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EFFECT OF SOME PHYSICAL AND CHEMICAL TREATMENTS ON THE FERMENTATION OF SOYBEAN MEAL USING GAS PRODUCTION TECHNIQUE.

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SUMMARY

The effect of heat (110 °C, 2 h), formaldehyde (30% w/v) and tannic acid (TA) at three levels (1, 3 and 5 % of DM) treatments of soybean meal were assessed by using the *in vitro* gas technique. *In vitro* gas production values were recorded at 3, 6, 9, 12, 24, 48 and 72 h incubation periods. Kinetics of gas production was fitted to an exponential model. Volatile fatty acids (VFA) and ammonia-N (NH_3-N) concentrations were determined at 16 h of incubation. The organic matter digestibility (OMD), metabolizable energy (ME) and net energy (NE) were calculated from gas production after 24-h incubation. Microbial protein (MP) was calculated from OMD. The maximum gas volume was recorded for the untreated SBM followed by heating SBM, SBM treated with 1 or 3% of TA, whereas the values were lowest for SBM treated with formaldehyde and 5% of TA, respectively. The concentrations of NH_3-N and VFA's were decreased ($P<0.05$) when SBM treated with formaldehyde or 5% of TA. The values of OMD, ME, NE and microbial nitrogen (MN) were decreased with formaldehyde treated SBM.

The present study concluded that formaldehyde treated SBM decreased the *in vitro* gas production and rumen fermentation, however, no significant effects of the three levels of TA (1, 3 and 5 % of DM) or heat treatments were noticed than untreated SBM..

Keywords: *soybean meal, heating SBM, formaldehyde, tannic acid, gas production, in vitro*

INTRODUCTION

Proteins are one of the most important constituents of ruminant diets. It is essential for growth, repair of old tissue, and milk production. For high-producing ruminants, there should be enough soluble readily fermentable protein to support microbial growth and fermentation in the rumen, plus a source of less fermentable protein, which can pass directly to the abomasum and small intestine for a normal proteolytic digestion and absorption process. The latter is often called rumen undegradable, or by pass protein. The increased undegradable protein in the rumen and its availability for absorption in the lower gastrointestinal tract may benefit rapidly growing calves and high-production dairy cows (Dove and Milne, 1994). Soybean meal (SBM) is the most commonly used protein supplement in beef and dairy rations. It is quite palatable and has a good amino acid balance with high availability, but the rumen degradability of its crude protein is high. It is estimated that only 34% of protein in SBM escaped from rumen fermentation (NRC, 1996). Therefore, improvement of ruminal escape characteristics of SBM is a major importance to ruminant nutritionists; especially ruminant animals, which have high requirements for undegradable dietary protein in certain physiological states (Agriculture Research Council, 1984). Various methods for treating proteins have been used to reduce their degradation in the rumen. These can be divided to chemical and physical treatments (Mir, *et al* 1984). Some of that methods, e.g. heat, extrusion, expeller, lignosulfonate and formaldehyde treatments have been successfully used to protect SBM from ruminal degradation (LjØkjel, *et al.* 2000, and Wulf and Súdekum, 2005). Also, tannins are polyphenolic compounds of plant origin which bind with proteins by hydrogen bound. The value of tannins to ruminant protein nutrition lies in the sensitively of the bonding to pH; over the normal pH range in the rumen, protein remains bound to the tannin, but at the low pH in abomasums the protein is released (Silanikove, *et al.* 2003, and Rubanza *et al.* 2005). Although, dietary protein is protected from degradation in the rumen by tannins, it is available for digestion in the abomasums and small intestine. However, there is still much need to perfect conditions for protein protection methods especially with tannins. The *in vitro* gas production technique is a useful method to evaluate the nutritional quality of feedstuffs and predict digestion kinetics in the rumen. Also, *in vitro* gas production technique can be used to predict animal performance at a much lower cost and less time-consuming, based on the strong relationship between measured digestibility and that predicted from gas production, regression equations have been developed and the method has been standardized. In fact, there is still need to perfect conditions for protein protection. Therefore, the objective of the current study was to determine the effects of soybean meal treated by heat, formaldehyde and tannic acid at three levels (1, 3 and 5 % of DM) on *in vitro* gas production, rumen fermentation, energy content and microbial protein.

MATERIALS AND METHODS

1. Soybean meal (SBM) treatments:

A suitable amounts of soybean meals (SBM) were kept untreated (UT), heat treated (HT) {heated for 2 h at 110 °C} or formaldehyde treated (FT), which prepared according to (Subuh, *et al.*, 1996) or SBM treated with three levels of tannic acid (TA) (1%, 3% and 5 % TA of DM). Briefly FT of SBM was done by 30 % w/v solution sprayed on to 4 mm thick layers of SBM in plastic bags at room temperature, followed by sealing of bags and shaking for 5 min before storage.

2. Chemical Analyses :

Soybean meal was analyzed according to AOAC, (1990) for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash.

3. In vitro gas production and rumen fermentation:

Evaluation of the effects of different treatments of SBM using *in vitro* gas production technique was carried according to the procedure described by Menke and Steingass

(1988). Buffer and mineral solution are prepared and placed in a water bath at 39°C under continuous flushing with CO_2. Rumen fluid was collected before the morning feeding from three fistulated sheep fed on timothy hay and commercial concentrate mixture diet into a pre-warmed thermos flask. The rumen fluid was mixed and filtered through four layers of cheesecloth and flushed with CO_2. The well mixed and CO_2 flushed rumen fluid was added to the buffered mineral solution (1:2 v/v), which was maintained in water bath at 39°C, and mixed. Samples (200±10 mg) of air-dry feedstuffs were accurately weighed into syringe fitted with plungers. Buffered rumen fluid (30 ml) was pipetted into each syringe. The syringes were immediately placed into the water bath at 39°C (Blümmel and Ørskov, 1993). Two syringes with only buffered rumen fluid are incubated and considered as the blank. The syringes are gently shaken every 1 h for the first 12 h of incubation. The gas production was recorded after 3, 6, 9, 12, 24, 48 and 72 h of incubation. Cumulative gas was expressed as milliliter of gas produced per 200 mg of dry matter and corrected for blanks. Cumulative gas production GAS (Y) at time (t) was fitted to the exponential model of Ørskov and McDonald (1979) as follows: Gas (Y) = a + b (1-exp^{-ct}),
where; a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time. Other syringes containing 400 mg protein sources samples and 45 ml buffered rumen fluid were incubated for determination of ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) concentrations at 16 h of incubation.

4. Estimation of energy values, organic matter digestibility and microbial protein:

The energy value and organic matter digestibility of protein sources were calculated from the gas produced of 200 mg feed dry matter after 24 h of incubation with the levels of crude protein, ash and crude fat (Menke *et al.*, 1979). Microbial protein was calculated as 19.3 g microbial nitrogen per kg OMD according to Czerkawski (1986).

5. Statistical analyses:

Data were subjected to analysis of variance (ANOVA) using the General Linear Model. Significant differences between individual means were identified using least significance difference (LSD) multiple range test (SAS, 2000).

RESULTS AND DISCUSSION

1. Gas production and estimated parameters:

Cumulative gas production profiles, corrected for blank, of the means of UT-SBM, HT-SBM, FT-SBM and TA-SBM at 1%, 3% or 5% condensed tannins (CT) are shown in Fig. (1). The data showed that gas production of FT-SBM during the first 24 h of incubation is very low compared to the control or other SBM treatments, then the gas production rate of that treatment increased after 24 h, may be the microorganisms were adapted or destroyed the formaldehyde after 24 h. Gas produced after 72 h incubation ranged between 44.00 and 59.00 ml/0.200 g DM of substrate (Table 1). The highest values during 72 h were obtained with UT-SBM followed by TA-SBM (with 1%, 3%, and 5% of TA) then, HT-SBM. The lowest value was obtained with FT-SBM (Table, 1). Kinetics of gas production obtained from the exponential model were significantly different ($P \leq 0.05$) among all substrates (Table, 1). The values of the soluble fractions (a) were 0.2, 5.3, 4.1, 5.0, 5.2 and 5.4 ml for FT-SBM, HT-SBM, TA-SBM (3% T), TA-SBM (5% T), TA-SBM

(1% T) and UT-SBM, respectively. The gas production of both soluble (a) and insoluble fractions (b) were significantly (P≤ 0.05) decreased with FT-SBM (Table 1). The value of (b) for FT-SBM on average was significantly (P<0.05) decreased when compared with UT-SBM or TA-SBM and this was probably reflected due to the FT-SBM as a low degradable protein sources (Table 1). The gas production rate constant for the insoluble fraction (c) of SBM was significantly (P<0.05) reduced with FT-SBM or TA-SBM while, the value was increased by heating (Table 1). The lowest value of (c) was observed with FT-SBM (0.05 ml/h). The values of TA-SBM were significantly (P<0.05) decreased when compared with UT-SBM, however no significant.

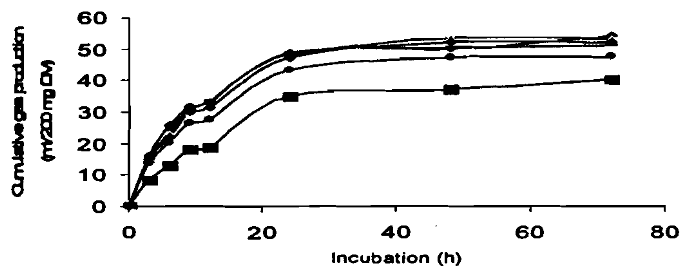

Fig. 1.The effect of soybean meal treated by heating, formaldehyde and different levels of tannic acid on cumulative gas production (ml/200 mg DM) for 72 h incubation, in vitro

Table (1): Cumulative gas produced at different incubation times of feedstuffs and parameters of gas production (mean ± SD).

Feedstuffs	Cumulative gas (ml) produced at				Parameters of gas production		
	12 h	24 h	48 h	72 h	*a* (ml)	*b* (ml)	*c* (ml/h)
SBM	35.7± 2.4	51.3± 2.5	54.0± 2.8	59.0± 2.2	5.4±0.39^{a}	52.1±2.4^{a}	0.08±0.006^{a}
HSBM	36.0± 1.6	51.0± 1.6	54.3± 1.7	56.0± 2.5	5.3±0.59^{a}	54.6±1.4^{a}	0.09±0.003^{a}
FSBM	20.3± 2.1	36.3± 2.1	39.3± 2.5	44.0± 1.6	0.2±0.03^{b}	43.2±2.4^{b}	0.05±0.005^{c}
SBM+1%T	34.0± 0.0	50.0± 0.0	57.0± 1.4	58.3± 0.9	5.2±0.28^{a}	53.7±1.3^{a}	0.07±0.002^{b}
SBM +3%T	33.7± 0.9	50.3± 1.3	56.7± 0.5	58.0± 1.6	4.1±1.42^{a}	54.5±1.5^{a}	0.07±0.002^{b}
SBM +5%T	32.0± 0.8	49.0± 1.6	55.3± 1.3	56.3± 0.5	5.0±1.08^{a}	52.3±1.7^{a}	0.06±0.005^{b}

SBM = soybean meal; HSBM = heated SBM; FSBM = SBM treated with formaldehyde; SBM+1%T=SBM treated with 1% tannic acid; SBM +3%T =SBM treated with 3% tannic acid; SBM +5%T =SBM treated with 5% tannic acid.

Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979), where; a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c the gas production rate constant for the insoluble fraction (b), t = incubation time.

a,b,c Means within a column bearing different superscripts differ (P<0.05).

Differences between the different tested levels of TA treatments. The (c) value of FT-SBM was also significantly ($P<0.05$) reduced when compared with UT-SBM or TA-SBM. El-Waziry *et al.* (2007) showed that autoclaving of SBM was significantly ($P<0.05$) reduced the degradation constant (a and b fractions) and the gas production rate (c). Similar results reported by LjØkjel, *et al.* (2000) they examined SBM autoclaved at 120 and 130°C for 30 min using *in situ* technique. Also, heat treatments of SBM by expelling and roasting have shown similar effects (Subuh *et al.* 1996, and Titgemeyer and Shirley 1997). In contrast, extrusion cooking of SBM does not seem to be effective (Daecon *et al.* 1988; Waltz and Stern, 1989), presumably due to the very short heating time during extrusion. Moreover, to obtain an effective heat treatment that protects protein from rumen degradation, moisture levels above 20 % is required (Cleale *et al.* 1982). On the other hand, when SBM was treated with CT, the estimated parameters of SBM degradability (a, b and c) using gas production technique were significantly ($P\leq 0.05$) decreased compared with UT-SBM. 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 rapidly protein sources such as SBM (Reid *et al.* 1973). The value of tannins to ruminant protein nutrition lies in the sensitivity of the bonding to pH; over normal pH range in the rumen, protein remains bound to the tannin, but at the low pH normally occurring in the abomasum, the protein is released (Broderick, *et al.* 1991). Protein is thus effectively protected from degradation in the rumen, but becomes available for digestion in the abomasum and small intestine. Barry and McNabb (1999) observed that CT substantially increased rumen escape of protein in fresh herbage, although levels greater than 4% of DM. Makkar *et al.* (1988) reported that if tannin concentration in the diet becomes too high, microbial enzyme activities including cellulase and intestinal digestion may be depressed. The present results showed that the *in vitro* digestibility and gas production parameters were not correlated negatively with phenolic compounds, in particular CT (1%, 3% and 5%). These results are in agreement with that reported by El-Waziry *et al.* (2007), while different than results reported by Ammar, *et al.* (2005). The effect of quebracho CTs extract on ruminal fermentation activity was clearly dose dependent, and the daily administration of dose Q1 (0.5 g/kg LW per day) did not produce any adverse effects on rumen fermentation (Hervas, *et al.* 2003). Stern *et al.* (1985) have reported that heating at temperatures did not overprotect protein and did not alter protein availability for intestinal supply. Zaman, *et al.* (1995) concluded that roasting of lupins or soybeans is a useful tool to increase available rumen by-pass protein and to reduce the effective degradation of N in the rumen of dairy cows. Roasting of beans resulted in a linear decrease in the rapidly soluble fraction, a linear increase of slowly degradable fraction, and a decrease in the estimated effective degradation of DM and N. Heat treatment of SBM (139°C, 2 h) resulted in a reduction ($P<0.001$) in effective degradability of DM and CP compared with non-heated SBM.

2. In vitro rumen fermentation:

The concentrations of NH_3-N and individual VFA's are shown in Table (2). The highest values were obtained by UT-SBM, HT-SBM, TA-SBM (1%T and 3%), while, the lowest values were obtained by FT-SBM and TA-SBM (5%T), respectively (Table 2). The concentrations of NH_3-N were significantly decreased ($P<0.05$) when SBM treated with formaldehyde or 5% of T (Table 2). This result may be associated with a decrease in protein degradation, which may depend on formaldehyde and increasing tannic acid levels. This corresponds to results of McAllister, *et al.* (1993) and Kanjanapruthipong, *et al.*

(2002), who found the lowest NH_3–N concentrations in xylose-treated canola meal and formaldehyde-treated soybeans. Wulf and Sudekum, (2005) suggested that concentrations of ammonia–N in ruminal fluid were lower for formaldehyde-treated (SBT) ground soybeans (SBT) than for untreated soybean (SB). Yoruk *et al.* (2006) reported that the formaldehyde levels (0.6% and 0.9% of CP content of SBM) significantly decreased the rumen NH_3-N concentration. There was no difference between non-heated and heated SBM for milk yield, 4% fat-corrected milk yield, milk composition, live weight changes, ruminal NH_3-N (Hadjipanayiotou, 1995). Tice, *et al.* (1993) reported that no decline in ruminal NH_3 N of cows fed diets containing heat-treated soybeans compared to cows fed whole raw soybeans. The lower ammonia concentrations were mainly due to reduce proteolysis, degradation of peptides and deamination of amino acids in the rumen (Newbold, *et al.* 1990). The VFA Concentrations were significantly ($P<0.05$) decreased when SBM treated by formaldehyde or 5% T (Table 2). This result may be associated with a decrease in the activity of rumen microorganisms and fermentation. El-Waziry, *et al.* (2007) observed that the VFA concentrations were significantly ($P<0.05$) decreased when SBM treated by autoclaving or quebracho. The present results showed that the lowest values of acetate, propionate and butyrate were observed with FSBM and SBM+5% (Table 2). Wulf and Sudekum, (2005) suggested that concentrations of propionic and butyric acids and total SCFA in ruminal fluid were lower for formaldehyde-treated (SBT) ground soybeans (SBT) than for untreated soybean (SB). Liu, *et al.* (2002) mentioned that 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, and their results are in agreement with the present study concerning gas production and VFA.

Table (2): Effect of soybean meal treatment on individual volatile fatty acids (mM) and ammonia-N (mg N/l) concentrations in rumen liquor of sheep fed timothy hay and concentrate mixture *in vitro* (mean ± SD).

Item	SBM	HSBM	FSBM	SBM+1%T	SBM+3%T	SBM+5%T
Acetate (A)	37.9 ± 0.83^{a}	35.73 ± 0.53^{ab}	8.31 ± 3.11^{d}	34.5 ± 1.27^{ab}	32.34 ± 0.83^{bc}	31.64 ± 2.02^{c}
Propionate(P)	14.6 ± 0.26^{a}	14.39 ± 0.25^{a}	3.67 ± 0.10^{c}	13.26 ± 0.33^{ab}	12.02 ± 0.27^{b}	10.96 ± 0.55^{b}
A to P ratio	2.60	2.48	2.26	2.60	2.69	2.89
Iso-butyrate	1.89 ± 0.03^{a}	1.99 ± 0.13^{a}	0.92 ± 0.02^{b}	1.70 ± 0.01^{a}	1.37 ± 0.08^{ab}	1.08 ± 0.02^{b}
Butyrate	9.12 ± 0.33^{a}	8.53 ± 0.29^{a}	2.06 ± 0.03^{b}	8.32 ± 0.18^{a}	8.29 ± 0.27^{a}	8.01 ± 0.57^{a}
Iso-valerate	3.67 ± 0.06^{a}	3.51 ± 0.13^{a}	0.85 ± 0.02^{c}	3.23 ± 0.01^{a}	2.63 ± 0.14^{a}	1.99 ± 0.06^{b}
Valerate	3.43 ± 0.11^{a}	3.25 ± 0.13^{a}	0.79 ± 0.02^{c}	3.05 ± 0.03^{a}	2.52 ± 0.11^{a}	1.93 ± 0.07^{b}
NH_3-N	99.85 ± 1.15^{a}	91.70 ± 4.70^{a}	11.20 ± 1.20^{c}	91.10 ± 4.10^{a}	85.85 ± 5.85^{a}	72.45 ± 7.55^{b}

SBM = soybean meal; HSBM = heated SBM; FSBM = SBM treated with formaldehyde; SBM+1%T=SBM treated with 1% tannic acid; SBM +3%T =SBM treated with 3% tannic acid; SBM +5%T =SBM treated with 5% tannic acid.

[a,b,c,d] Means within a column bearing different superscripts differ ($P<0.05$).

Energy contents, organic matter digestibility and microbial protein synthesis:

The predicted metabolizable energy (ME, MJ/kg DM), net energy (NE, MJ/kg DM), organic matter digestibility (OMD) and microbial nitrogen (MN, mg/kg DM) are presented in Table (3). The predicted ME and NE which calculated from gas production after 24 h incubation were significantly ($P\leq 0.05$) decreased when SBM was treated by formaldehyde (Table, 3). The values of NE were on average of 6.50 MJ/kg DM for FT-SBM and 7.80 MJ/kg DM for UT-SBM, with significantly differences ($P<0.05$). The OMD (%) was higher in UT-SBM and HT-SBM and lowest in FT-SBM (Table, 3). The measured organic matter digestibility is closely correlated with that predicted from gas production and the crude protein and ash contents of feeds (Menke and Steingass, 1988). The values of MN, which predicted from OMD, are presented in Table (3). The values of MN were on average of 13.45 g/kg OMD for FT-SBM and 16.02 g/kg OMD for UT-SBM, with significantly differences (P<0.05). These results are in agreement with the results of Yoruk, *et al.* (2006), who reported that the amount of microbial protein decreased in rams fed with SBM treated with both 0.3% and 0.6% formaldehyde (P< 0.05).

Table (3): Effect of soybean meal treatment on the predicted of metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD) and microbial protein (MN) *in vitro*.

Feedstuffs	ME (MJ/Kg) DM	NE (MJ/kg DM)	OMD %	MN g/kg OMD
SBM	13.19 ± 0.39^a	7.80 ± 0.22^a	83.00 ± 2.22^a	16.02 ± 0.43^a
HSBM	13.13 ± 0.29^a	7.77 ± 0.14^a	82.73 ± 1.45^a	15.96 ± 0.28^a
FSBM	10.83 ± 0.32^b	6.50 ± 0.18^b	69.66 ± 1.83^b	13.45 ± 0.35^b
SBM +1%T	12.98 ± 0.00^a	7.68 ± 0.00^a	81.81 ± 0.00^a	15.79 ± 0.00^a
SBM +3%T	13.03 ± 0.20^a	7.71 ± 0.11^a	82.11 ± 1.11^a	15.85 ± 0.22^a
SBM +5%T	12.82 ± 0.26^a	7.60 ± 0.14^a	80.93 ± 1.45^a	15.61 ± 0.28^a

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

In conclusion, the present study concluded that the treatment of soybean meal by formaldehyde decreased the gas production, rumen fermentation, protein degradation and energy value while, no significant effect of the three levels of tannic acid (1, 3 and 5 % of DM) or heating.

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تأثير بعض المعاملات الطبيعية و الكيميائية في تخمير كسب فول الصويا باستخدام طريقة إنتاج الغاز معمليا

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تم دراسة تأثير معاملة كسب فول الصويا بالحرارة (١١٠ م – ٢ ساعة) و بالفورمالدهيد (٣٠٪ وزن/حجم) و كذلك بثلاث مستويات من حامض التانيك (١، ٣، ٥٪ من المادة الجافة) و لقد تم تحضين عينات كسب فول الصويا المعامل و غير المعامل مع سائل الكرش و استخدم في الدراسة طريقة إنتاج الغاز معمليا.

سجل إنتاج الغاز الناتج عند ازمنة مختلفة من التحضين ٣، ٦، ٩، ١٢، ٢٤، ٤٨، ٧٢ ساعة و باستخدام المعادلة الاسية أمكن حساب حركية إنتاج الغاز. و لقد قدر تركيز الاحماض الدهنية الطيارة و الامونيا بعد ١٦ ساعة من التحضين بينما معامل هضم المادة العضوية و الطاقة الميتابولزمية و الصافية فتم حسابهم بمعلومية انتاج الغاز بعد ٢٤ ساعة من التحضين أما النيتروجين الميكروبي فقدر بمعلومية معامل هضم المادة العضوية.

أوضحت النتائج أن أعلى انتاج للغاز كان على الترتيب كسب فول الصويا غير المعامل – المعامل حراريا – المعامل بحامض التانيك ١٪ ثم ٣٪. أنخفض تركيز الامونيا و الاحماض الدهنية الطيارة نتيجة لمعاملة فول الصويا بالفورمالدهيد و حامض التانيك(٥٪). انخفضت قيم معامل هضم المادة العضوية و الطاقة الميتابوليزمية و الصافية نتيجة معاملة كسب فول الصويا بالفورمالدهيد. أستنتج من الدراسة أن معاملة كسب فول الصويا بالفورمالدهيد أدى إلى حدوث انخفاض في انتاج الغاز و تخمرات الكرش معمليا بينما لا يوجد تأثير معنوي نتيجة للمعاملة الحرارية و كذلك تركيزات حامض التانيك المستخدمة.