubation were transferred to the centrifuge tube and centrifuged at 3000g for 10 min. Five milliliter from the supernatant were transferred into 25 ml glass bottle and stored in a freezer until they were analyzed for NH₃-N and VFA determination. Volatile fatty acids and ammonia concentration were determined according to Warner, (1964) and Preston, (1995), respectively. True dry and organic matter digestibility were determined for the half of the syringes at 24 h incubation according to the procedure described by Van Soest, (1994) incubating the syringe residue with neutral detergent solution (NDS) at 110°C for 3 h (the purpose of this treatment is to solubilize the microbes and to only obtain the undegraded feed). The contents were filtered through crucibles; the residue was washed with distilled water then with acetone. The crucibles were dried at 110°C for 24 h for determining truly dry matter degradation. Then the crucibles were transferred to a muffle furnace for 2 h at 600°C for determining truly organic matter degradation.

Predicted parameters

A new approach to evaluate feeds from gas parameters was adopted where, gas production from fermentation of the soluble fraction (GPSF) was estimated from gas produced after 3 h (GP3) of incubation. Gas production from fermentation of the insoluble fraction (GPNSF) was estimated from the gas production between 3 h (GP3) and 24 h (GP24) of incubation according to Van Gelder *et al.* (2005). The energy values were calculated from the amount of gas produced at 24 h of incubation with supplementary analyses of crude protein, ash and crude fat. This approach was developed by the research group in Hohenheim (Germany) and is based upon extensive *in vitro* incubation of feedstuffs (Menke *et al.*, 1979; Menke and Steingass, 1988).

$$\text{ME (MJ /kg DM)} = 1.06 + 0.157\text{GP} + 0.084\text{CP} + 0.22 \text{EE} - 0.081*\text{A}$$

$$\text{OMD (\%)} = 14.88 + 0.889 \text{GP} + 0.45\text{CP} + 0.0651*\text{A}$$

Where: ME is the metabolizable energy, OMD is organic matter digestibility, GP is 24 h net gas production (ml/200 mg DM), CP is crude protein (% of DM), A is ash (% of DM)

$$\text{NE (Mcal/lb)} = (2.2 + (0.0272*\text{Gas}) + (0.057*\text{CP}) + (0.149*\text{EE})) / 14.64$$

Where: NE is the net energy, Gas is 24 h net gas production (ml/g DM), CP is crude protein (% of DM), EE is ether extract (% of DM)

Three-step procedure *in situ*/ *in vitro*

Ruminal and post-ruminal protein digestion of the various treatment feeds or diets were evaluated using and three-step *insitu/invitro* procedure (Calsamiglia and Stern, 1995). This part of the experiment followed the procedure of Calsamiglia and Stern (1995). Approximately, (4.0 g) of feed sample were weighed into a 6 cm×10 cm Dacron polyester bag with a pore size of 52µm (four bags per sample) and suspended in the rumen of a cannulated sheep for 16 h. Bags were then removed and washed with cold tap water using a washing machine, frozen for 48 h and later washed and dried in a forced-air oven at 55°C for 48 h and weighed. Samples from each bag were taken for N analysis using the Kjeldahl method. Samples of ruminal undegraded residue (0.5 g) were weighed into a 50 ml polypropylene centrifuge tube. Two blank tubes were also prepared to correct for N contribution of the enzymes. Ten milliliters of pre-warmed (37°C) HCl-pepsin solution (1 g of pepsin (Sigma P-7012) dissolved into 1 l of 0.1N HCl) were added into each tube. Tubes were vortexed and incubated for 1 h in a shaking water bath at 39°C. After the 1 h incubation, 0.5 ml of 1N NaOH solution was added and tubes were again vortexed. The procedure continued by adding 13.5 ml of phosphate-pancreatin buffer (0.5 M KH₂PO₄ buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g / L of pancreatin (Sigma 7545). Tubes were vortexed and incubated for 24 h in a shaking water bath at 39°C. After incubation, 3 ml of trichloro acetic acid (TCA, 100%) solution were added to each tube, then vortexed. The tubes were left for 15 min and then centrifuged at 10,000×g for 15 min. Part of the supernatant (5 ml) was pipetted from each tube for N concentration determination by the Kjeldahl method.

Statistical analysis

Data were analyzed by the generalized linear model procedure in SAS (2002). The following model was assumed: $Y_{ij} = \mu + F_i + e_{ij}$. Where: μ is the overall mean, F_i is the treatment diets, e_{ij} is the random error term. Gas production runs were considered as the experimental units. Each run was repeated three times, and replicates within the same run were considered as repetitions. Differences among means were tested using Duncan multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The mean values of the proximate analysis and tannin content on dry matter basis of the untreated SBM, treated SBM and mixed SBM with *A. saligna* leaves are presented in Table (1). Ash content did not significantly differ among treatment diets. Crude protein (CP) ranged from 25.1% in SBM+AS (1:3) to 44.6% in SBM. Ether extract content ranged from 1.9% in SBM+AS (3:1) to 4.2% in treated SBM with ethanol. The fiber fractions did not significantly differ between untreated or treated SBM. The highest value for NDF and ADF were observed in SBM+AS (1:3), SBM+AS (1:1) and SBM+AS (3:1) but did not differ significantly among untreated SBM, roasted SBM, treated SBM with ethanol and autoclaving SBM respectively. The highest value for TP, TT and CT were observed in SBM+AS (1:3) and SBM+AS (1:1).

Table (1). Proximate analyses and tannins content of the treated, untreated SBM and mixed SBM with *Acaciasaligna* (AS) leaves (DM Basis)

Item	CP	EE	Ash	NDF	ADF	TP	TT	CT
SBM	44.6	2.0	6.6	19.0	12.9	0.19	0.12	0.03
Ethanol-treated SBM	43.8	4.2	5.6	20.9	14.6	0.43	0.14	0.11
Roasting SBM	44.2	2.2	6.2	19.8	12.7	0.12	0.03	0.13
Autoclaving SBM	44.4	2.2	5.8	19.5	11.7	0.36	0.04	0.09
SBM+AS (3:1)	37.28	1.9	6.9	23.6	18.3	0.43	0.32	0.65
SBM+AS (1:1)	30.8	3.9	7.6	28.2	23.5	1.12	0.62	1.56
SBM+AS (1:3)	25.1	2.5	8.7	35.1	33.8	2.85	2.00	1.86

SBM: Soybean meal, AS: *Acacia saligna*, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, TP: total phenols (eq-g tannic acid /100g DM), TT: total tannins (eq-g tannic acid /100g DM), CT: condensed tannins (eq-g leucocyanidin/100g DM).

Figure (1) shows the cumulative gas production profiles, corrected for blank, for untreated, treated SBM and mixed SBM with *A.saligna* leaves incubated in buffered rumen fluid for 96 h *in vitro*. The highest cumulative gas production was observed in SBM followed by treated SBM with ethanol, roasting SBM and SBM+AS (3:1). On the other hand, roasting SBM and SBM+AS (3:1) did not differ significantly, while the lowest mean values was obtained by SBM+AS (1:3), autoclaving SBM and SBM+AS (1:1).

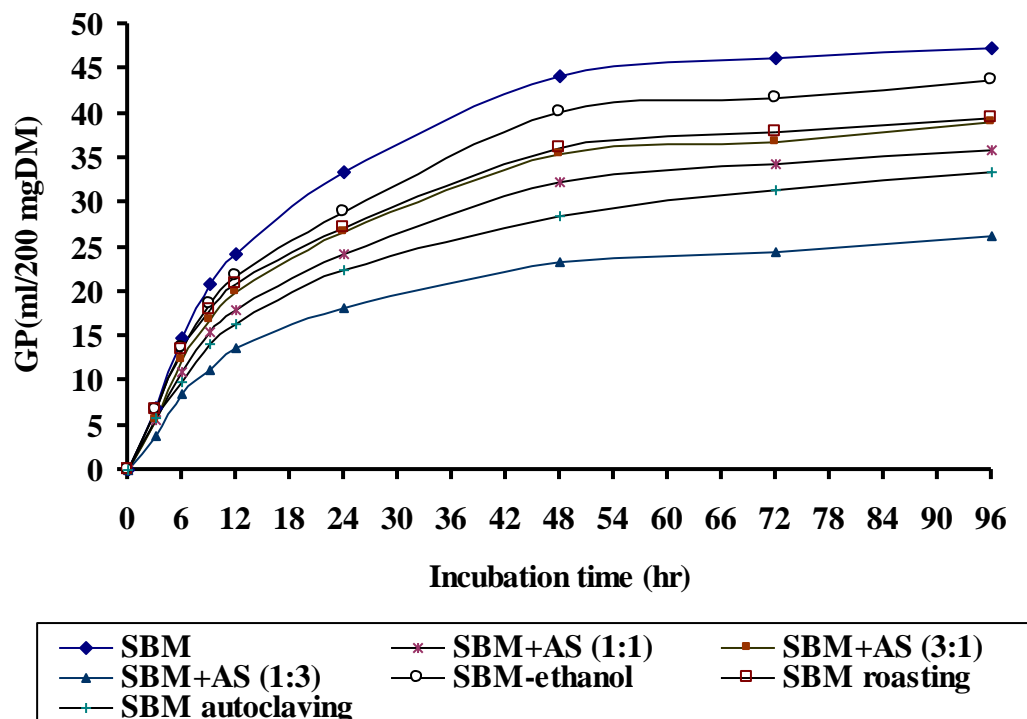


Figure (1). Cumulative gas production profiles for untreated and treated SBM and mixed SBM with *Acacia saligna* (AS) leaves incubated 96 h *in vitro*.

The least square means of the estimated parameters obtained from exponential model, GPSF and GPNSF (ml/ g DM) are given in Table 2. Estimated constant rate of gas production (c) did not significantly differ among untreated, treated SBM and mixed SBM with *A. saligna* leaves. The fitted a+b values ranged from 25.3 in SBM+AS (1:3) to 47.0 ml in SBM. There were wide variations among untreated, treated SBM and mixed SBM with *A. saligna* leaves in terms of GPSF and GPNSF. The results showed that SBM+AS (1:3) had the lowest GPSF and GPNSF followed by SBM+AS (1:1), SBM+AS (3:1) and autoclaving SBM. In concern of GPSF, there were no significant effects for roasting or ethanol-treated SBM in comparison to untreated SBM.

In the present study, heat treatment of SBM significantly ($P < 0.05$) reduced the rapidly soluble fraction and the gas production rate. Similar results were reported by Konishi *et al.* (1999) who examined roasted SBM at 133, 143 and 153°C for 3 h using nylon bag technique. Heating creates crosslinkages between peptide chains and, in the presence of carbohydrate, forms complexes between free amino and aldehyde groups through Maillard reaction. These reactions lower the protein solubility. Heat treatment of SBM is reported to reduce protein degradation in the rumen resulting from a lower solubility of protein in the rumen and these results are in agreement with the present study for decreasing soluble fraction (Table 2).

In the present study, heat treatment of SBM (autoclaving SBM) significantly ($P < 0.05$) reduced the degradation constant (a and b fractions) and the gas production rate (c). Similar results were reported by Ljøkjælet *et al.*, (2000) who tested autoclaved SBM at 120 and 130°C for 30 min using *in situ* technique. Also, heat treatment of SBM by expelling and roasting has shown the same effect (Titgemeyer and Shirley, 1997 and El-Waziry *et al.*, 2005). When mixing SBM with *A. saligna* leaves in different proportions, the estimated parameters using gas production technique was significantly ($P < 0.05$) decreased compared with untreated SBM. The beneficial effects of feeding tanniferous plant species with protein-rich diet are generally described to the protection of proteins from degradation in the rumen due to the presence of small amounts of condensed tannins (CT) in the plant species. Condensed tannins

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 protein sources such as SBM. When tannin-containing plants are eaten, most binding appears to take place during chewing, but additional binding can occur in the rumen, including binding of proteins from other dietary components (Waghorn and Jones, 1989). There is concern that if tannin concentration in the diet becomes too high, microbial enzyme activities (Makkaret *et al.*, 1988) and intestinal digestion (Horigomeet *et al.*, 1988) may be depressed. Treated SBM with tannic acid or CT (Frutos *et al.*, 2000; El-Waziry *et al.*, 2005, 2007) decreased the degradation of SBM. Min *et al.* (2003) reported in their review that dietary concentrations of CT, ranging from 20 to 45 g/kg DM, improved efficiency of N use and increased the daily weight gain in lambs on temperate fresh forages like *Lotus corniculatus*.

Table (2). Least square means (\pm SE) of gas production kinetics for the treated and untreated SBM and mixed SBM with *Acaciasaligna* (AS) leaves incubated in vitro 96 h.

Item	a+b	c	GPSF	GPNSF
SBM	47.0 \pm 3.4 ^a	0.060 \pm 0.004	31.6 \pm 4.4 ^a	136.2 \pm 8.3 ^a
Ethanol-treated SBM	43.1 \pm 3.7 ^a	0.054 \pm 0.005	30.7 \pm 4.8 ^a	115.2 \pm 9.1 ^{ab}
Roasting SBM	38.5 \pm 3.4 ^b	0.061 \pm 0.004	30.1 \pm 4.4 ^a	106.4 \pm 8.3 ^b
Autoclaving SBM	32.2 \pm 3.4 ^{bc}	0.061 \pm 0.004	26.1 \pm 4.4 ^{ab}	86.8 \pm 8.3 ^c
SBM+AS (1:1)	35.1 \pm 3.4 ^b	0.057 \pm 0.004	24.6 \pm 4.4 ^b	97.3 \pm 8.3 ^b
SBM+AS (3:1)	38.1 \pm 3.4 ^b	0.059 \pm 0.004	24.9 \pm 4.4 ^b	109.2 \pm 8.3 ^b
SBM+AS (1:3)	25.3 \pm 3.4 ^c	0.061 \pm 0.004	16.2 \pm 4.4 ^c	74.9 \pm 5.7 ^d

Means within a column bearing different superscripts differ ($P < 0.05$)

SBM: Soybean meal, AS: *Acacia saligna*, a: gas production from the soluble fraction (ml), b: gas production from the insoluble fraction (ml), c: gas production rate (ml/hr), GPSF: gas production from soluble fractions (ml/g DM), GPNSF: gas production from non-soluble fractions (ml/g DM), SE: standard error.

Fukushima (1969) described the mechanism of protein denaturation by ethanol solutions. The addition of a solvent, such as ethanol, that is miscible with water but less polar, lowers the dielectric constant of the system. This tends to increase the strength of all electrostatic interactions between protein molecules in contact with water. The presence of the less polar solvent weakens the hydrophobic bonds inside the proteins. In this situation, the structure of the protein changes and, hence, it is denatured. Treatment of SBM with ethanol solutions decreased the washout fraction of CP. It is important to note that effects of heat and ethanol treatments on protein structures are different. Heat processing can result in protein denaturation and aggregation, formation of cross-linkages between amino acids and reducing sugars (*i.e.* the Maillard reaction), or between proteins (*i.e.* iso-peptide bonds). These two latest reactions will probably make the protein more resistant to degradation in the rumen and small intestine. In this study, ethanol treatments decreased intestinal digestibility of CP compared to untreated SBM. In native structure of globular proteins, many hydrophobic amino acids are buried inside the protein. Ethanol solution induces the unfolding of the protein and denaturation, thus exposing non-polar groups that were previously blocked (Taha and Mohamed, 2004). Ethanol processing of SBM exposed hydrophobic amino acids (especially aromatics) that are position groups for active site of pepsin and trypsin enzymes (Murray *et al.*, 2003).

The least square means of metabolizable energy (ME, MJ/kg DM), net energy (NE, MJ/kg DM), organic matter digestibility (OMD%) and microbial protein (MP, g/kg DOM) from gas production and chemical analyses for untreated, treated SBM and mixed SBM with AS leaves are presented in Table (3). The predicted ME varied widely in substrates and was particularly high in SBM, while SBM+AS (1:3) had significantly lower values of ME and NE. Microbial protein content varied from 82.8 g/kg DOM in SBM+AS (1:3) to 125.4 g/kg DOM in SBM. Roasting autoclaving and ethanol-treated SBM decreased energy content, OMD and MP while the differences were significant for ME and OMD in comparison with untreated SBM. There were negative associative effects on SBM in terms of SCFA, energy content, OMD and MP. The lowest values for the predicted parameter were recorded with SBM+AS (1:3).

Table (3). Least square means for the predicted parameters from gas production and chemical analyses for untreated SBM, treated SBM and mixing SBM with *Acacia saligna* (AS) leaves by different proportions *in vitro* (\pm SE).

Item	ME	NE	OMD	MP
SBM	9.29 \pm 0.32 ^a	5.98 \pm 0.21 ^a	65.0 \pm 2.1 ^a	125.4 \pm 4.1 ^a
Ethanol-treated SBM	8.66 \pm 0.35 ^b	5.79 \pm 0.23 ^a	60.7 \pm 2.3 ^b	117.2 \pm 4.5 ^{ab}
Roasting SBM	8.44 \pm 0.32 ^b	5.85 \pm 0.21 ^{ab}	59.3 \pm 2.1 ^b	114.5 \pm 4.1 ^{ab}
Autoclaving SBM	7.81 \pm 0.32 ^b	5.35 \pm 0.21 ^b	55.2 \pm 2.1 ^b	106.6 \pm 4.1 ^b
SBM+AS(1:1)	7.47 \pm 0.32 ^b	5.00 \pm 0.21 ^b	52.2 \pm 2.1 ^c	100.8 \pm 4.2 ^b
SBM+AS (3:1)	8.11 \pm 0.32 ^b	5.22 \pm 0.21 ^b	56.8 \pm 2.1 ^b	109.6 \pm 4.1 ^b
SBM+AS (1:3)	6.15 \pm 0.32 ^c	4.04 \pm 0.21 ^d	42.9 \pm 2.1 ^d	82.8 \pm 4.3 ^c

Means within a column bearing different superscripts differ ($P < 0.05$)

NE: Net energy (MJ/kgDM); ME: metabolizable energy (MJ/kgDM); OMD: organic matter degradation (%); MP: microbial protein (g/kg DOM), SE: standard error.

The digestibility of measured organic matter is closely correlated with that predicted from gas production and the crude protein and ash contents of feeds. Therefore, the method can be used to predict the extent of digestion for various feeds. The predicted ME and NE which was calculated from gas production after 24 h incubation were reduced when SBM was treated by heating (autoclaving and roasting) or mixed with *A. saligna* leaves (Table 3). The values of MP were ranged between 125.4 to 82.8 g/kg OMD for SBM and SBM plus AC (1:3), respectively. These results are in agreement with the results of (Czerkawski, 1986) who found that a highly significant correlation between MP syntheses was predicted from OMD in the rumen and that predicted from VFA production. The decrease in DOM, ME and NE in which diets was probably due to increased Maillard products which also decreased rumen degradability and formation of complexes between tannins and dietary proteins and carbohydrates, as well as reduced rumen microbial proteolytic, ureolytic and cellulolytic enzyme activities, general fermentative activities and cell multiplication (Makkar *et al.*, 1988 and Muhammed *et al.*, 1994).

The mean values of true and apparent dry matter and organic matter degradation at 24 and 96 h incubation *in vitro* for untreated, treated SBM and mixed SBM with *A. saligna* leaves in are given in Table (4). The results showed that treating SBM by autoclaving decreased ($P < 0.05$) the degradation of dry and organic matter (true or apparent) while, the roasting or ethanol treatment did not affect ($P > 0.05$) degradation of either truly or apparently. Combining SBM with AS leaves decreased ($P < 0.05$) the dry and organic matter degradation (true and apparent) compared to untreated SBM except the true degradation of dry matter of SBM+AS (3:1) except TDDM of SBM+AS (3:1). The lowest degradation values were observed with SBM+AS (1:3).

Table (4). Least square means (\pm SE) of dry and organic matter degradation (truly and apparently) for untreated and treated SBM and mixed SBM with *Acacia saligna* leaves.

Item	Degradation at 24 h		Degradation at 96 h	
	TDDM	TDOM	ADDM	ADOM
SBM	96.1 \pm 3.0 ^a	94.8 \pm 3.4 ^a	85.6 \pm 4.0 ^a	82.5 \pm 4.2 ^{ab}
Ethanol-treated SBM	95.6 \pm 3.7 ^a	92.0 \pm 2.8 ^{ab}	92.9 \pm 4.0 ^a	91.8 \pm 3.6 ^a
Roasting SBM	94.9 \pm 4.6 ^a	93.5 \pm 2.8 ^{ab}	95.1 \pm 3.6 ^a	93.0 \pm 3.6 ^a
Autoclaving SBM	82.6 \pm 3.2 ^b	78.1 \pm 2.4 ^c	85.6 \pm 3.6 ^a	83.3 \pm 3.6 ^{ab}
SBM+AS (1:1)	74.9 \pm 2.6 ^c	73.7 \pm 2.4 ^c	74.4 \pm 3.6 ^c	69.6 \pm 2.5 ^c
SBM+AS (3:1)	87.6 \pm 3.5 ^a	86.0 \pm 2.9 ^b	82.9 \pm 3.6 ^b	82.0 \pm 3.6 ^{ab}
SBM+AS (1:3)	63.4 \pm 2.6 ^d	62.1 \pm 2.4 ^d	62.1 \pm 2.7 ^d	60.9 \pm 2.4 ^d

Means within a column bearing different superscripts differ ($P < 0.05$)

SBM: Soybean meal, AS: *Acacia saligna*, TDDM: truly digested dry matter, TDOM: truly digested organic matter, ADDM: Apparently digested dry matter, ADOM: Apparently digested organic matter, SE: standards error.

Ammonia-N concentration (NH₃-N) for the untreated-, treated SBM and mixed SBM with AS leaves in different proportions incubated *in vitro* for 24 hr are presented in Figure (2). There were significant differences among substrates in the NH₃-N concentration. The highest mean value of NH₃-N concentration was obtained with untreated SBM. The lowest mean value of NH₃-N concentration was

obtained with autoclaving SBM followed by SBM+AS (1:3), ethanol-treated SBM and roasted SBM, respectively.

The concentrations of NH₃-N were significantly (P<0.05) decreased in SBM treated by heating or mixed with *Acacia saligna* leaves (Figure 2). In agreement, other researchers has shown that ruminants fed diets containing soybean or cottonseed meal (autoclaved at 121°C for 45 and 90 min; Sherrod and Tillman 1962), extruded whole soybeans (155°C; Block *et al.*, 1981), roasted SBM (115, 130, or 145°C; Plegge *et al.*, 1985) or mixed with *Acacia saligna* leaves (Ben Salem *et al.*, 2005) had a lower ruminal NH₃-N concentration than ruminants fed a non-heated control. Contradictory, with the present results, Tice *et al.*, (1993) fed cows diets containing heat-treated soybeans (whole soybeans extruded at 132 and 149°C, or roasted to 143°C and steeped for 30 min, respectively) with no decline in ruminal NH₃-N compared to cows fed whole raw soybeans. The studies of Wallace *et al.* (1981) and Newbold *et al.* (1990) have concluded that the lower ammonia concentrations were mainly due to reduced proteolysis, degradation of peptides and deamination of amino acids in the rumen.

The volatile fatty acids (VFA) for the untreated, treated SBM and mixed SBM with *A.saligna* leaves by different proportions incubated *in vitro* for 24 h is shown in Figure (3). The highest mean values for VFA concentrations were obtained with SBM+AS (3:1) and SBM treated with ethanol followed by SBM plus *A. saligna* leaves (1:3). The lowest mean value of VFA concentration was obtained by autoclaving SBM or roasting SBM. The concentrations of VFA were significantly (P<0.05) decreased when SBM treated by heating or when mixed with *A. saligna* 50% SBM plus 50% *A. saligna* (Figure 3).

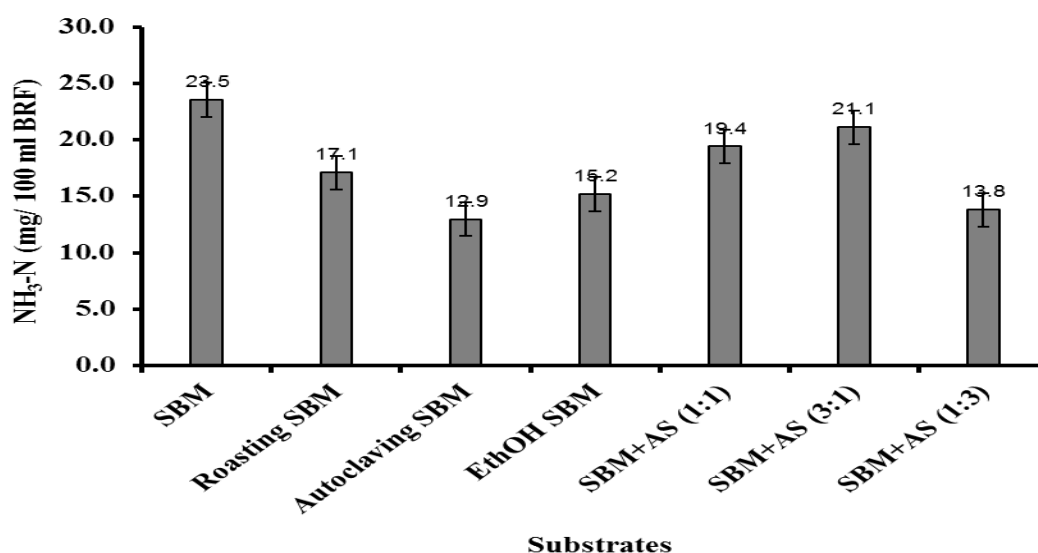


Figure (2). Ammonia-N concentration (NH₃-N) for untreated, treated SBM and mixed SBM with *Acacia saligna* leaves (AS) by various proportions incubated *in vitro* for 24h.

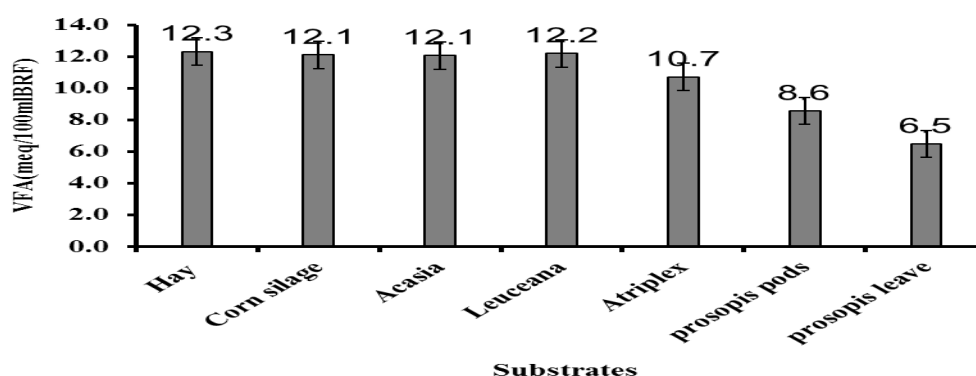


Figure (3). Volatile fatty acids (VFA) concentration for untreated, treated SBM and mixed SBM with *Acacia saligna* (AS) leaves by various proportions incubated *in vitro* for 24h.

Gas production is an indirect measure of substrate degradation, and is a good predictor for the production of VFA, which is positively related to microbial mass production. The lack of consensus among experiments may be due to differences in the level of heat treatment applied to the oilseeds and meal or to the quantity of soluble N provided by the non-oilseed portion of the diet. Getachew *et al.* (1998) stated that in heating, free amino-groups (mainly the epsilon amino-group of lysine) react with other groups to form enzyme-resistant, interand intra-molecular bonds, thereby reducing the solubility and digestibility of the protein.

The mean values of ruminal and intestinal protein degradation using the three step *in situ/ in vitro* procedure for the untreated, treated SBM and mixed SBM with *A.saligna* leaves in are presented in Table (5). The RDP content varied from 33.6% in autoclaving SBM to 62.4% in SBM+AS (3:1). The RUP differed significantly among feedstuffs. The highest value for RUP was observed in autoclaving SBM but the lowest value observed in SBM+AS (3:1). The IPD content was highest in autoclaving SBM and lowest in SBM+AS (1:1).

Table (5). Least square means (\pm SE) *in situ/ in vitro* values of rumen degradable protein, rumen undegradable protein and intestinal protein digestion for treated and untreated SBM using the three step procedure.

Item	RDP%	RUP%	IPD%
SBM	52.9 \pm 8.8 ^b	47.1 \pm 8.8 ^c	61.1 \pm 7.3 ^b
Ethanol- treated SBM	43.5 \pm 7.2 ^c	56.5 \pm 7.2 ^b	52.5 \pm 7.3 ^c
Roasting SBM	54.6 \pm 7.2 ^b	45.4 \pm 7.2 ^c	62.5 \pm 6.4 ^b
Autoclaving SBM	33.6 \pm 7.2 ^d	66.4 \pm 7.2 ^a	70.3 \pm 7.3 ^a
SBM+AC (1:1)	58.9 \pm 7.2 ^{ab}	41.1 \pm 7.2 ^{cd}	48.5 \pm 7.3 ^d
SBM+AC (3:1)	62.4 \pm 7.2 ^a	37.6 \pm 7.2 ^d	54.7 \pm 7.3 ^c
SBM+AC (1:3)	45.7 \pm 4.4 ^c	54.3 \pm 4.4 ^b	51.5 \pm 4.5 ^c

Means within a column bearing different superscripts differ (P<0.05)

RDP: Rumen degradable protein, RUP: Rumen undegradable protein, IPD: Intestinal protein digestion, SE: standard error.

Lynch *et al.* (1987) noted that ethanol treatments appeared to be superior to dry heat treatments, because they altered ruminal degradability of SBM protein without reducing its intestinal digestibility. Sadeghi and Shawrang, (2007) found that treatment of SBM with 600 ml/l ethanol solution was optimal and had the highest potential for increasing RUP of SBM, making higher quality dietary protein available to the ruminant. Observed intestinal digestibility of CP reaching the duodenum were low, probably due to the presence of tannins, and the high proportion of acid detergent insoluble nitrogen (ADIN) which is not easily digested by intestinal enzymes.

The three-step procedure has been developed to estimate intestinal digestion of ruminal undegraded feed protein in ruminants (Calsamiglia and Stern, 1995) as an alternative to using animals with cannulae in the duodenum and ileum. Stern *et al.* (1997) compared results of the three-step procedure with *in vivo* intestinal protein digestion and found a high correlation ($r^2 = 0.91$). They concluded that the *in vitro* method provides an excellent estimate of *in situ* mobile bag protein degradability and is sensitive to the effects of heat treatment. Mixing *A. saligna* leaves with SBM decreased ruminal NH₃-N and *in situ* degradation of SBM. These findings suggest that Acacia tannins impaired wastage of dietary proteins, mainly from SBM, through microbial degradation in the rumen.

CONCLUSION

It is concluded that autoclaving SBM, ethanol- treated SBM and mixing SBM with Acacia leaves (1:3) could be used advantageously to reduce RDP, and increase the bypass protein but the autoclaving SBM showed the best treatment on improving the intestinal protein digestion of SBM.

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الهضم المعمل للبروتين في الكرش والأمعاء لكسب فول الصويا غير المعامل والمعامل

مروة فايز عبد الخالق عطيه، محمد عماد عبد الوهاب ناصر، خالد عبد السلام الشاذلي، صبحي محمد عبد الله سلام
قسم الإنتاج الحيواني والسمكي، كلية الزراعة، جامعة الإسكندرية، الإسكندرية، مصر.

أجريت هذه التجربة لتقييم خصائص تخمرات الكرش وهضم البروتين في الكرش والأمعاء لكسب فول الصويا غير المعامل والمعامل بالتعقيم، التخميص، الإيثانول، والخلط بنسب مختلفة مع أوراق الأكاسيا بنسبة (1:3 ، 1:1 ، 3/1) باستخدام تقنية إنتاج الغاز و Three step *in situ* / *in vitro* procedure. و أوضحت النتائج أن أعلى إنتاج للغاز لوحظ مع كسب فول الصويا غير المعامل، كسب فول الصويا المعامل بالإيثانول، التخميص، ثم كسب فول الصويا المخلوط مع أوراق الأكاسيا بنسبة 3:1 على الترتيب. أقل إنتاج غاز لوحظ مع كسب فول الصويا المخلوط مع الأكاسيا بنسبة (1:3)، ثم كسب فول الصويا المعامل بالتعقيم ثم كسب فول الصويا المخلوط مع الأكاسيا بنسبة (1:1). كانت الطاقة الممتلئة والصافية المحسوبة مرتفعة مع كسب فول الصويا غير المعامل بينما كانت منخفضة مع كسب فول الصويا المخلوط مع أوراق الأكاسيا بنسبة (1:3). وكان أعلى تركيز للأمونيا في حالة كسب فول الصويا غير المعامل بينما أقل تركيز للأمونيا مع كسب فول الصويا المعامل بالتعقيم، كسب فول الصويا المخلوط مع الأكاسيا بنسبة (1:3)، كسب فول الصويا المعامل بالإيثانول ثم المعامل بالتخميص على الترتيب. تراوحت نسبة البروتين المتحلل في الكرش من 33.6% مع كسب فول الصويا المعامل بالتعقيم إلى 62.4% مع كسب فول الصويا المخلوط مع الأكاسيا بنسبة (3:1). وكانت نسبة البروتين المهضوم في الأمعاء أعلى مع كسب فول الصويا المعامل بالتعقيم، ثم كسب فول الصويا المعامل بالتخميص وكسب فول الصويا غير المعامل وأقل قيمة للبروتين المهضوم في الأمعاء كانت مع كسب فول الصويا المخلوط مع الأكاسيا بنسبة (1:1). من هنا نستنتج أن: معاملة كسب فول الصويا بالتعقيم والإيثانول والخلط مع أوراق الأكاسيا بنسبة (1:3) تؤدي إلى خفض البروتين المتحلل في الكرش ولزيادة البروتين المار من الكرش للأمعاء، لكن معاملة كسب فول الصويا بالتعقيم أوضحت أنها أفضل معاملة لتحسين هضم البروتين في الأمعاء.