Research Journal of Biological Sciences 4 (9): 1068-1072, 2009
ISSN: 1815-8846

The Influence of Exogenous Enzyme, Formaldehyde and/or Sodium Hydroxide on *in vitro* Gas Production Parameters of Sunflower Meal

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Abstract: This experiment was conducted to evaluate the effect of exogenous enzyme (3 g kg^{-1} DM), formaldehyde (30 and 60 g kg^{-1} DM) and or sodium hydroxide (40 g kg^{-1} DM) on *in vitro* gas production parameters of sunflower meals (25 and 165 g fat kg^{-1} DM) were with gas production technique and using fistulated sheep rumen fluid. Kinetics of gas production was fitted to an exponential model. Sodium hydroxide and enzyme treatments significantly increased gas production (B), Metabolizable Energy (ME), Organic Matter Digestibility (OMD), Net Energy Lactation (NEL), ammonia-N (NH_3-N) and Short Chain Fatty Acid (SCFA) but formaldehyde decreased them. The lowest of gas production was estimated for low fat sunflower meal treated with 60 g formaldehyde/kg DM (75.9 mL/500 mg DM). There was significant difference among samples for gas production rate Constant (C). Sodium hydroxide treated high fat sunflower meal had the highest of ME, OMD, NEL and SCFA (35.8 MJ kg^{-1} DM, 207.5 g kg^{-1} OM, 2.42 Mcal kg^{-1} DM and 1.53 μmol L^{-1}, respectively). Ammonia-N concentration of high fat sunflower meal were the highest (40.9 mg/100 mL). The values of B, ME, OMD, NEL, NH_3-N and SCFA for low fat sunflower meal was less than high fat sunflower meal. The results showed, it may be that gas production parameters of low fat and high fat sunflower meal are influenced by exogenous enzyme, formaldehyde and NaOH.

Key words: Enzyme, formaldehyde, sodium hydroxide, sunflower meal, *in vitro*, gas production

INTRODUCTION

Gas production technique used for assessing the digestible value of the ruminant feeds. The gas production is basically by the fermentation of carbohydrates and protein can be fermented but is not the same as that of carbohydrates (Blu¨mmel and Ørskov, 1993). Sunflower meal is used as supplemental protein in dairy rations and is classified as highly degradable (Economides, 1998). Formaldehyde treatment that formed cross-links with proteins, make protein less degradable in the rumen (Ashes *et al*., 1984). Sodium hydroxide (NaOH) treatment, caused to reduce the degradation of protein in the rumen and to dissolve hemicellulose, to hydrolyze ester bonds between lignin (i.e., ferulic and p-coumaric acids) and hemicellulose and to swell cellulose microfibrils and improve digestibility (Canale *et al*., 1992). Also, Euna *et al*. (2006) reported using of exogenous enzymes increase digestibility.

The objective of current study was to determine *in vitro* gas production and estimated parameters of low fat (25 g kg^{-1} DM) and high fat (165 g kg^{-1} DM) sunflower meal, (LSM and HSM, respectively) as untreated and treated with exogenous enzyme, formaldehyde and or NaOH.

MATERIALS AND METHODS

Experimental samples: Two level of 30 and 60 g kg^{-1} DM of formaldehyde (37% solution of commercial grade formalin) was mixed with the sunflower meals (low and high fat) for 30 min and were transferred inside sealed PVC bags and shaken for 5 min and kept for 5 days at room temperature.

After 5 days treated meals were spread in thin layers (4 mm) on the plastic and were air-dried. Treating sunflower meals with 4% solution of NaOH (40 g kg^{-1} DM) was done the same but samples were kept for 48 h. Also, 3 g kg^{-1} DM exogenous enzyme used for processing sunflower meals. The enzyme mixture composition was Cellulase, Xylanase, Betaglucanase, Alpha amylase, Pectinase, Phytase, Protease and Lipase

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as 0.03, 6.6, 10, 0.7, 0.7, 0.07, 0.5 and 3 MU kg^{-1}, respectively; Bioproton Pty. Ltd. Co. Therefore, experimental samples were; ULSM (Untreated LSM), SHLSM (40 g kg^{-1} DM NaOH treated LSM); F30LSM (30 g formaldehyde/kg DM treated LSM); F60LSM (60 g formaldehyde/kg DM treated LSM); ELSM (3 g exogenous enzyme/kg DM treated LSM); UHSM (Untreated HSM); SHHSM (40 g kg^{-1} DM NaOH treated HSM); F30HSM (30 g formaldehyde/kg DM treated HSM); F60HSM (60 g formaldehyde/kg DM treated HSM); EHSM (3 g exogenous enzyme/kg DM treated HSM).

Gas production method: Rumen fluid was supplied from two fistulated sheep in prior to the morning meal. Animals fed a 40:60 concentrate: forage (2 kg alfalfa hay and 4.5 kg corn silage) diet. About 500 10 mg sample (1.0 mm screen) incubated with 35 mL buffered rumen fluid under continuous CO_2 reflux in 100 mL calibrated glass syringes for 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h, in a water bath maintained at 39°C, according to Menke and Steingass (1988). Samples were incubated in triplicate together with 3 syringes containing only incubation medium (blank). After 96 h of incubation, the medium of each syringe used for determination ammonia-N (NH_3-N) concentration. Content of NH_3-N was determined using distillation method (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden). Cumulative gas production data were fitted to the exponential equation:

$$Y = B (1-e^{-Ct})$$

Where:

B = The gas production from the fermentable fraction (mL)
C = The gas production rate constant C (mL h^{-1})
t = The incubation time (h)
Y = The gas produced at time (t)

The values of Organic Matter Digestibility (OMD) and Metabolisable Energy (ME) of samples were calculated by the equation of (Menke and Steingass, 1988), OMD (g kg^{-1} OM) = 148.8 + 8.89 GP + 4.5 CP + 0.651A and ME (MJ kg^{-1} DM) = 2.20 + 0.136 GP + 0.057 CP + 0.0029 CP^2. Short Chain Fatty Acids (SCFA) were determined by the equation reported by Getachew *et al.* (1999). SCFA (μmol L) = 0.0239×GP B 0.0601. The net energy of lactation (NEL, Mcal kg^{-1} DM) = (1.64 + 0.0269 GP + 0.00078×GP^2 + 0.0051CP + 0.01325CL)/4.186 (Seker, 2002). Value of CP, A and CL were crude protein, ash and crude lipid in g/100 g DM. GP was the net gas production (mL/200 mg DM) after 24 h incubation.

Statistical analysis: Data on *in vitro* gas production, ME, OMD, NEL, NH_3-N and SCFA were subjected to analysis as a completely randomized design using the General Linear Model (GLM). Duncan's multiple range test was used to compare treatment means at $p<0.05$.

RESULTS AND DISCUSSION

Exogenous enzyme, formaldehyde and NaOH had significant effect on *in vitro* gas production kinetic, ME, OMD and NEL of samples ($p<0.05$) (Table 1). Enzyme and NaOH treatments increased B and the highest of B (185 mL/500 mg DM) and C (0.1 mL h^{-1}) was for SHHSM, but formaldehyde resulted to decrease these parameters and this decrease for 60 level was >30 level. Alkali to cleave esterified bonds within cell wall structure, reduce the physical enmeshment of cellulose and solubilize the inhibitory phenolic compounds; thereby enhancing enzymatic saccharification and facilitates microbial colonization of plant cell walls and improve the ruminal degradation (Euna *et al*., 2006). Chen *et al.* (2007) reported alkali treatment increased colonization of *F. succinogenes in situ* experiments. The researchers reported alkali makes carbohydrates more accessible to the action of rumen microorganisms and organic matter digestibility improved (Rezaeian *et al*., 2005). Berger *et al.* (1979) concluded solubilization of hemicellulose with NaOH caused to improvement in DM digestibility. Exogenous enzymes work in synergy with rumen microbial enzymes, which containing xylanases and esterases had stimulatory effects on fibre degradation of alfalfa hay *in vitro* (Nsereko *et al*., 2000), whereas Colombatto *et al.* (2003)

Table 1: Gas production and estimated parameters of sunflower meal treated with exogenous enzyme, NaOH and or formaldehyde

Samples[1]	B (mL)	C (mL h^{-1})	ME (MJ kg^{-1} DM)	OMD (g kg^{-1} OM)	NEL (Mcal kg^{-1} DM)
ULSM	140.8f	0.091b	21.4h	179.3g	1.48f
SHLSM	156.6d	0.03f	24.5f	187.4d	2.09d
F30LSM	104.2h	0.06e	19.8i	165.8h	1.24g
F60LSM	75.9j	0.126a	17.8j	150.8i	1.17h
ELSM	147.5e	0.06e	22.1g	182.3f	1.71e
UHSM	167.1c	0.08c	29.4c	185.9e	2.24c
SHHSM	185.2a	0.13a	35.8a	207.5a	2.42a
F30HSM	116.9g	0.07d	27.4d	189.7c	2.09d
F60HSM	94.9i	0.08dc	25.7e	182.6f	1.51f
EHSM	173.1b	0.07d	31.2b	203.2b	2.32b
SEM	0.8	0.002	0.02	0.12	0.002

[1]ULSM (Untreated LSM), SHLSM (40 g kg^{-1} DM NaOH treated LSM); F30LSM (30 g formaldehyde/kg DM treated LSM); F60LSM (60 g formaldehyde/kg DM treated LSM); ELSM (3 g exogenous enzyme/kg DM treated LSM); UHSM (Untreated HSM); SHHSM (40 g kg^{-1} DM NaOH treated HSM); F30HSM (30 g formaldehyde/kg DM treated HSM); F60HSM (60 g formaldehyde/kg DM treated HSM); EHSM (3 g exogenous enzyme/kg DM treated HSM); B: Gas production from fermentable fraction; C: Gas production rate constant; ME: Metabolizable Energy; OMD: Organic Matter Digestibility; NEL: Net Energy Lactation; SEM: Standard Error of Mean; Means within each column with different letters are significantly different ($p<0.05$)

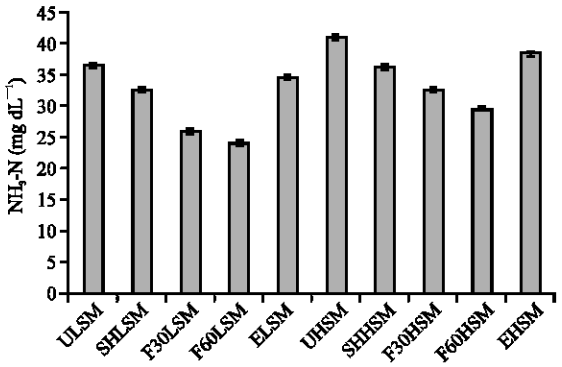

Fig. 1: The effect of processing of sunflower meal with exogenous enzyme, NaOH and or formaldehyde on *in vitro* NH_3-N content. ULSM (Untreated LSM), SHLSM (40 g kg^{-1} DM NaOH treated LSM); F30LSM (30 g formaldehyde/kg DM treated LSM); F60LSM (60 g formaldehyde/kg DM treated LSM); ELSM (3 g exogenous enzyme/kg DM treated LSM); UHSM (Untreated HSM); SHHSM (40 g kg^{-1} DM NaOH treated HSM); F30HSM (30 g formaldehyde/kg DM treated HSM); F60HSM (60 g formaldehyde/kg DM treated HSM); EHSM (3 g exogenous enzyme/kg DM treated HSM)

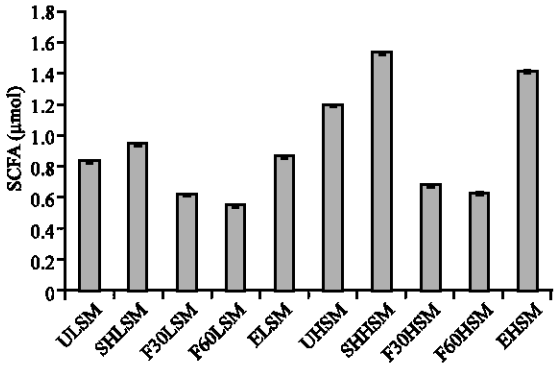

Fig. 2: The effect of processing of sunflower meal with exogenous enzyme, formaldehyde and or NaOH on *in vitro* Short Chain Fatty Acids (SCFA). ULSM (Untreated LSM), SHLSM (40 g kg^{-1} DM NaOH treated LSM); F30LSM (30 g formaldehyde kg^{-1} DM treated LSM); F60LSM (60 g formaldehyde/kg DM treated LSM); ELSM (3 g exogenous enzyme/kg DM treated LSM); UHSM (Untreated HSM); SHHSM (40 g kg^{-1} DM NaOH treated HSM); F30HSM (30 g formaldehyde/kg DM treated HSM); F60HSM (60 g formaldehyde/kg DM treated HSM); EHSM (3 g exogenous enzyme/kg DM treated HSM)

increases their hydrolytic potential within the rumen and increase digestibility of plant cell wall (Morgavi *et al*., 2000). The researchers reported that enzyme products indicated that some xylanases and proteases improved *in vitro* degradation of alfalfa hay. The effect of enzyme, NaOH and formaldehyde on C fraction was significant ($p<0.05$) that El-Waziry *et al*. (2005) concluded protected soybean meal decreased C. Protect of samples by formaldehyde decreased B that El-Waziry *et al*. (2005) reported decrease B for protected soybean meal. Fraction of C of F30HSM and F60HSM was significantly higher than F30LSM and F60LSM. Value of B of HSM was higher than LSM that prove the findings of Getachew *et al*. (2001) reported addition yellow grease and corn oil increased *in vitro* gas production (MachMueller *et al*., 1998) explained, the reduction of protozoa (rumen protozoa is importance in methane formation) by sunflower seed might be partly decrease gas production.

Thomas *et al*. (1979) reported a reduction in DM degradability of meals and seed by formaldehyde (10-20 g kg^{-1} CP). Reduce of ME and NE for treated soybean meal reported by El-Waziry *et al*. (2005). Despite of high fat of HSM, OMD value of HSM was more than LSM but MachMueller *et al*. (1998) concluded the inclusion of fatty feeds (4-5%), caused to decrease 4% fermentation and reduced the quantity OMD.

Proccessing of sunflower meal with enzyme and NaOH increased NH_3-N content, but formaldehyde decreased it compared with untreated sunflower meal and 60 level of formaldehyde had the lowest of NH_3-N content (Fig. 1). This result suggested by Nishino *et al*. (1995) for aldehydes. McNiven (1995) observed, little change was shown in ruminal ammonia by 25 and 50 g NaOH kg DM. Fat content had no effect on NH_3-N production but Getachew *et al*. (2001) showed depression in NH_3-N by fat, due to reduce proteolysis and deamination of amino acids in the rumen. Low NH_3-N concentration with coconut oil probably was partly a result of the absence of the protozoa and a suppression of the fiber-degrading methanogenic bacteria (MachMueller *et al*., 1998). Treatment of enzyme and NaOH increased SCFA value and formaldehyde decreased it (Fig. 2). There is positive correlation between SCFA and gas production, about 54% of the total gas volume, CO_2 was produced from buffering the SCFA (Menke and Steingass, 1988).

CONCLUSION

The result showed exogenous enzyme and NaOH improved B, OMD, ME, NEL, NH_3-N and SCFA and formaldehyde decreased these parameters in compared with untreated samples. Therefore, based on the present

data, formaldehyde is not recommended to treat SFM, when much fermentation is a goal of the feeding strategy. Also, this experiment showed ether extract content of sunflower meal had not negative effect on gas production and the other estimated parameters.

REFERENCES

Ashes, J.R.., J.L. Mangan and G.S. Sidhu, 1984. Nutritional availability of amino acids from protein cross-linked to protect against degradation in the rumen. Br. J. Nutr., 52: 239-247. http://www.ncbi.nlm.nih.gov/pubmed/6433969.

Berger, L., T. Klopfenstein and R. Britton, 1979. Effect of sodium hydroxide on efficiency of rumen digestion. J. Anim. Sci., 49: 1317-1323. http://jas.fass.org/cgi/content/abstract/49/5/1317.

Blu¨mmel, M. and E.R. Ørskov, 1993. Comparison of gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Anim. Feed Sci. Technol., 40: 109-119. http://www.fao.org/agris/search/display.do?f=./1993/v1903/NL9300946.xml;NL9300946.

Canale, C.J., B.P. Glenn and J.B. Reeves, 1992. Chemically treated alfalfa: Lignin composition and *in situ* disappearance of neutral detergent fiber components. J. Dairy Sci., 75: 154-155. http://jds.fass.org/cgi/content/abstract/75/6/1543.

Chen, X.L., J.K. Wang, Y.M. Wu and J.X. Liu, 2007. Effects of chemical treatments of rice straw on rumen fermentation characteristics, fibrolytic enzyme activities and populations of liquid and solid-associated ruminal microbes *in vitro*. Anim. Feed Sci. Technol., 141 (1-2): 1-14. DOI: 10.1016/j.anifeedsci.2007.04.006.

Colombatto, D., G. Herv´as, W.Z. Yang and K.A. Beauchemin, 2003. Effects of enzyme supplementation of a total mixed ration on microbial fermentation in continuous culture, maintained at high and low pH. J. Anim. Sci., 81: 2617-2627. http://jas.fass.org/cgi/content/abstract/81/10/2617.

Economides, S., 1998. The nutritive value of sunflower meal and its effect on replacing cereal straw in the diets of lactating ewes and goats. Livest. Product. Sci., 55: 89-97. http://cat.inist.fr/?aModele=afficheN&cpsidt=10642367.

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. Applied Sci. Res., 1: 313-320. http://www.insinet.net/jasr/313-320.pdf.

Euna, J.S., K.A. Beauchemin, S.H. Hong and M.W. Bauer, 2006. Exogenous enzymes added to untreated or ammoniated rice straw: Effects on *in vitro* fermentation characteristics and degradability. Anim. Feed Sci. Technol., 131: 86-101. http://cat.inist.fr/?aModele=afficheN&cpsidt=18246694.

Getachew, G., H.P.S. Makkar and K. Becker, 1999. Stoichiometric relationship between short chains fatty acid and *in vitro* gas production in presence and absence of polyethylene glycol for tannin containing browses. EAAP Satellite Symposium, Gas Production: Fermentation Kinetics for Feed Evaluation and to Assess Microbial Activity, Aug. 18-19. Wageningen, The Netherlands, pp: 18-19.

Getachew, G., E.J. DePeteres, P.H. Robinson and S.J. Taylor, 2001. *In vitro* rumen fermentation and gas production: Influence of yellow grease, tallow, corn oil and their potassium soaps. Anim. Feed Sci. Technol., 93: 1-15. http://cat.inist.fr/?aModele=afficheN&cpsidt=1130105.

MachMueller, A., D.A. Ossowski, M. Wanner and M. Kreuzer, 1998. Potential of various fatty feeds to reduce methan release from rumen fermentation *in vitro* (Rusitec). Anim. Feed Sci. Technol., 71: 117-130. http://cat.inist.fr/?aModele=afficheN&cpsidt=2239461.

Menke, K.H. and H. Steingass, 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev., 28: 7-55.

Mcniven, M.A., 1995. Influence of roasting or sodium hydroxide treatment of barley on digestion in lactating cow. J. Dairy Sci., 78: 1106-1115. http://jds.fass.org/cgi/content/abstract/78/5/1106.

Morgavi, D.P., K.A. Beauchemin, V.L. Nsereko, L.M. Rode, A.D. Iwaasa, W.Z. Yang, T.A. McAllister and Y. Wang, 2000. Synergy between ruminal fibrolytic enzymes and enzymes from *Trichoderma longibrachiatum*. J. Dairy Sci., 83: 1310-1321. http://jds.fass.org/cgi/content/abstract/83/6/1310.

Nsereko, V.L., D.P. Morgavi, L.M. Rode, K.A. Beauchemin and T.A. McAllister, 2000. Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms *in vitro*. Anim. Feed Sci. Technol., 88: 153-170. http://cat.inist.fr/?aModele=afficheN&cpsidt=986876.

Nishino, N., S. Uchida and M. Ohshima, 1995. Formation of lysinoalanine following alkaline processing of soya bean meal in relation to the degradability of protein in the rumen. J. Sci. Food Agric., 68: 59-64. DOI: 10.1002/jsfa.2740680110.

Rezaeian, M., G.W. Beakes and A.S. Chaudhry, 2005. Relative fibrolytic activities of anaerobic rumen fungi on untreated and sodium hydroxide treated barley straw in *in vitro* culture. Anaerobe, 11: 163-175. http://www.ncbi.nlm.nih.gov/pubmed/16701547.

Seker, E., 2002. The determination of the energy values of some ruminant feeds by using digestibility trial and gas test. Rev. Med. Vet., 153: 323-328.

Thomas, E., A. Trenkle and W. Burroughs, 1979. Evaluation of protective agents applied to soybean meal and fed to cattle. Laboratory measurements. J. Anim. Sci., 49: 1337-1345. http://Jas.fass.org/cgi/content/abstract/49/5/1346.