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The Effect of Tannic Acid on *in vitro* Gas Production and Rumen Fermentation of Sunflower Meal

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Abstract: The aim of this study was to investigate the effect Tannic Acid (TA, 20 and 30 g kg^{-1} DM) on *in vitro* gas production and rumen fermentation of low and high fat sunflower meal (25 and 165 g kg^{-1} DM, respectively). Kinetics of gas production was fitted to an exponential model. The results showed that tannic acid caused to reduce the fermentable fraction (b) and gas production rate constant for the insoluble fraction (c) ($p<0.05$) and the lowest (b) and (c) was for low fat Sunflower Meal (SML) treated by 30 g kg^{-1} DM TA (102.5 and 0.01 mL h^{-1}, respectively). The Organic Matter Digestibility (OMD) and Metabolizable Energy (ME) were decreased by TA treatments. Untreated SML had the highest ME and OMD (29.4 MJ kg^{-1} DM, 185.6 g kg^{-1} OM, respectively).The ammonia-N (NH_3-N) concentrations and Short Chain Fatty Acid (SCFA) decreased ($p<0.05$) when SM treated with TA. Content of NH_3-N was lowest for SML treated 30 g kg^{-1} DM TA (12.3 mg dL^{-1}). Untreated sunflower meal had the lowest SCFA concentration and the highest Microbial Biomass (MB). The results showed, it may be that *in vitro* fermentation, gas production parameters and nutritive value of sunflower meal are influenced by tannic acid content.

Key words: Tannic acid, gas production, rumen fermentation, sunflower meal

INTRODUCTION

Fermentation of substrate and carbohydrate by rumen microbes *in vitro* results in production fermentative gases (specially methane). The researchers reported gas production is an indirect measure of substrate degradation (Liu *et al*., 2002). The *in vitro* gas production is more efficient than the *in sacco* method in evaluating the effects of tannins (El-Waziry *et al*., 2007). Tannins are polyphenolic compounds of plant origin, which bind with protein and used as chemical additives for protecting and decreasing ruminal degradation of protein sources such as soybean meal (El-Waziry *et al*., 2007). At normal pH of the rumen, protein remains bound to the tannin, but at the low pH of abomasum, the protein is released, so protein becomes available for digestion in the small intestine (El-Waziry *et al*., 2005). Effects of tannin from different sources in reducing ruminal gas production and ammonia levels have been reported. The researchers reported treating soybean meal with tannic acid (hydrolysable tannins) using an *in vitro* gas production technique was reduced protein degradation of soybean meal and kinetics of gas production (EL-Waziry *et al*., 2005). Therefore, the aim of this study was to determine the effects of different levels of tannic acid (20 and 30 g kg^{-1} DM) on gas production parameters and rumen fermentation of low fat (SML) and high fat (SMH) sunflower meal.

MATERIALS AND METHODS

Gas production: Parameters of *in vitro* Gas Production (GP) was determined according to the Menke and Steingass (1988). Rumen fluid was supplied from two fistulated sheep were fed a 40:60 concentrate: forage (3 kg concentrate: 2 kg alfalfa hay and 4.5 kg corn silage) in prior to the morning meal, homogenized in a laboratory blender, filtered through three layers of cheese-cloth and purged with CO_2. The well mixed and CO_2 flushed rumen fluid was added to the buffered rumen fluid solution (1:2 v v^{-1}), which was maintained in a water bath at 39°C. Experimental samples were; untreated SML (T0SML), 20 g kg^{-1} DM Tannic Acid Treated SML (T1SML), 30 g kg^{-1} DM Tannic Acid Treated SML (T2SML), untreated SMH (T0SMH), 20 g kg^{-1} tannic acid treated SMH (T1SMH), 30 g kg^{-1} tannic acid treated SMH (T2SMH). About 200 mg experimental sample (1.0 mm screen) incubated with 35 mL buffered rumen fluid under continuous CO_2 reflux in 100 mL calibrated glass syringes

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for 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h, in a water bath maintained at 39°C. Samples were incubated in triplicate together with three syringes containing only incubation medium (blank).

Calculation and statistical analysis: After 96 h of incubation, the medium of each syringe used for determination ammonia-N (NH_3-N) concentration 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 (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. 651 XA 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. SCFA (μmol L^{-1}) = 0.0239 GP-0.0601. CP and XA were crude protein and ash in g/100 g DM and GP was the net gas production (mL/200 mg DM) after 24 h incubation. Method of Bl¨ummel was adopted to determine the Microbial Biomass production (MB).

Data of *in vitro* gas production, ME, OMD, NH_3-N, SCFA and MB 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

Gas production and estimated parameters: Gas production parameters, ME and OMD of tannic acid treated sunflower meal shown in Table 1. Tannic acid significantly decreased b and c fractions of sunflower meal and this decrease for 30 g kg^{-1} tannic acid was the highest ($p<0.05$). The researchers concluded that treatment with tannic acid decreased the degradation (b and c) of soybean meal (El-Waziry *et al*., 2005). Reductions in digestibility have been observed *in vivo* only when forages containing over 5% DM condensed tannin are fed (Waghorn *et al*., 1990). Tabacco *et al*. (2006) showed tannins significantly depressed gas production, probably hampering rumen microorganisms. It is reported that if tannin concentration in the diet becomes too high, microbial enzyme activities including cellulase and intestinal digestion may be depressed (Horigome *et al*., 1988). McMahon *et al*. (2000) concluded that tannins do not simply inhibit cellulose digestion by ruminal fluid *in vitro* and *in vivo*, but the inhibitory effects of tannins involved the bacterial cells themselves. Inhibiting both microbial enzymes in the rumen and mammalian enzymes in the small intestine and inhibition of cellulose digestion by tannins reported by Lyford *et al*. (1967). Condensed tannins have effect on microbial adhesion, penetration, colonization and consortium formation, processes which are essential for the ruminal digestion of feed.

Table 1: The effect of various amounts of tannic acid on *in vitro* gas production parameters of sunflower meal

Treatments	b (mL)	c (mL h^{-1})	OMD (g kg^{-1} OM)	ME (MJ kg^{-1} DM)
T0SML	140.8c	0.09b	179.6b	21.6d
T1SML	118.2e	0.05d	154.3d	15.5e
T2SML	102.5f	0.01f	136.3f	12.6f
T0SMH	167.3a	0.11a	185.6a	29.4a
T1SMH	150.2b	0.07c	165.3c	24.2b
T2SMH	137.3d	0.04e	149.2e	22.5c
S.E.M.	0.8	0.002	0.02	0.12

Untreated SML (T0SML), 20 g kg^{-1} DM tannic acid Treated SML (T1SML), 30 g kg^{-1} DM tannic acid treated SML (T2SML), untreated SMH (T0SMH), 20 g kg^{-1} tannic acid treated SMH (T1SMH), 30 g kg^{-1} tannic acid treated SMH (T2SMH), SEM: Standard Error of Mean, means within each low with different letters are significantly different ($p<0.05$)

This study showed predicted ME, which were decreased when SM was treated by TA and this decrease for 30 g kg^{-1} tannic acid was the highest ($p<0.05$). The mean values of ME and OMD were highest for T0SMH (24.2 MJ kg^{-1} DM and 185.6 g kg^{-1} OM, respectively). This result proves the result of El-Waziry *et al*. (2007) that reported ME were decreased when soybean meal was treated by querbracho tannin. These results are in agreement with the results of Tabacco *et al*. (2006) that explained, the tannin reduced OM digestibility by about 5.1%. Muhammed *et al*. (1994) reported decrease in OMD and ME was probably due to decreased rumen degradability and formation of complexes between tannins and dietary proteins and carbohydrates, as well as reducing rumen microbial proteolytic, ureolytic and cellulolytic enzyme activities. Tannin caused to altered microbial profiles in the fermentation, reduced microbial numbers and or enzyme production from the microbes available to ferment substrate (Apajalahti *et al*., 2004) and reduced microbial degradation of carbohydrates (Muhammed *et al*., 1994).

Rumen fermentation: The effect of tannic acid treated sunflower meal on NH_3-N and Microbial Biomass (MB)

shown in Fig. 1. Procesing of sunflower meal with tannic acid decreased NH_3-N and increased MB and 30 g kg^{-1} level of tannic acid had the lowest NH_3-N content and the highest MB ($p<0.05$). Sliwinski *et al.* (2002) found that adding chestnut wood extract containing tannins to a basal diet for dairy cows reduced the level of rumen ammonia in comparison to the without tannin diet. Newbold *et al.* (1990) concluded that the lower ammonia concentrations were mainly due to reduce proteolysis, degradation of peptides and deamination of amino acids in the rumen.

Frutos *et al.* (2000) concluded that quebracho tannins could be used for improving the digestive utilization of protein-rich feeds in sheep. Makkar (2003) suggested that several tannin-rich legumes could be used advantageously to increase bypass protein to improve ruminant performance. Therefore, dietary proteins fixed to tannins escape rumen degradation and are released in the abomasum.

Values of Short Chain Fatty Acid (SCFA) in different treatments given in Fig. 2. The mean concentration of SCFA were lowest and highest for 30 g kg^{-1} level of tannic acid and untreated sunflower meal, respectively. This result prove EL-Waziry *et al.* (2005) that reported the volatile fatty acid concentrations were significantly decreased when soybean meal treated by tannic acid.

There is positive correlation between SCFA and gas production (Menke and Steingass, 1988) and gas production is a good predictor for the production of volatile fatty acid, which is positively related to microbial mass production (Liu *et al.*, 2002).

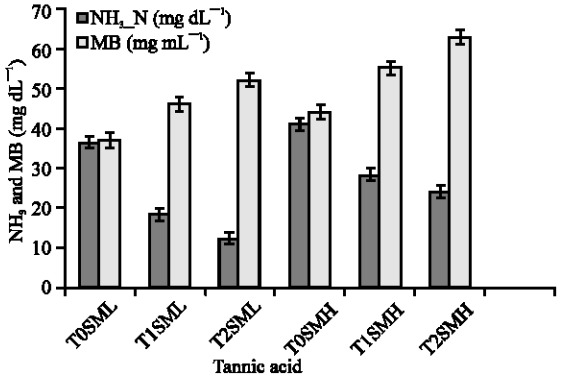

Fig. 1: The effect of various amounts of tannic acid on *in vitro* NH_3 and MB of sunflower meal. Untreated SML (T0SML), 20 g kg^{-1} DM tannic acid treated SML (T1SML), 30 g kg^{-1} DM tannic acid treated SML (T2SML), untreated SMH (T0SMH), 20 g kg^{-1} tannic acid treated SMH (T1SMH), 30 g kg^{-1} tannic acid treated SMH (T2SMH)

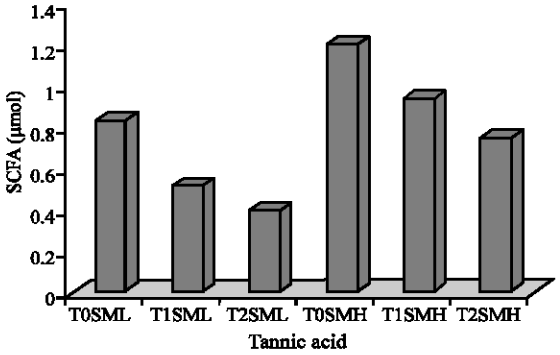

Fig. 2: The effect of various amounts of tannic acid on *in vitro* SCFA of sunflower meal. Untreated SML (T0SML), 20 g kg^{-1} DM tannic acid treated SML (T1SML), 30 g kg^{-1} DM tannic acid treated SML (T2SML), untreated SMH (T0SMH), 20 g kg^{-1} tannic acid treated SMH (T1SMH), 30 g kg^{-1} tannic acid treated SMH (T2SMH)

CONCLUSION

Processing of low and high fat sunflower meal with tannic acid dacreased B, OMD, ME, SCFA and NH_3-N, but this treatment increased MB and 30 g kg^{-1} level of tannic acid had the most effect. Therefore it is recommended to use 30 g kg^{-1} tannic acid on SM that protect protein from degradation in the rumen.

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