EFFECT OF TANNIN TREATMENT ON PROTEIN PROTECTION OF SOYBEAN MEAL AND PERFORMANCE OF BUFFALO HEIFERS

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

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(Minor Subject: VETERINARY BIOCHEMISTRY)

By

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CERTIFICATE − I

This is to certify that the thesis entitled, "**Effect of tannin treatment on protein protection of soybean meal and performance of buffalo heifers**" submitted for the degree of M.V.Sc. in the subject of **Animal Nutrition** (Minor subject: **Veterinary Biochemistry**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Chamadia Bilal (L-2017-V-07-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE − II

This is to certify that the thesis entitled, "**Effect of tannin treatment on protein protection of soybean meal and performance of buffalo heifers**" submitted by **Chamadia Bilal (L-2017-V-07-M)** to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.V.Sc.** in the subject of **Animal Nutrition** (Minor subject: **Veterinary Biochemistry**) has been approved by the student's advisory committee after oral examination on the same, in collaboration with an external examiner.

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ABSTRACT

The present study was conducted to investigate the effect tannin on protein protection of Soybean meal on *in vitro* degradability, *in vivo* nutrient utilization, blood profile, and growth performance of buffalo heifers. The study was conducted in two phases. In the phase I SBM was treated with quebracho and tannic acid, chemical composition of treated SBM in comparison to control SBM was determined. *In vitro* studies were conducted using treated SBMs, and TMRs (containing treated SBM) as substrates. In Phase II, *in vivo* study was carried out to determine the effect of tannic acid treated SBM on nutrient utilization, performance of buffalo heifers and blood metabolites. The tannic acid lowered ammonical nitrogen significantly ($P<0.05$) compared to Quebracho and control. Tannic acid improved partitioning factor non-significantly ($P<0.05$). The TMR prepared by replacing 50% SBM with 1% tannic acid treated SBM was selected for *in vivo* trial on the basis of *in vitro* because it reduced ammonical nitrogen compared to control significantly, there was higher EMMP than control and PF was numerically higher. A 90-day growth trial was conducted on buffalo heifers. Sixteen animals were divided in to two groups. Animals were fed total mixed ration with dry roughage, non-leguminous silage and concentrate in the proportion of 10:50:40. There was no significant ($P>0.05$) difference between control and treatment group in total DM but CP intake was significantly ($P>0.05$) lower in treatment group. *In vivo* digestibility of CP, EE and cellulose decreased significantly ($P<0.05$). The tannic acid treatment of SBM gave encouraging results in terms of growth in buffalo heifers. Tannic acid significantly ($P<0.05$) increased weight gain/kg CP intake. It also improved FCR. Tannic acid reduced alkaline phosphatase and alanine transaminase significantly ($P<0.05$).

Keyword: buffalo heifer growth, tannin treated SBM, ruminal protein protection

Signature of Major Advisor **Signature of the Student**

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LIST OF ABBREVIATIONS USED

AA	:	Amino Acid
ADF	:	Acid Detergent Fibre
ADG	:	Average Daily Gain
ADIN	:	Acid Detergent Insoluble Nitrogen
ADL	:	Acid Detergent Lignin
ADS	:	Acid Detergent Solution
aNDF	:	Amylase-Treated Neutral Detergent Fiber
aNDFom	:	Amylase-treated Neutral Detergent Fiber organic matter
ANOVA	:	Analysis of variance
AR	:	Analytical Reagent
ATTD	:	Apparent Total Tract Digestibility
BCG	:	Bromocresol Green
BSA	:	Bovine serum albumin
BUN	:	Blood Urea Nitrogen
BW	:	Body weight
CF	:	Crude Fibre
CFM	:	Concentrate Feed Mixture
CHO	:	Carbohydrates
CP	:	Crude Protein
CT	:	Condensed Tannins
CTAB	:	Cetyl Trimethyl Ammonium Bromide
D	:	Days
DM	:	Dry Matter
DMD	:	Dry Matter Digestibility
DMI	:	Dry Matter Intake
DNA	:	Deoxyribonucleic Acid
EDTA	:	Ethylenediaminetetraacetic acid
EE	:	Ether Extract
EMMP	:	Efficiency of Microbial Mass Production
EPG	:	Eggs per gram
FCR	:	Feed Conversion Ratio
GP	:	Gas Production
IVDMD	:	*In vitro* Dry Matter Digestibility
IVOMD	:	*In vitro* Organic Matter Digestibility
Kg	:	Kilogram
ME	:	Metabolisable energy

MJ	:	Megajoule
mM	:	milimole
mm	:	millimeter
MMP	:	Microbial Mass Production
N	:	Nitrogen
NADH	:	Nicotinamide adenine dinucleotide
NADPH	:	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NaHCO3	:	Sodium Bicarbonate
NaOH	:	Sodium Hydroxide
NDF	:	Neutral Detergent Fiber
NDFD	:	Neutral Detergent Fiber Digestibility
NDFom	:	Neutral Detergent Fiber organic matter
NDS	:	Neutral Detergent Solution
NE	:	Net Energy
NGP	:	Net Gas Production
NH_3-N	:	Ammonical Nitrogen
NH_4^+-N	:	Ammonical Nitrogen
NH_4HCO_3	:	Ammonium Hydrogen Carbonate
OM	:	Organic Matter
OMD	:	Organic Matter Digestibility
PEG	:	polyethylene glycol
PF	:	Partitioning Factor
QT	:	Quebracho Tannin
RDP	:	Rumen Degradable Protein
RNA	:	Ribonucleic acid
SBM	:	Soybean meal
SCFA	:	Short Chain Fatty Acids
SPSS	:	Statistical Package for Social Sciences
SRL	:	Strained Rumen Liquor
TA	:	Tannic acid
TCHO	:	Total Carbohydrates
TDN	:	Total Digestible Nutrients
TMR	:	Total Mixed Ration
TP	:	Total Phenols
TT	:	Total Tannins
TVFA	:	Total Volatile Fatty Acids

CHAPTER – I

INTRODUCTION

In India contribution of buffalo milk is 49.1% whereas it is more than 72% in Punjab. The total population of buffaloes is 51.60 lakh in the state compared to 24.28 lakh cows (Singh and Kaur 2018). The age at first calving in buffalo heifers under field conditions is quite high (>42 months), impacting the economics of buffalo farming. Protein is one of the limiting and costly major nutrient for weight gain in growing animals. Rumen degradability of crude protein (CP) in commonly used feedstuffs like mustard cake often exceeds 80% and a large part of the ammonia is produced during ruminal deamination which is partially incorporated into a microbial protein, while the rest is absorbed and converted into urea in the liver and is excreted from the body via urine, i.e. lost from the animal. Protection of proteins can lead to higher protein availability in the small intestine in the scenario of high RDP rations.

Oil cakes left after milling of mustard, groundnut, linseed, cottonseed and soybean seeds are the major protein sources for feeding livestock in India. The cakes vary in their protein contents depending on the cultivar and methods of oil extraction and their quality depending on the degradability, solubility and chemical composition. Economic and efficient use of protein through a controlled extent and rate of protein degradation in the rumen to provide balance amino acid supply is of significance in ruminant protein nutrition. Additionally, rumen undegradable protein contribution to rumen outflow of total protein and its amino acid composition influences the pattern of amino acids available for absorption in the small intestine (Gupta *et al* 2011).

To decrease the degradation of the protein in the rumen many methods have been tried. These include heat processing, chemical treatments or a combination of both. Heat processing of the proteinaceous feed has received commercial acceptance. It denatures protein and forms protein-carbohydrate (Maillard reaction) and protein-protein cross-link. Overheating causes significant absolute loss of lysine, cysteine and arginine. In chemical methods aldehydes introduces cross-linkages in the protein. Formaldehyde is carcinogenic in nature so, harmful for the handler. Some chemicals like acids, alkalis, and ethanol denature protein structure and make it rumen undegradable. Another type of chemical like tannins bind with protein but with little or no alteration and makes it rumen undegradable.

Tannins are a heterogeneous group of high molecular weight phenolic compounds and divided into two groups: the condensed and hydrolysable tannins. The high affinity of tannins for proteins is due to the presence of a large number of phenolic groups. Tannins provide many points at which bonding may occur with the carbonyl groups of peptides. Hydrolysable tannins consist of a carbohydrate core with phenolic carboxylic acids bound by ester linkages. Whereas, condensed tannins, consists of oligomers of flavan-3-ols and related flavanol residues which typically produce anthocyanidins on acid degradation. (Mueller-Harvey & McAllan 1992)

Tannin increases utilization of the protein for ruminants by binding proteins at ruminal pH of 5.5 to 7.0 and thus decreasing the microbial degradation of proteins. The tannin-protein complexes dissociate in the acidic pH of the abomasum (i.e., pH 2.5 to 3.5) and releases protein for digestion and absorption (Barry *et al* 1986). Condensed tannins (CT) increase the passage of plant protein to the intestine and have increased the availability and absorption of essential amino acids by 60% compared with equivalent CT-free forage (Waghorn *et al* 1990). Values exceeding 5.5% of DM inhibit microbial activity excessively and depress voluntary intakes.

If the concentration of tannins is below about 4% of dry matter (DM), they improve the nutritive value of herbage by binding to plant proteins and protecting them from excessive degradation in the rumen (Waghorn 1990). Ruminant production of milk, meat and wool could be increased by 10-15% and reduce the occurrence of bloat if grazed pasture contained 2-3% CT (Waghorn 1990). CT also reduces nematodal load and important in rumen methane mitigation. Tannins as organic protein protectants can be used at safer levels in livestock farming for sustaining the production and health of domestic ruminants (Bunglavan and Dutta 2013).

Many studies have been conducted on the effect of tannins on the animal performance but most of them are done with plant extract which is generally having many secondary metabolites and it is difficult to assign the positive effect to a single plant constituent. Although few studies have been conducted with tannic acid and quebracho as the source of tannin but no research has been conducted in the buffaloes for the growth performance. So the components like Tannic acid (as a source of hydrolysable tannins) and Quebracho extract (as a source of condensed tannins) needs to be explored for their effect on protein protection and buffalo heifer performance.

There are very few researches that are comparing condensed and hydrolysable tannin effect for the protein protection from the ruminal degradation.

Keeping in view the above observation the study was planned with the following objectives:

1. To study the effect of varying levels of tannins on protein protection of soybean meal through *in vitro* protein degradability.
2. To study the effect of tannin treatment of soybean meal on growth performance of buffalo heifers.

CHAPTER – II

REVIEW OF LITERATURE

2.1 Laboratory evaluation of tannin treated protein sources

Gunun *et al* (2017) studied the effect of rambutan peel powder (RPP), a tannin rich material, supplementation on *in vitro* gas production, rumen fermentation characteristics and methane production. They supplied RPP at 0, 4, 8, 12, 16 and 20 mg/0.5 g DM. Results of their study revealed that RPP supplementation did not affect gas production kinetics and *in vitro* digestibility ($p > .05$). The concentration of NH_3-N decreased linearly with the increasing levels of RPP supplementation ($p < .05$). Propionate increased ($p < .05$) when supplemented with RPP at 16 mg, while acetate and butyrate remained the same. Supplementation of RPP decreased methane production ($p < .05$).

Anantasook *et al* (2013) conducted *in vitro* study on supplementation of *Terminalia chebula* which contains tannins and saponins. They supplied *T. chebula* at 0, 4, 8, 12, 16 and 20mg with 0.5 g of roughage and concentrate ratio at 60:40. They found that cumulative gas production (96 h of incubation) were higher ($P<0.01$) with *T. chebula* supplementation at 12, 16 and 20mg than other treatments. However, *in vitro* dry matter degradability (IVDMD) and *in vitro* organic matter digestibility (IVOMD) were not significantly different among treatments ($P<0.05$). They found NH_3-N concentrations tended to quadratically increase with increasing levels of *T. chebula* in the diet. In addition, total volatile fatty acids (VFA) and propionate concentrations were increased ($P<0.01$), while acetate concentration, acetate-to-propionate ratio, CH_4 production and protozoal populations were decreased ($P<0.01$) when supplemented with *T. chebula* at 8, 12 and 16mg, respectively.

Bueno *et al* (2015) studied *in vitro* gas production and digestibility of condensed tannins in five ruminant species. They evaluated Four roughages as substrates: maize silage (*Zea mays*), fresh elephant grass (*Pennisetum purpureum*), Tifton-85 hay (*Cynodon spp*.) and fresh alfalfa (*Medicago sativa*) and used Acacia (*Acacia molissima*) tannin extract as an external CT source to raise dietary CT content to 50 eq-g of leucocyanidin per kg of DM. They found the effect of the inclusion of tannin extract on IVDMD ($P<0.05$) but not on IVOMD and reported that the inclusion

of CT from acacia extract lowered gas production. They obtained higher partitioning factor when CT was included in diets ($P<0.001$). The proportion of SCFA did not change ($P<0.05$) with the inclusion of CT in the diet.

Gemeda and Hassen (2015) investigated *in vitro* digestibility, gas production (GP) characteristics and methane production of tanniferous browse plants. There was significant ($P<0.05$) variation in the chemical composition of studied browses. They reported that the tannin decreased ($P<0.05$) GP. The study revealed tanniferous browse plants significantly ($P<0.05$) decreased IVOMD and ME content. Tannin decreased ($P<0.05$) total volatile fatty acid and methane production.

Ishlak *et al* (2015) evaluated the effects of different feed additives (cinnamaldehyde, monensin, and quebracho condensed tannin extract) on fermentation, trans-fatty acids (FA) formation and selected strains of rumen bacteria. Treatment diets in their study were: control diet (44:56 forage to concentrate; CON), control plus cinnamaldehyde (CIN) at 400 mg/L, control plus monensin (MON) at 12 mg/L, and control with quebracho condensed tannin extract (QTAN) at 100 g/kg of diet (DM basis). They didn't find any effect ($P<0.05$) of feed additives on an apparent dry matter (DM), neutral detergent fiber (NDF) and organic matter (OM) digestibility but apparent protein digestibility decreased ($P<0.01$) with the QTAN and CIN diets. They found reduction ($P<0.05$) in the concentration of acetate and C18:0 in the treatment diets. They reported increment ($P<0.05$) in Concentration of trans C18:1 and vaccenic acid (VA) with the MON and CIN diets and was greatest with the MON diet. They found improvement ($P<0.05$) in the concentration of propionate with the treatment diets and was greatest with the MON diet. They found reduction ($P<0.01$) in the Ammonia-N concentration with all feed additives and was least with the QTAN diet. They reported DNA abundance of *Butyrivibrio proteoclasticum* decreased ($P<0.05$) with the MON and CIN diets. They found no effect ($P<0.05$) of Feed additives on the DNA abundance of *Anaerovibrio lipolytica* and *Butyrivibrio* SA.

Jayanegara *et al* (2015a) added purified tannin sources and polyethylene glycol and evaluated methane emission and rumen fermentation *in vitro*. They incubated Hay and concentrate mixture (70:30 w/w, 380 mg) was incubated in Hohenheim glass syringe. They injected purified tannins into the syringes at a concentration of 1.0 mg/mL each, either without or with PEG 6,000 addition in three

replicates. Their results revealed that methane emission decreased (20%-27%) when the purified tannins were added into the basal diet as compared to control ($P<0.05$), and PEG addition increased methane emission ($P<0.05$). All purified tannins decreased total gas and TVFA production ($P<0.05$). They concluded that tannins mitigate methane emission while PEG neutralizes such effect.

Jayanegara *et al* (2015b) studied effects of Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population *in vitro*. They found absorbance 0.043, 0.067, 0.421 and 0.167 for the chestnut, sumach, mimosa and quebracho tannins respectively for condensed tannins. Protein precipitation capacity of the hydrolysable tannins (chestnut and sumach) was higher than that of the condensed tannins (mimosa and quebracho) as it was estimated as 5.8, 7.1, 4.6 and 4.1 for the chestnut, sumach, mimosa and quebracho tannins, respectively. All the tannins decreased total gas production and IVOMD by following a linear and a quadratic pattern, respectively ($P\leq0.05$). Higher levels of all purified tannins linearly improved PF ($P\leq0.05$). Levels of tannins generally decreased total SCFA ($P\leq0.05$) except for sumach tannins. The proportion of acetate decreased Total SCFA production showed an interaction effect between tannin sources and doses ($P\leq0.05$). Increasing with increasing levels of tannin additions while the proportion of propionate increased, and as a consequence, the ratio of acetate to the propionate also decreased. All the purified hydrolysable and condensed tannins decreased total methanogen population than that of control when added at 1.0 mg/ml; the decrease ranged from 22.3 to 36.7% from control. However, there was no difference for the lower levels of purified tannin additions except for mimosa tannins at 0.75 mg/ml.

Bhatta *et al* (2014) evaluated effects of graded levels of tannin-containing tropical tree leaves on *in vitro* rumen fermentation, total protozoa and methane production. They incubated 200 mg equilibrated leaf samples. They incubated leaf samples at 2.5, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0% (DM basis) of TMR. They reported that highest total phenol (TP; g kg 1 DM) was recorded in *Azardirachta indica* (108) followed by *Ficus bengalensis* (103) and *Autocarpus integrifolis* (76), and total tannin (TT) content also showed a similar trend. However, condensed tannin (CT) was highest in *F. bengalensis* (260) followed by *Au. integrifolis* (186) and *Az.*

indica (138). When *Au. integrifolis* was incubated with TMR at graded levels, 24-h gas production was similar to that of control up to 5% and thereafter linearly decreased. However, in the case of *Az. indica* and *F. bengalensis*, reduction in gas production ($P<0.05$) was observed even at a 2.5% level primarily due to their higher TP content. There was a significant ($P<0.05$) reduction in the NH_3 (mg/dl) concentration from 56 (TMR) to 41.6 in *F. bengalensis*, 35.7 in *Au. integrifolis* to 31.5 in *Az. indica*. *Autocarpus integrifolis* tannins did not cause inhibition of TVFA concentration, but it reduced ($P<0.05$) at >5% inclusion level in *Az. indica* and >2.5% in *F. bengalensis*, respectively. The protozoal count was similar at all levels in *Au. integrifolis*, but reduced ($P<0.05$) in *F. bengalensis* and *Az. indica*.

Adamczyk *et al* (2012) studied the precipitation of proteins by tannins, effects of concentration, protein/tannin ratio and pH. They have mixed 0.5 mL of TA (dissolved in water) with 0.7 mL of BSA in the buffer (pH 4.7 or 7); three different ratios of compounds were used, (i) 'medium BSA/TA ratio' (containing 5 mg TA and 11.55 mg BSA), (ii) high TA ratio (10 mg TA and 4.62 mg BSA), and (iii) high BSA ratio (2.5 mg TA and 23.1 mg BSA). At pH 4.7 medium and high TS treatments were having about 100% protein precipitation but with high BSA protein precipitation was only about 50%. But at pH 7 only high TA was having near 100 protein precipitation and the rest two treatments had precipitated below 7% of protein. It is often assumed that high precipitation can be obtained only at pH close to the isoelectric point. However, their study suggested that at a certain tannin /protein ratio (high TA ratio), BSA can also be precipitated at pH 7, far from the isoelectric point. At such a pH, tannins are still able to precipitate proteins. Tannins lose this ability at pH 9 and higher, which is above the pKa of their phenolic groups. On the basis of observations, they concluded that substantial precipitation of proteins at high pH (pH 7) requires a relatively large amount of tannins and a small amount of protein.

Mohammadabadi T and Chaji M (2012) studied the influence of the plant tannins on *in vitro* ruminal degradation. They evaluated untreated SBM (USM), SBM treated with 30 g/kg DM oak leaves tannin (OLSM), oak fruit tannin (OFSM), pistachio hull tannin (PHSM) and pistachio leaves tannin (PLSM). Tannin contents of OL, OF, PH and PL were 53, 79, 48, and 65 g/kg DM respectively. The result of their experiment showed that the tannin of OL and PH did not reduce the fermentable fraction but, tannin of OF and PL reduced these parameters ($P<0.05$) i.e. net gas

production which was 149.8, 124.2, 108.2, 126.2 and 119.2 for USM, OLSM, OFSM, PHSM and PLSM respectively. Gas production rate also remained constant. The values of ME were 12.3, 11.2, 8.1, 10.9 and 9.2 MJ/kg DM for USM, OLSM, OFSM, PHSM and PLSM, respectively. The value of OMD and ME of sunflower meal were decreased by tannin of oak fruit and pistachio leaves, but oak leaves and pistachio hull did not influence these parameters ($P<0.05$). All tannin sources decreased ($P<0.05$) the concentration of SCFA and NH_3-N. The mean values of SCFA concentrations were 0.82, 0.73, 0.48, 0.74 and 0.64 µmol/L for USM, OLSM, OFSM, PHSM and PLSM, respectively. The mean values of NH_3-N concentrations were 31.6, 19.2, 15.3, 19.5 and 18.2 mg/dL for USM, OFSM, OLSM, PHSM and PLSM, respectively.

Niderkorn *et al* (2012) studied the potential role of condensed tannins (CT) *in vitro* rumen fermentation characteristics. They incubated Cocksfoot and sainfoin in different proportions (in g/kg, 1000:0, 750:250, 500:500, 250:750 and 0:1000) under anaerobic conditions in culture bottles containing buffered rumen fluid from sheep. In their study Incubations were carried out using artificial saliva with and without polyethylene glycol (PEG), which binds and thus inactivates CT. When the sainfoin level increased from 0 to 1000 g/kg, the ammonia concentration in the medium quadratically decreased from 3.20 to 0.53 mmol/l in absence of PEG ($P<0.01$) but not in its presence. They found lower total gas and methane productions in mixtures incubated in the absence of PEG than in the presence of PEG ($P<0.001$). The presence of PEG increased gas production ($P<0.001$) and NH_3-N concentration in the medium ($P<0.001$).

Seresinhe *et al* (2012) conducted *in vitro* experiments to evaluate the suitability of several mixtures of high tanniniferous non-legumes with low tanniniferous legumes on *in vitro* parameters. The results of their study revealed that supplementing high tannin non-leguminous forages by incremental substitution of legume forage increased gas production parameters, NH_3-N, IVDMD and microbial population in the fermentation liquid. They didn't find any significant effects on methane production by the presence of CT or different levels of CP in forage mixtures.

Gupta *et al* (2011) evaluated pellets of groundnut cake, cottonseed cake, mustard seed cake, linseed cake and soybean cake and used Acacia catechu leaves (ACL) as tannin source with different tannin levels i.e. 2-8% for *in vitro* dry matter

degradability (IVDMD) and ammonia production in cattle inoculums. Results of their study showed that Irrespective of protein sources, Oil cake-ACL pellets with 2, 4, 6 and 8% tannin level had lowered IVDMD by 54.37, 48.91, 46.09 and 46.46%, respectively compared to control which had IVDMD of 70.50%. NH_3 -N production from oil cake-ACL pellets was 21.46, 17.64, 13.61 and 14.46 mg/100ml at 2, 4, 6 and 8% tannin level against 30.52 mg/ 100 ml at 0% tannin level.

Getachew *et al* (2008a) evaluated the influence of tannic acid application on alfalfa hay in *in vitro* rumen fermentation, serum metabolites and nitrogen balance in sheep. They fed Alfalfa hay with and without TA to sheep. In their study, TA was sprayed on chopped alfalfa at three concentrations which were 0, 30, 60 and 90 g TA per kg DM. They reported that the addition of TA had no influence on the extent and rate of gas production but significantly decreased NH_4-N concentration at 30 ($P<0.05$), 60 and 90 ($P<0.0001$) g/kg DM. They reported TA significantly decreased ($P<0.05$) isovalerate. Whereas, it did not affect the total and individual SCFA acid production. Tannic acid significantly ($P<0.05$) decreased *in vitro* true degradability of DM after 24 h incubation at 60 and 90 g TA per kg DM. The effect of TA on IVTD was not significant ($P<0.05$) after 48 h of incubation. N digestibility was significantly reduced with all three levels of TA additions. They reported a reduction in the proportion of urine-N to total N output by adding 60 g ($P<0.05$) and 90 g ($P<0.01$) TA per kg DM. Serum metabolites and liver enzymes were not affected by TA ($P<0.05$). Higher faecal N as the TA level increased indicates incomplete dissociation of tannin-protein complexes post ruminally.

Getachew *et al* (2008b) evaluated influence of addition of gallic acid, tannic acid and quebracho tannins to alfalfa hay on *in vitro* rumen fermentation and microbial protein synthesis and found that QT and TA decreased gas production ($P<0.001$), IVTD ($P<0.001$), and total SCFA ($P<0.001$). QT addition resulted in a decrease in acetate to propionate ratio while TA increased it compared to control. Total purines were not affected by the addition of QT and TA although it tended to be higher in the presence of QT and TA. The efficiency of microbial protein synthesis increased when QT and TA were added compared with the control. Addition of QT and TA at the rate of 50, 100, and 150 g/kg resulted in a reduction of NH_4^+-N by 12%, 31%, and 51%, respectively, while tannic acid resulted in a reduction of NH_4^+-N by 13.8%, 25.9% and 46.5% at the same dose.

El-Waziry *et al* (2007) studied the effect of autoclaving and quebracho tannin (QT) treated-soybean meal (SBM) on gas production and rumen fermentation *in vitro* and they reported cumulative gas production during 72 h was lower in treated SBM compared to control and results found were as 43.41, 40.98, 36.89, 39.62, 31.67 and 27.87 for the SBM, SBM treated with 1% QT, SBM treated with 2% QT, SBM treated with 3% QT, autoclaved SBM and gelatin, respectively. The mean values of NH_3-N concentrations of sampling times were 10.17, 8.76, 7.68, 7.47 and 6.43 mM for untreated SBM, SBM treated with 3% QT, SBM treated with 1% QT, SBM treated with 2% QT and autoclaving SBM, respectively. The mean values of ME were 9.63, 8.85, 8.72, 8.38 and 7.10 MJ/kg DM for untreated SBM, SBM treated with 1% QT, SBM treated with 3% QT, SBM treated with 2% QT and autoclaving SBM, respectively. The OMD (%) was higher in untreated SBM and gelatin and lowest in autoclaved SBM and SBM treated with 2, 1 or 3% QT. They concluded that there were no significant differences between each other so they recommended to spray 1% quebracho tannins on SBM or feeding with tanninferous plant species that protect the protein from degradation in the rumen due to the presence of small amounts of condensed tannins in the plant species.

Martinez *et al* (2006) studied the effect of moderate amounts of tannins on the utilization of protein contained in forages. The effects of commercial tannic acid (hydrolysable tannins) and quebracho tannins (condensed tannins) (50 g kg−1 DM) on the *in vitro* fermentation of ground wheat and corn grains by mixed ruminal bacteria were examined. They reported regardless of the source of tannin, microbial fermentation was inhibited in both grains, as demonstrated by a decline in gas production, DM disappearance, volatile fatty acids and ammonia production. However, they found effects more pronounced for wheat than corn grain, mostly during the initial stages of the incubation. Scanning electron microscopy revealed that both sources of tannins inhibited the microbial hydrolysis of the endosperm protein matrix. Tannins did not prevent bacterial attachment to starch granules, but they reported slower starch hydrolysis indirectly as a result of a tannin-mediated reduction in the degradation of the surrounding protein matrix.

El-Waziry *et al* (2005) studied the effect of Roasting and Tannic Acid Treated-soybean meal on gas production and rumen fermentation. *In vitro* treatments

included were as Soybean meal (SBM), roasted SBM at 146ºC for 30 min, SBM treated with 1% tannic acid (TA) of DM, SBM treated with 2% TA of DM and SBM treated with 3% TA of DM as a low degradable protein source. They reported that the highest mean values of cumulative gas production during 72 h was obtained by SBM followed by roasting SBM, SBM treated with 1%TA, SBM treated with 2%TA and SBM treated with 3% TA. The concentrations of NH_3-N were significantly ($P<0.05$) decreased when SBM treated by heating or TA. The mean values of NH_3 -N concentrations of sampling times were 10.17, 8.99, 3 8.89, 7.69 and 9.15 mM for SBM, SBM treated with 1% TA, SBM treated with 2% TA, SBM treated with 3% TA and roasting SBM, respectively. They found concentrations of VFA were significantly ($P<0.05$) decreased when SBM treated by heating or TA. The mean values of VFA concentrations of sampling times were 86.39, 81.22, 81.26, 75.27 and 84.08 mM for SBM, SBM treated with 1% TA, SBM treated with 2% TA, SBM treated with 3% TA and roasting SBM, respectively. The OMD was higher in SBM and roasting SBM and lowest in SBM treated with 2 or 3% TA. They found a reduction in ME and NE values when SBM was treated by heating or TA. The mean values of ME were ranged from 8.10 to 9.63 MJ/kg DM for SBM treated with 3% TA and untreated SBM, respectively.

Wina and Abdurohman (2005) compared ruminal bypass protein (*in vitro*) by adding tannins isolated from *Calliandra calothyrsus* leaves or formaldehyde. They conducted two experiments. The first experiment aimed to obtain the optimum level of added tannins to decrease the dry matter digestibility of protein sources. In the first experiment, a protein source (*Gliricidia* leaves, milled soybean meal or casein) was weighed into an *in vitro* tube. Crude tannins were added at the level of 0, 10, 20, 30, 40 and 50 mg to each tube. They conducted the second experiment to compare the ability of tannin to formaldehyde to form a complex with protein *in vitro*. They incubated one set of tubes containing tannin-protein or formaldehyde-protein complex with rumen liquor for 48 h and another set with rumen liquor (48h) and followed by pepsin-HCl for 24 h (total incubation time: 72 h). They found that the addition of tannin isolate to a protein source in the *in vitro* incubation led to a reduction of dry matter digestibility of the protein source in a dose-dependent manner. However, the decline of dry matter digestibility was in a different rate with a different protein source; casein had the highest reduction rate (slope = - 0.139), followed by soybean

meal (SBM) (slope = - 0.105) and *G. sepium* had the lowest (slope = -0.0975). DM digestibility of soybean meal, casein and *G. sepium* was not significantly different when the levels of tannin were above 80, 60 and 60 mg/g substrate, respectively. In the rumen fermentation (48 h incubation), both DM and CP digestibilities of casein, SBM and *G. sepium* leaves were much higher when reacted with tannin than with formaldehyde. High digestibility of DM or CP at 72 h of incubation also occurred in the presence of tannin, from that they concluded that tannin at the maximum level of 80 mg/g protein source did not affect the intestinal (pepsin) digestion of tannin-treated protein. Both DM and CP digestibilities at 48 and 72 h of incubations, which were lower in the presence of formaldehyde suggesting that formaldehyde (2 g/100 g protein source) protects protein not only from the degradation in the rumen but also partly from the hydrolysis by pepsin in the lower gut.

Getachew *et al* (2002) evaluated *in vitro* gas production of tropical browses. Total phenol (TP), tannins (T) and condensed tannins (TP and T as tannic acid equivalent; CT, as leucocyanidin equivalent) ranged from 17–250, 7–214, and 0–260 g/kg DM respectively in the browses used by them. They reported increment in *in vitro* gas production on the addition of PEG to tannin-containing browses.

Singh *et al* (2001) studied biodegradation of tannic acid in an *in vitro* ruminal system. They compared blank rumen liquor with 2gms starch, starch + TA and only TA and found that tannic acid caused an increase in the gas volume and a decrease in the ammonia concentration which was more apparent after 24 h of the buffered rumen liquor during incubation for 24–72 h. The thin layer chromatographic profile of the supernatant obtained on anaerobic incubation of tannic acid with rumen liquor showed gallic acid and pyrogallol were present in the samples collected at 24 h of incubation. Gallic acid or residual tannic acid could not be detected in the samples collected at 48 and 72 h.

Makkar *et al* (1995) studied *in vitro* effects of tannins. They reported a reduction in the *in vitro* true digestibility of dry matter by 3, 6 and 7% for *Q. incana*, *D. cinerea* and *A. barteri*, respectively, at a tannin concentration of 0.47 mg/ml, and 17, 21 and 27%, respectively, at 0.93 mg/ml. They reported that tannins also decreased the production of short-chain fatty acid (SCFA) and their molar proportions (acetate decreased whereas propionate increased). The efficiency of microbial protein

synthesis, expressed as the ratio of N incorporation per unit of SCFA production, was higher with the tannins.

2.2 Effect of tannin on ruminal degradation and animal performance

Molina-Botero *et al* (2019) evaluated the effect of adding increasing levels of ground pods of *Enterolobium cyclocarpum* blended with foliage of *Gliricidia sepium* on emissions of ruminal methane (CH_4), volatile fatty acid proportions, rumen pH and microbial population in cattle. They used four heifers and fed (13 days) 0, 15, 30, and 45% of pods of *E. cyclocarpum* blended with foliage of *G. sepium*. They found no significant (P=0.272) difference in dry matter intake (DMI). They reported a reduction in the apparent fiber digestibility (81 g/kg). They found a linear effect on molar proportions of butyric acid and acetic to a propionic acid ratio (P<0.05) on the incorporation of legume foliage and pods. They reported no effect on Rumen population of total bacteria, methanogenic archaea, and total protozoa by the increasing levels of condensed tannins and saponins in rations (P<0.05). They reported substitution of 15 and 30% of pods of *E. cyclocarpum* mixed with foliage of *G. sepium* in the ration, decreases annual methane emissions per unit product, without affecting dry matter intake or rumen microbial population.

Zhou *et al* (2019) evaluated effects of dietary tannic acid on rumen fermentation and nutrient digestion in beef cattle. They used eight growing Simmental cattle (male castrated, initial live weight 350 ± 25 kg) as experimental animals. They reported adding TA at 16.9 g/kg decreased the ruminal concentration of TVFA (P<0.05) and the molar proportions of iso-valerate ($p < 0.001$) and valerate (P<0.05), increased the molar proportion of acetate and the molar ratio of acetate to propionate (P<0.05). Furthermore, adding TA decreased the ruminal concentration of NH_3-N ($p < 0.001$). Increasing dietary CP level or adding TA did not affect the rumen pH. The metabolites of TA including gallic acid, pyrogallol and resorcinol were detected in the rumen fluid only when TA was added ($p < 0.001$). They reported adding TA decreased the digestibility of the dietary DM (P<0.05), OM (P<0.01) and CP ($p < 0.001$) at both dietary CP levels but did not affect the digestibility of EE, NDF and ADF.

Brown *et al* (2018) studied the effects of tanniniferous *Acacia karroo* leaf to evaluate the performance. They fed goats with different level of *Acacia karroo* leaf

meal at 20%, 25%, 30% 40% and 50% of the total diet. Daily dry matter intakes were similar across the treatments. *Acacia karroo* leaf meal inclusion improved dry matter, organic matter, crude protein, neutral detergent fibre and acid detergent fibre digestibility coefficients. They reported there were no effects of dietary treatments on final live weights of goats. However, live weight gains were higher in goats fed a diet containing 50% *Acacia karroo* leaf meal inclusion level.

Gerlach *et al* (2018) studied the effect of condensed tannin supplementation on *in vivo* nutrient digestibilities and energy values of concentrates in sheep. They evaluated four treatments consisting of grass hay and concentrate containing different concentrations of the CT-rich extract (CON, without CT; CT1, CT3 and CT5, with 1, 3 and 5% CT-rich extract in ration DM), resulting in CT concentrations of 0, 2.03, 6.19 and 10.2 g/kg DM, respectively. The *in vivo* digestibility of organic matter and crude protein of the concentrates was unaffected by CT1 and decreased significantly with CT3 (−21%) and CT5 (-28%; $P < 0.05$). The decrease was even more pronounced for digestibility of aNDFom. For aNDFom and ADFom, the digestibility was reduced with CT1 ($P<0.05$), whereas ether extract digestibility was unaffected. The *in vitro* gas production of rations with 1, 3 and 5% CT supplementation was reduced by 3.0, 9.2 and 18.2% which exceeds the CT concentration in the ration. The metabolizable energy concentration of the concentrates decreased markedly (−25%) from 12.9 to 9.7 MJ/kg DM ($P<0.05$), for CON and CT5, respectively.

Koenig *et al* (2018) conducted an experiment to determine the effects of feeding a condensed tannin extract (CT) on dry matter intake (DMI), growth performance, carcass traits, and NH_3-N emissions of beef feedlot cattle. They divided 36 crossbred steers to 4 diets with increasing concentrations of a CT extract from *Acacia mearnsii* at 0%, 1.2%, 2.4%, and 3.5% of DM. They reported DMI was not affected at 1.2% and 2.4% but tended to decrease at 3.5% CT extract. They noted no effect ($P \geq 0.12$) of increasing CT extract on ADG. They reported a reduction in the NH_3 -N emissions by 23% by feeding 2.5% CT.

Ali *et al* (2017) studied the effect of hydrolysable tannin supplementation on production performance of dairy crossbred cows. In the experiment, they selected twelve cross-bred (Friesian and Sahiwal/ Cholistani cross) dairy cows (average milk yield 10 litres/day, average lactation number 3-4, average age 6±2 years). They

divided cows into four groups; A, B, C and D (3 cows per group) and each cow act as replicate. They fed Group A (control) without hydrolysable tannin and supplemented hydrolysable tannin in the diet of groups B, C and D at 20, 30 and 40 g /day, respectively. Total milk production was found highest and varied significantly from the control and group B in group D ($P<0.01$). Milk protein, fat, lactose and total solids were varying non-significantly between groups. Milk urea content was decreased in supplemented groups. Somatic cell count of milk decreased in hydrolysable tannin supplemented groups whereas it was unchanged in the control group from the beginning of the trial.

Ebert *et al* (2017) studied the effect of condensed tannin extract supplementation on growth performance and nitrogen balance of beef steers. They used 27 Angus-crossbred steers (350 ± 32 kg initial BW) to determine the effects of CT supplementation during the finishing period on growth performance and N balance. Steers were stratified by initial BW and assigned randomly to 1 of 3 treatments: CT added at 0% of diet DM, 0.5% of diet DM (0.5% CT), or 1.0% of diet DM (1.0% CT) and fed for 126 d. Dry matter intake did not differ ($P = 0.57$) across treatments. No differences ($P \geq 0.86$) among treatments were observed in final BW, average daily gain, and gain/feed intake. Hot carcass weight, dressing percent, KPH percent (Percent Kidney, Pelvic, Heart Fat), and marbling score were not different ($P \geq 0.26$). No differences ($P \geq 0.13$) were observed in OM intake, starch intake, and fecal OM among treatments. Fecal starch excreted ($P = 0.03$) linearly increased with the inclusion of CT in the diet. Nitrogen intake did not differ ($P = 0.16$) among treatments. Fecal N linearly increased ($P = 0.01$) with CT inclusion in the diet. Urinary N and retained N were not different ($P \geq 0.39$) among treatments.

Junior *et al* (2017) evaluated the effect of monensin and *Acacia mearnsii* tannins on ruminal fermentation efficiency in cattle. They gave treatments as Control, Monensin (300 mg per animal) and a tannin-rich extract from Acacia mearnsii (100 g per animal). They reported that there was no effect ($P<0.05$) of additives on pH, ruminal DM disappearance rate, the concentrations of NH_3-N, the production of acetic or butyric acids or the total ruminal SCFA. Monensin treatment was responsible for reducing CH_4 production by 10.7%, whereas tannin inclusion reduced it by 8.0% when compared to the control treatment.

Pathak *et al* (2017) studied the effect of condensed tannins from *Ficus infectoria* and *Psidium guajava* leaf meal mixture on nutrient metabolism, methane emission and performance of lambs. They took twenty-four lambs of ~6 months age and gave 4 dietary treatments (CT-0, CT-1, CT-1.5, and CT-2 containing 0, 1.0, 1.5, and 2.0 percent CT through LMM, respectively) for 3 months. They reported that intake of dry matter and organic matter (g/d) was higher in CT-1.5 than control. Digestibility of various nutrients did not differ irrespective of treatments. Nitrogen retention and microbial nitrogen synthesis (g/d) were significantly higher in CT-1.5 and CT-2 groups relative to CT-0. Total body weight gain (kg) and average daily gain (g) were significantly higher in CT-1.5 followed by CT-1 and CT-0, respectively. Feed conversion ratio (FCR) in lambs was significantly (linear, $p<0.01$) higher for CT-1.5 than CT-0. Methane emission was linearly decreased in CT-2 followed by CT-1.5 and CT-1. Methane energy (kcal/d) was linearly decreased in CT groups. The CT supplementation at 1% to 2% of the diet through *Ficus infectoria* and *Psidium guajava* LMM significantly improved nitrogen metabolism, growth performance, wool yield, FCR and reduced methane emission by lambs.

Rivera-Mendez *et al* (2017) studied the effect of level and source of supplemental tannin on growth performance of steers during the late finishing phase. They took ninety-six Holstein steers (478 ± 6.5 kg) in an 84-day trial to evaluate the effects of level (0%, 0.2%, 0.45, and 0.6% of dry matter (DM) basis) of supplemental condensed tannin (Quebracho) on feedlot growth performance during the late finishing phase. Supplemental tannin increased (6.5%, P=0.05) average daily gain (ADG), with a tendency (linear effect, P= 0.15) for ADG to increase with the level of supplementation. DMI likewise tended to increase (linear effect, P= 0.14) with a level of supplementation. Tannin supplementation increased gain efficiency (5.5%, P = 0.04) and dietary NE (3.2%, P= 0.06). However, neither gain efficiency nor dietary NE was improved (P> 0.74) by feeding more than 0.2% of supplemental tannin. They conducted another study with Ninety-six Holstein steers (392 ± 4 kg) in an 84-d feeding trial to evaluate the effect of tannin sources on feedlot cattle performance during the late finishing phase. Treatments consisted of the basal diet supplemented with no supplemental tannin, 0.6% condensed tannin, 0.6% hydrolysable tannin and a combination of 0.3% condensed and 0.3% hydrolysable tannin. Inclusion of 0.6% of tannin in diet tended to increase ADG (6.8%, P = 0.08). Tannin supplementation

likewise tended to increase DMI (4%, P = 0.06). However, compared to controls, DMI was greater (7.1%, P<0.05) for steers fed the 50:50 combination of the condensed and soluble tannins than when tannin sources were fed singly (2.4%, P > 0.10).

Supapong *et al* (2017) studied the effect of supplementing *Delonix regia* (DR) seed meal on feed intake, digestibility, rumen fermentation, nitrogen balance and CH_4 production in Thai native beef cattle fed on rice straw. They assigned four Thai native beef cattle to receive DR seed meal supplementation at 0, 90, 180 and 270 g/d. Their investigation revealed that the total intake was significantly increased with the inclusion of seed meal at 270 g (P<0.05). DM and OM digestibility were decreased when increasing DR seed meal were incorporated into the diet (P<0.05). Ruminal NH_3-N concentration increased in beef cattle receiving DR seed meal. Supplementation of DR seed meal did not alter fungal zoospores' concentration (P<0.05), whereas the protozoal population was at 0, 4 h post-feeding, and the mean values reduced when increasing the levels of DR seed meal supplemented (P<0.05). The concentration of propionic acid at 4 h post-feeding and its average concentration were significantly highest when 270 g DR seed meal was supplemented (P<0.05). N absorption, N retention and proportion of N retention to N intake were enhanced when 270 g DR seed meal was supplemented (P<0.05).

Aguerre *et al* (2016) studied the effect of quebracho-chestnut tannin extracts on performance, rumen fermentation, and nitrogen partitioning in dairy cows. They fed 0, 0.45, 0.90, or 1.80% of a tannin mixture in the basal diet of Holstein cows. They found DM intake decreased (25.5 to 23.4 kg/d) linearly, as well as a linear increase in milk/DM intake (1.62 to 1.75) and a trend for a linear decrease in fat-and-protein-corrected milk (38.4 to 37.1 kg/d) with increasing levels of tannin supplementation. They found a negative linear effect for milk urea N (14.0 to 12.9 mg/dL), milk protein yield (1.20 to 1.15 kg), and concentration (2.87 to 2.83%). They reported a change in milk protein concentration tended to be quadratic and predicted maximum was 2.89% for a tannin mixture fed at 0.47% of dietary DM. They found a reduction in ruminal NH_3-N (11.3 to 8.8 mg/dL) with tannin supplementation, total branched-chain volatile fatty acid concentration (2.97 to 2.47 mol/100 mol), DM, organic matter, CP, and neutral detergent fiber digestibility was also reduced. Dietary

tannin had no effect on intake N (587 ± 63 g/d), milk N (175 ± 32 g/d), or N utilization efficiency (29.7 ± 4.4%). However, their results showed that feeding tannin extracts linearly increased fecal N excretion (214 to 256 g/d), but reduced urinary N (213 to 177 g/d) and urinary urea N (141 to 116 g/d) excretion. Decreasing dietary CP did not influence milk production, but increased N utilization efficiency (milk N/N intake; 0.27 to 0.33), and decreased milk urea N (15.4 to 11.8 mg/dL), ruminal NH_3-N (11.0 to 9.3 mg/dL), apparent digestibility of DM (66.1 to 62.6%), organic matter (68.2 to 64.3%), and CP (62.9 to 55.9%), as well as urinary N excretion (168 vs. 232 g/d).

Gunun *et al* (2016) investigated the effect of supplementation of mao (*Antidesma thwaitesianum*) seed meal (MOSM) containing condensed tannins (CT) on rumen fermentation, nitrogen (N) utilization and microbial protein synthesis in goats. They supplemented MOSM at 0%, 0.8%, 1.6%, and 2.4% of total dry matter (DM) intake, respectively in goats diet. Results of their study revealed that supplementation with MOSM did not affect feed intake, nutrient intakes and apparent nutrient digestibility ($P<0.05$). They didn't find the effect of MOSM supplementation on ruminal pH and ammonia nitrogen (NH_3-N). They reported a reduction in blood urea nitrogen was in goats supplemented with MOSM at 2.4% of total DM intake. They reported propionate was increased linearly with MOSM supplementation, whereas acetate and butyrate remained the same. They found a reduction in ruminal methane (CH_4) when goats were fed with MOSM at 1.6% and 2.4% of total DM intake. They didn't find any effect of treatment on numbers of bacteria and protozoa ($P<0.05$). They reported that there were linear decreases in urinary N ($P<0.01$) and total N excretion ($P<0.01$) by MOSM supplementation. N retention was increased linearly ($P<0.05$) when goats were fed with MOSM supplementation at 1.6% and 2.4% of total DM intake. Microbial protein synthesis was not significantly different among treatments ($P<0.05$).

Kai *et al* (2016) studied the effect of purple prairie clover (*Dalea purpurea*) hay and its condensed tannins on growth performance, wool growth, nutrient digestibility, blood metabolites and ruminal fermentation in lambs fed total mixed rations. They selected thirty-six Canadian Arcott ram lambs (39.5 ± 7.4 kg initial body weight (BW); 4.1 ± 0.3 months) by BW. They fed 40% (DM basis) of a barley

grain-based concentrate and either 60% alfalfa hay (AH), 60% PPC hay (PH) or PH diet supplemented with polyethylene glycol (PH-p). They reported lower dry matter intake of lambs fed PH diet (P<0.01) than those of lambs fed AH and PH-p diet and reported that there was no difference in DMI between AH and PH-p fed lambs. The wool yield, strength, length, fibre diameter, comfort factor, curvature and spinning fineness were not found affecting (P<0.05) by dietary treatments. They found Lambs consuming PH exhibited greater apparent total tract digestibility of DM (P<0.05) and OM (P<0.05) and tended (P=0.059) to have greater CP digestibility compared to those fed AH, but lower digestibility of CP (P<0.001), aNDF (P<0.01) and ADF (P<0.05) than those fed PH-p. Digestion of starch was nearly complete (99.9%) for all lambs and there was no difference in starch digestion among lambs fed the three diets. Rumen fluid collected from lambs fed PH had lower (P<0.05) concentrations of ammonia, VFA and protozoa as compared to lambs fed AH or PH-p. Animals fed PH-p had greater Glucose (P<0.05) and Blood urea nitrogen (P<0.001) than those fed PH. Concentrations of Creatinine and Blood urea nitrogen were lower (P<0.05) for lambs fed PH than lambs fed AH. In contrast, lambs fed PH and PH-p had greater serum Total antioxidant capacity (TAC) (P<0.01) and Catalase (P<0.001) than those fed AH with TAC.

Rivera-Mendez *et al* (2016) assessed the influence of tannins supplementation on growth performance, dietary net energy and carcass characteristics of yearling steers fed finishing diet containing dried distillers grains with solubles. Dietary treatments consisted of steam- flaked corn-based growing-finishing diet containing 15% DDGS supplemented with 0, 0.32, and 0.64% CT (70% condensed tannin; CT) which correspond to 0, 2.2 and 4.4 g of condensed tannins/kg DM. They found that the tannin supplementation did not affect (P=0.97) daily gain weight gain (ADG), averaging 1.77 kg. Tannin supplementation increased (linear effect, P <0.01) dry matter intake (DMI). They found dietary NE decreased (linear effect, P < 0.01) with tannin supplementation. They reported that there were no treatment effects on carcass characteristics.

Yang *et al* (2016) studied the effects of dietary supplementing tannic acid (TA) on nutrient digestibility and plasma biochemical parameters. Four levels of TA, that is 0, 6.5, 13.0 or 26.0 g/kg dry matter (DM), were added to the basal ration

(composed of corn silage and concentrate mixture) as experimental treatments respectively. Each experimental period consisted of a 12-day adaptation phase followed by a 3-day sampling phase. They found that the supplementing TA at 26.0 g/kg DM decreased the DM and OM digestibility ($P<0.05$), and at 6.5, 13.0 or 26.0 g/kg DM decreased the CP digestibility ($P<0.01$) whereas, did not affect EE, NDF and ADF digestibility ($P<0.05$). Supplementing TA at 6.5, 13.0 or 26.0 g/kg DM did not affect the plasma concentration of glucose, total cholesterol, triglyceride, total protein, albumin, aspartate aminotransferase, alanine amino-transferase ($P<0.05$).

Ahnert *et al* (2015) studied the influence of ruminal Quebracho tannin extract (QTE) infusion on apparent nutrient digestibility, nitrogen balance, and urinary purine derivatives excretion in heifers. They used non-growing and non-pregnant six adult heifers fitted with ruminal cannulae (Jersey × German Black Pied Lowland) and fed at maintenance energy and protein level with a constant DM intake across the trial. The QTE product was a spray-dried moistened powder containing 68.6% total tannins and 15.9% condensed tannins (on fresh matter basis) was used. They reported that the mean body weight increased linearly with incremental QTE infusion ($P<0.001$) from 475 kg (standard deviation (SD) 33) at control to 504 kg (SD 35) at 6% QTE ($P\leq0.045$). The average total tract digestibility (ATTD) of DM ($P\leq0.045$), OM ($P\leq0.044$), and NDF ($P\leq0.020$) were lower at 4 and 6% QTE compared to CONTROL and 1 and 2% QTE. Moreover, ATTD of ADF was lower at 4 and 6% QTE than at control and 2% QTE (SE 0.02; $P\leq 0.026$). A linear increase in fecal N excretion with increasing QTE dosage was found ($P<0.001$), whereas urinary N excretion linearly decreased with increasing QTE dosage ($P<0.001$). With each additional %-unit of QTE infused, fecal N excretion increased by 2.0 g/d and excretion of urinary N decreased by 2.5 g/d. The absolute decrease in urinary N excretions (g/d) exceeded the increase in fecal N excretion, in particular at 1 and 2% QTE. The partitioning of fecal N to urinary N linearly increased from 0.50 at control to 0.96 at 6% QTE ($P<0.001$). The ATTD of CP decreased by 17% from 0.698 at control to 0.576 at 6% QTE (SE 0.01; $P<0.001$). At 4% QTE fecal excretion of ADIN was slightly higher than ADIN intake resulting in a negative coefficient of ATTD. Urinary excretion of PD declined linearly with increasing QTE level ($P<0.001$). The infusion of QTE at 4 and 6% reduced urinary PD excretion by about 9 and 19%, respectively, compared to control.

Jolazadeh *et al* (2015) studied the effects of soybean meal (SBM) treated with various amounts of tannins extracted from pistachio hulls on performance, ruminal fermentation, nutrient digestibility, and blood metabolites in Holstein bulls. They fed treatment group one of four SBM treatments included in a total mixed ration (TMR) at a constant rate of 105 g/kg DM. they treated SBM with pistachio extract concentrate (PEC), which contained 111.4 g/kg total phenol and 71.3 g/kg total tannin per dry matter of extract, at four experimental treatment rates of 0 (SBM-0), 5 (SBM-5), 10 (SBM-10), and 15 (SBM-15) kg PEC per 100kg SBM on a dry matter basis. They reported there was no effect of treatment of SBM with PEC on final body weight or dry matter intake (DMI), but adding PEC linearly increased the average daily gain (L, P=0.001) and feed efficiency (L, P=0.001). Inclusion of PEC linearly decreased ruminal ammonia nitrogen (L, P=0.02) and total protozoal population (L, P=0.002), but did not affect concentrations of total volatile fatty acids (VFA), individual VFA or pH in the rumen. They found that the total tract nutrient digestibility and blood metabolites were unaffected by SBM treatment with PEC, except for concentrations of albumin and total protein in plasma, which increased linearly (L, P=0.004 and P=0.001, respectively) with increasing PEC treatment.

Orlandi *et al* (2015) studied digestibility, ruminal fermentation and duodenal flux of amino acids in steers. They took Four Holstein steers fitted with a duodenal cannula and rumen catheter. They offered diet at a restricted amount of 20 g dry matter (DM)/kg BW and consisted of oat (*Avena strigosa*) plus concentrate, in a proportion of 0.55:0.45 (DM basis) respectively. Their treatments were no tannin (Control) or inclusion of tannin extract in the concentrate at a rate of 20, 40 or 60 g/kg DM (i.e. 9, 18 or 27 g/kg of total dietary DM). The apparent total-tract organic matter (OM) digestibility was not affected whereas the neutral detergent fiber (aNDF) digestibility tended to linearly decrease ($P<0.10$) at increased levels of tannin extract inclusion. Tannin extract linearly reduced ($P<0.05$) the total-tract digestibility of N compounds, as well as the urinary N excretion, and linearly increased ($P<0.05$) the fecal N excretion, N retention and the efficiency of N utilization by steers. The concentration of ammonia N in ruminal fluid linearly decreased ($P<0.05$) with tannin extract inclusion. Linear positive responses ($P<0.05$) to tannin treatments were observed for duodenal flux of total N, -amino N and non-ammonia non-microbial N. The microbial N supply tended to be negatively affected ($P<0.10$) whereas both the

ruminal OM digestibility and ruminal degradability of feed N compounds linearly decreased ($P<0.05$) with increasing tannin extract levels. The efficiency of ruminal microbial protein synthesis tended to increase quadratically ($P<0.10$) at increased levels of tannin extract. The dietary inclusion of tannin extract positively impacted ($P<0.05$) or tended to impact ($P<0.10$) the duodenal flux of all amino acid groups, as well as of most individual amino acids. They concluded dietary inclusion of *Acacia mearnsii* tannin extract up to a level of 18 g/kg DM decreased the urinary N excretion and improved the amino acid supply in steers fed fresh-frozen oat forage plus concentrate without significantly affecting the total OM digestibility.

Raju *et al* (2015) studied Effect of feeding oak leaves (*Quercus semecarpifolia* (QS) vs *Quercus leucotricophora* (QL)) on nutrient utilization, growth performance and gastrointestinal nematodes of goats in temperate sub-Himalayas. They conducted *in vivo* experiment with eighteen 7–9-month old crossbred (Chegu × Jamunapari) male goats (15.99 ± 1.53 kg) having strongyle infections (average EPG 4216). They randomly divided animals into three dietary treatments of six in each. They offered similar concentrate mixture to the animals and fed roughage source different in which local green grass, *Pennisetum clandestinum* (PC) in group T1 and tanniferous oak tree leaves were fed in groups T2 and T3, respectively. They conducted feeding trial for 141 days duration including the first 21 days for adaptation and subsequent 120 days for measurement of growth trial. They conducted digestion and nitrogen (N) balance trial of 6-day duration preceded by a 3 days adaptation period after 90 days of post-feeding. DM intake through roughage and total DM intake in oak leaves fed groups (T2 and T3) were higher ($P<0.05$) than the control group (T1). The nutrient intake (g/ kg $W^{0.75}$) through DM, OM, CP and TDN was also higher ($P<0.01$) in oak leaves fed group than grass fed control. In their study, the feeding of QL containing higher level of CT showed depressing effect on DM, OM, CP, NDF, ADF and TCHO digestibility and feeding of QS with lower level of CT lowered DM and OM digestibility only. Daily intake (g/d) of N and faecal excretion of N were higher ($P<0.05$) in oak leaves fed groups than control being attributed to higher DM intake. Average daily gain (g/d) was higher ($P<0.01$) in T2 (32.5) and T3 (25.7) groups compared to (13.5) Feed gain ratio (DMI/unit body weight gain) was comparatively better in T2 (22.9) than T1 (39.5) which was at par with T3 (29.9) indicating better feed efficiency in oak leaves

fed group. Mean Hb (g/dl) and PCV (%) levels were higher in T2 and T3 than T1. The mean serum urea level (mg/dl) was lower ($P<0.05$) in T3 as compared to T1 but at par with T2. Mean faecal egg counts (FEC) were reduced linearly with the advancement of feeding period ($P<0.01$) in both the oak leaves fed groups (T2 and T3) as compared to control group (T1).

Francis *et al* (2014) conducted study to determine the effectiveness of tannic acid in protecting dietary protein in soybean meal (SBM) from excessive microbial degradation in the rumen to extrapolate its bypass protein potential. They have done Tannic acid treatment by spraying plain water at 5% of the as-fed weight of SBM then mixed thoroughly for about 30 min, and sprinkled with tannic acid powder at 9% of the as-fed weight of SBM then thoroughly mixed for another 30 min. They incubated treated SBM samples for 24, 48, and 72 hr in rumen fistulated cattle. There was a significant reduction in DMD for the treated SBM as compared with the untreated at 24-hr, 48-hr, and 72-hr incubation. Dry matter disappearance rate (%/h) for un-treated SBM was 3.84, 2.06 and 1.38 whereas, for tannin treated SBM it was 2.82, 1.91 and 1.31 at 24, 48 and 72 hours incubation, respectively. Crude protein degradation rate (%/h) for un-treated SBM was 3.90, 2.08 and 1.39 whereas, for tannin treated SBM it was 2.76, 1.81 and 1.29 at 24, 48 and 72 hours incubation respectively.

Cieslak *et al* (2012) used four rumen cannulated dairy cows were to determine the effects of source of tannins on rumen microbial fermentation. Their treatments were either no supplemental tannin or supplemental *Vaccinium vitis idaea* (VVI). The dose of VVI was 140 g of extract containing the equivalent of 2 g of tannins/kg dietary DM. They found VVI reduced rumen CH_4 production, ammonia concentration and protozoa microbial populations (protozoa and methanogens) by 8, 46, and 35 and 21%, respectively. They found a reduction in acetate to propionate ratio but the proportion of total VFA was not affected.

Dschaak *et al* (2011) studied the influence of quebracho condensed tannin extract (CTE) on ruminal fermentation and lactation performance of dairy cows. They fed four dietary treatments: High Forage (HF) without CTE, HF with CTE (HF+CTE), Low Forage (LF) without CTE, and LF with CTE (LF+CTE) to lactating Holstein cows. Commercial quebracho CTE was added to the HF+CTE and the LF+CTE at a rate of 3% of dietary DM. They reported that supplementing CTE

decreased intakes of DM and nutrients regardless of forage level. They didn't find an effect on digestibility of DM and nutrients CTE supplementation. They reported that the yield of milk and milk component were not influenced by CTE supplementation. Negative effects of CTE supplementation on feed intake resulted in increased feed efficiency (milk yield/DM intake). They found a drop in the concentration of milk urea N (MUN) with the CTE in the diets. They reported no effect of supplementation of CTE in the diets on ruminal pH. Supplementing CTE decreased total volatile fatty acid concentration regardless of the level of forage. With CTE supplementation increased molar proportions of acetate, propionate, and butyrate in the HF diet, but not in the LF diet. They found a reduction in the concentration of ammonical-N with supplementation of CTE.

Mezzomo *et al* (2011) studied the influence of condensed tannin on intake and digestibility in beef steers. They selected four Bos indicus non-castrated male cattle with average body weight (BW) of 407±12 kg and 24 months of age. All the animals were cannulated in the rumen and abomasum. They fed a diet containing 13% roughage and 87% concentrate on a dry matter (DM) basis. Their diets were composed of corn grain cracked, whole cottonseed and sugarcane bagasse in natura, plus 1 of 4 supplements: soybean meal with CT, soybean meal without CT, CT with no soybean meal, and treatment with no soybean meal and CT. They didn't find the effect of experimental treatments on ruminal digestibility of DM, OM, and NDF. They reported an interaction of SBM×CT ($P<0.10$) on ruminal degradability of crude protein but, intestinal digestibility of CP remained unaffected ($P>0.10$). Interaction of CT×SBM inclusion found significant ($P<0.10$) on RUP flux, metabolizable protein (MP) flux, and on the ratio of MP: CP. They observed greater ($P<0.10$) protein flow from rumen to abomasum in case of animals fed diets with SBM and CT, resulting in increased amount of metabolizable protein in the small intestine and improved conversion of dietary CP into MP, which was not observed with the inclusion of CT in diets containing whole cottonseed as the main source of true protein. On the basis of results obtained from ruminal digestibility, associated to total tract apparent digestibility of CP and nitrogen balance, have indicated that animals fed diets with CT had lower N loss, no differences were found ($P>0.10$) among treatments on microbial protein content in the abomasal digesta.

Liu *et al* (2011) conducted the study to evaluate the effects of chestnut tannins (CT) and coconut oil (CO) on growth performance, methane (CH_4) emission, ruminal fermentation, and microbial populations in sheep. They fed control diet (CTR), 10 or 30 g of CT/ kg of diet (CT10 and CT30), 25 g of coconut oil/kg of concentrate (CO25), and 10 or 30 g of CT/kg of diet + 25 g of CO/kg of concentrate (CT10CO25 and CT30CO25). Results of their study revealed that the addition of CT, CO, and CT + CO had no significant effects on growth performance of sheep but reduced CH_4 emission. They found a reduction in the NH_3-N concentration in rumen fluid in CT30 whereas, Addition of CO decreased the concentration of total volatile fatty acids in rumen fluid. They didn't find any significant differences in pH and molar proportion of volatile fatty acids among treatments. Addition of CT, CO, and CT + CO significantly decreased methanogen and protozoa populations. They reported a reduction in the counts of *Fibrobacter succinogenes* with the supplementation of CO. No significant differences were obtained in populations of fungi, *Ruminococcus flavefaciens*, or *Ruminococcus albus* among treatments.

Toral *et al* (2011) studied effects of tannins in ewes and they made diet mixture (1:1, w/w) of two commercial extracts of quebracho condensed tannins and chestnut hydrolysable tannins to a diet and they reported neither DM intake nor milk yield was affected by diet supplementation with tannin. The same lack of differences occurred for milk fat, CP and total solid contents, as well as for Bodyweight change.

Alipour *et al* (2010) studied the effects of several levels of extracted tannin from grape pomace (GP) on digestibility of soybean meal. They diluted purified tannins in aqueous solution (100 g/l). Then added that solution to SBM to obtain levels of 15, 30, 45 and 60 g/kg DM of tannins. They used three rumen-fistulated Ghezel sheep to determine the rate of disappearance of N from tannin-treated SBM. Five grams of each sample was placed into the *in sacco* bags and incubated in the rumen for 2, 6, 8, 24 and 48 h. Simulated intestinal digestibility of the ruminal undegraded protein was determined by the *in sacco*/*in vitro* procedure. They reported in all incubation times gas production was visibly decreased due to the incorporation of tannin extract. The gas parameters Ap (potential gas production (ml/g OM)) and μ (the fractional rate of gas production at T/2 (/h)) decreased (linear and quadratic; $P<0.01$), and lag time and T/2 increased (linear and quadratic; $P<0.01$), with increased

tannin levels. The ammonia concentration at 24 h of incubation decreased with the increased addition of tannin extract ($P<0.01$). Increasing the level of tannin extract decreased the *in sacco* a fraction, and increased the b fraction, of N (linear and quadratic; $P<0.01$), while the disappearance rate of the b fraction of N decreased as the level of tannin extract increased ($P<0.01$). Effective disappearance of N (linear and quadratic; $P<0.01$) decreased as the tannin dose increased. They found the incorporation of tannin extract increased intestinal digestibility of CP which was 0.45 at the control and increased to 0.49, 0.51, 0.52, 0.53 at 1.5, 3, 4.5, 6 (linear and quadratic; $P<0.01$).

Krueger *et al* (2010) studied the effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers. In their study, the diet offered contained 14.9 g/kg DM of supplemental tannin (HT or CT), which resulted in approximately 0.38g tannin/kg BW consumed per day. They found that there was no effect ($P<0.05$) of tannin supplementation on rumen pH and ammonia concentrations. Control calves had higher hot carcass weight (HCW) ($P<0.05$) than CT treated calves with HT treated calves having an intermediate HCW; however, there was no effect ($P<0.05$) of tannin treatment on ribeye area (REA), 12th rib fat thickness, kidney, pelvic, and heart fat (KPH), yield grade, and marbling score. Dressing percent was not affected ($P<0.05$) by tannin. Control calves had higher ($P<0.05$) empty body weight (EBW) than CT treated calves with EBW intermediate for HT treated calves. There was no effect of tannin supplementation on BW at harvest (HBW). Empty body weight as a proportion of harvest BW was similar ($P<0.05$) for all calves (923.2, 912.4, and 920.8±6.2 g/kg HBW for control, HT, and CT, respectively). Tannin supplementation had no effect on the organ mass as a proportion of EBW for the liver, heart, kidney, small intestine, large intestine, and dissectible GIT fat.

Soltan (2009) studied rumen fermentation characteristics and lactation performance in dairy cows fed different rumen-protected soybean meal products. Their study included one hundred lactating Holstein dairy cows 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 17-25 week after

calving. 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. Ruminal pH reduction (P<0.05) was observed with inclusion of different treated HS, EP, PA or HPA SBM products 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. The VFA concentrations were reduced (P<0.05) when treated SBM (HS, EP, PA and HPA) replaced the untreated SBM in dairy cow diets by about 1.7, 0.5, 1 and 1.5%, respectively. Higher (P<0.05) milk fat% and milk fat yield g/cow/day 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. 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%.

Tiemann *et al* (2008) conducted trial to see the effects of *Calliandra calothyrsus* and *Flemingia macrophylla* on methane mitigation and metabolic protein supply at unchanged energy supply to sheep. The apparent total tract digestibility of organic matter, NDF and ADF were reduced when the tannin-rich plants were incorporated. They reported that faecal N losses increased linearly relative to N intake with increasing proportion of the CT-rich legumes in the diet (P<0.001) while the proportionate urinary N losses decreased for both CT-rich species (P<0.05). They also reported a reduction in the methane emission per day and per unit of feed and energy intake by up to 24% with the inclusion of the tannin-rich plants.

Frutos *et al* (2002) conducted a study with eight shrub species (*Cytisus purgans, C. scoparius, Genista florida, G. occidentalis, Calluna vulgaris, Erica*

arborea, E. australis and Juniperus communis). They found that the CT was negatively correlated (P<0:05) with OM degradation and cumulative gas production. It was noted that the CT was positively correlated with lag time, which is consistent with the extensively reported suppressive effect of condensed tannins on rumen degradation and on the interference of these compounds with microbial attachment to feeds. The positive correlation between plant secondary compounds and the partitioning factor was found.

Martinez *et al* (2005) used tannic acid to protect barley meal from ruminal degradation. They reported at 2, 4, 8 and 12 h, disappearances of DM and CP from barley were lower (P<0.05) in all TA-treated samples than in the controls, and the response became more pronounced as TA treatment level increased At 24h, the difference was significant only at the 5% treatment level, and by 48h no differences among treatments were evident (P<0.05). Effective degradabilities of DM and CP decreased (P<0.05) as TA treatment level increased, and they were also lower when outflow rates were set at 0.05/h and (or) 0.08/h when compared with 0.02/h. All levels of TA treatment decreased (P<0.05) the soluble fraction (a), the potentially degradable fraction (b) and the degradation rate (c) of barley DM. The impact of TA on the kinetics of *in situ* digestion of CP was similar to the effect on DM digestibility, but decreases in slowly degradable fraction (b) and digestibility rate (c) were significant (P<0.05) only at TA treatment levels 2.5% and 5% barley. They conducted Scanning electron micrographs of untreated and TA-treated barley meal after 24h of *in situ* incubation, only the cuticle and sub-aleuronic endosperm tissue remained in untreated samples, but a considerable amount of the endosperm persisted in TA-treated barley.

Salem *et al* (2005) evaluated benefits from the association of small amounts of tannin-rich shrub foliage (*Acacia cyanophylla*) with soya bean meal as supplements to Barbarine sheep fed on oaten hay. In trial they evaluated three diets: D1 (control): hay (ad libitum) + 200 g SBM; D2 (mixed strategy): D1 and 100 g acacia fed with the SBM; D3 (sequential strategy): as D2, but the SBM fed 1 h later than acacia when acacia was consumed completely. Irrespective of dietary treatment, hay intake was not affected (P<0.05) by acacia supply (526, 567 and 497 g DM/day, respectively for D1, D2 and D3). The three diets (D1, D2 and D3) had similar (P<0.05) DM (0.730,

0.680 and 0.675, respectively), OM (0.749, 0.700 and 0.704, respectively) and NDFom (0.712, 0.680 and 0.692, respectively) digestibilities. CP digestibility was similar among D1 and D2 ($P<0.05$) but decreased ($P<0.05$) in ewes given D3. They obtained the same trend for the concentration of plasma urea. Acacia feeding decreased NH_3-N with a maximum at 4 h post-feeding. Compared to D1 (TVFA= 120.8 mmol/L), the average concentration of TVFA decreased by 18 ($P<0.05$) and 28% ($P<0.05$) in ewes receiving D2 and D3, respectively. Compared to the control diet (D1), only diet D3 reduced ($P<0.05$) DM and N degradability of SBM.

Hervas *et al* (2000) studied the effect of tannic acid on rumen degradation and intestinal digestion of treated soya bean meals in sheep. Samples of SBM were prepared by spraying 100 g SBM with 100 ml distilled water containing 0, 1, 5, 10, 15 or 25 g of commercial tannic acid. They used three ruminally cannulated ewes to determine *in situ* degradability of tannic acid-treated SBM in their experiment. They estimated intestinal digestibility of protein remaining after 16 h rumen incubation by *in vitro*. They found that the tannic acid treatment of SBMs had a negative effect on both DM and N disappearances from bags, this effect being clearly dependent on the incubation time and the dose of tannic acid used to treat the meals. Tannic acid treatment significantly decreased the rate of degradation ($P<0.001$) but only the three soya bean meals treated with the highest doses of tannic acid (10, 15 and 25%) showed significantly lower rates of degradation than the control meal, both for DM and N. While all doses of tannic acid used in their experiment decreased the extent of N degradation, only meals 10, 15 and 25% showed significantly lower values of the extent of DM degradation than the control.

Santos *et al* (2000) studied the effects of tannic acid on composition and ruminal degradability of bermudagrass and alfalfa silages they treated forage with 21% (vol/wt) tannic acid solution. They sprayed the tannic acid solution over the chopped forage with a final tannic acid solution to protein ratio of 1:10. They used two lactating Holstein multiparous cows, fitted with a ruminal cannula. They introduced duplicate bags over time to have incubation periods of 6, 9, 24, 28, 72, and 96 h. They reported that the percentage of CP solubilized at initiation was higher for the control alfalfa (38.5%) than the tannic acid treated alfalfa (24.9%) and similarly, it decreased when tannic acid was added to the bermudagrass silage from 45.3% to

37.7% in control and tannic acid bermudagrass silage. whereas, b fraction (the percentage of CP potentially degradable in the rumen) was increased on tannic acid addition in the silages. The fraction c (the constant rate (percentage per hour) of the disappearance of fraction b) was increased for the alfalfa silage on tannic acid addition whereas, it decreased for the bermudagrass silage.

Salawu *et al* (1999) studied the effects of tannins as silage additives. They studied the total tract disappearance of DM of the silages in Friesian dairy cows using the mobile bag technique and found that the silage containing tannins had lower ($P<0.05$) DM disappearance in the rumen than the control silage. Similarly, the proportion of rumen undegradable DM which was lost in the lower tract was higher ($P<0.05$) in silages containing tannins (19.6%). They reported treatment with tannins reduced the soluble nitrogen (SN) and ammonia content of the silages.

Makkar *et al* (1988) studied the effect of oak leaves (*Quercus incana*) on various microbial enzyme activities of the bovine rumen. They used a Jersey bullock (150 kg) with a permanent rumen cannula for the nylon bag (150 x 90 mm, 35 (SE 5) pm pore size) incubations. They dried and ground leaves of oak, khirk (*Celtis australis*) and bamboo (*Dendrocalamus hamiltonii*) through a 1 mm screen and incubated in nylon bags in the rumen for 48 h. They selected *C. australis* because the chemical composition of its leaves was similar to that of *Q. incana* leaves except for tannins and ash. They found that the urease and carboxymethylcellulase activities were significantly lower for the *Q. incana* group than the *C. australis*. Protease activity was significantly lower for *Q. incana* leaves. Aspartate ammonia-lyase and glutaminase activities were not detectable. Both NADH and NADPH-linked glutamate dehydrogenase activities were significantly lower for the *Q. incana* group. The differences for other ammonia-assimilation enzymes, i.e. glutamate ammonia ligase and alanine aminotransferase were also significant. The activities of glutamate ammonia ligase were significantly higher for the tannin-rich group, unlike the other enzymes. The activities of alanine dehydrogenase and aspartate dehydrogenase (NADH-linked) could not be detected. With the addition of tannin-rich plant protein: RNA ratio decreased significantly; however, there was no difference in protein: DNA ratio. A significant decrease was also observed in microbial protein for the *Q. incana* group. However, the effects of some other constituents of the leaves on these

activities could not be ruled out. Significant differences were observed for urease, glutamate dehydrogenase (NADH-linked), glutamate ammonia ligase (both y-glutamyl transferase and forward reaction) and alanine aminotransferase. The activities of urease, protease, glutamate dehydrogenase (both NADH- and NADPH-linked) and alanine aminotransferase were inhibited increasingly with increasing quantities of the extract of *Q. incana* leaves in the assay mixture. The amount of extract required to inhibit aspartate aminotransferase was higher. Glutamine synthetase (forward reaction) was activated by up to 200 pl of the extract in the assay mixture and above this level, the activation was found to decrease. They obtained a similar trend for the glutamyl transferase reaction of glutamate ammonia ligase.

Waghorn *et al* (1994) studied the effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. They evaluated its effect on digestion by giving an intraruminal infusion of polyethylene glycol (PEG) to six of the sheep (PEG group). They reported that the digestibility of lotus DM was 68%, and the digestibility of fibre was not affected by CT. Infusion of PEG increased rumen concentrations of NH_3 and volatile fatty acids ($P < 0.001$). They reported a reduction in the feed intake by 12% than those of the PEG sheep at the end of the trial ($P < 005$). Growth rate and wool production were similar for sheep on both treatments.

CHAPTER – III

MATERIALS AND METHODS

The present study was conducted in the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. A brief description of the experimental techniques and procedures of analysis adopted during the study are reported in this chapter.

Quebracho ATO powder (*Schinopsis spp.*) (Argentinian Origin, ATLAS Quebracho Tannino Industries) containing tannins was procured from Krishna Enterprises, Chennai. Tannic Acid of AR grade (LOBA Chemie) was procured from Global Scientific Traders, Ludhiana.

3.1 Treatment of Soybean meal

Soybean meal was treated with different levels of tannins with 1, 2, 3 and 4% with two different tannin sources i.e. quebracho and tannic acid. 1% quebracho treated SBM was made by diluting 1 gm of quebracho in 100 ml of distilled water and then mixing 100 gms of ground SBM with the quebracho solution. Then treated SBM was kept overnight by closing lead and then on the next day it was dried at 70^{o} C in a hot air oven and then treated SBM was finely ground and stored. Likewise, for each level of tannin source was weighed and mixed with SBM as mentioned above. Along with tannin treatment, one sample of soybean meal was treated with distilled water without tannin for the control soybean meal to nullify the effect of water. Total nine types of soybean meals were obtained by different treatments which are enlisted in Table 1.

After treating SBM, different concentrate mixtures were made by replacing untreated SBM in basal concentrate mixture with different levels of tannin treated SBM, which were made by the earlier mentioned method. For each level of tannin treatment, two concentrate mixtures were made by replacing either 25% (lower level - L) or 50% (higher level - H) treated SBM.

Table 1: Treatment of SBM with different levels and sources of tannins

Treatment	Abbreviation
Soybean meal	SBM
water treated Soybean meal	W-SBM
1% Quebracho treated Soybean meal	1QSBM
2% Quebracho treated Soybean meal	2QSBM
3% Quebracho treated Soybean meal	3QSBM
4% Quebracho treated Soybean meal	4QSBM
1% Tannic Acid treated Soybean meal	1TSBM
2% Tannic Acid treated Soybean meal	2TSBM
3% Tannic Acid treated Soybean meal	3TSBM
4% Tannic Acid treated Soybean meal	4TSBM

3.2 Preparation of Total Mixed Rations (TMR) and analysis

The control concentrate mixture was prepared with maize 35, wheat bran 12, de-oiled rice bran 14, rice polish 12, soybean meal 25, mineral mixture 2, urea 1, and salt 1% (Table-2). The ingredient composition of the formulated concentrate mixtures for *in vitro* analysis is given in Table 2.

Table 2: Ingredient composition of the concentrate mixtures

Ingredients	Control	Treatment- L	Treatment- H
Maize	35	35	35
Wheat Bran	12	12	12
De-oiled rice polish	14	14	14
Rice polish	12	12	12
SBM	25	18.75	12.5
Treated SBM	0	6.25	12.5
Mineral mixture	2	2	2
Salt	1	1	1
Urea	1	1	1

Total sixteen concentrate mixtures were prepared by replacing SBM with treated SBM either 25% (lower level - L) or 50% (higher level - H) for each treatment. The ingredient composition of concentrate mixtures was similar for the different level and source of tannins just with the different level of replacement with tannin treated SBM. So, the total seventeen concentrate mixtures were made including control which are as mentioned in Table 3.

Table 3: Concentrate mixtures (CM) prepared and their abbreviations

Concentrate mixture	**Abbreviation**
Control CM	CONC
CM containing 25% SBM replaced with 1% quebracho treated SBM	1QCL
CM containing 50% SBM replaced with 1% quebracho treated SBM	1QCH
CM containing 25% SBM replaced with 2% quebracho treated SBM	2QCL
CM containing 50% SBM replaced with 2% quebracho treated SBM	2QCH
CM containing 25% SBM replaced with 3% quebracho treated SBM	3QCL
CM containing 50% SBM replaced with 3% quebracho treated SBM	3QCH
CM containing 25% SBM replaced with 4% quebracho treated SBM	4QCL
CM containing 50% SBM replaced with 4% quebracho treated SBM	4QCH
CM containing 25% SBM replaced with 1% Tannic acid treated SBM	1TCL
CM containing 50% SBM replaced with 1% Tannic acid treated SBM	1TCH
CM containing 25% SBM replaced with 2% Tannic acid treated SBM	2TCL
CM containing 50% SBM replaced with 2% Tannic acid -treated SBM	2TCH
CM containing 50% SBM replaced with 3% Tannic acid treated SBM	3TCL
CM containing 50% SBM replaced with 3% Tannic acid treated SBM	3TCH
CM containing 50% SBM replaced with 4% Tannic acid treated SBM	4TCL
CM containing 50% SBM replaced with 4% Tannic acid treated SBM	4TCH

Total mixed rations (TMRs) were made with dry roughage, non-leguminous silage and concentrate in the proportion of 10:50:40. For each level and source of tannin, TMRs were made with respective concentrate mixture. So total 17 TMRs were made which are mentioned in Table 4.

Table 4: Types of TMRs and their abbreviations

Total mixed ration	**Abbreviation**
TMR containing CONC	COT
TMR containing 1QCL	1QRL
TMR containing 1QCH	1QRH
TMR containing 2QCL	2QRL
TMR containing 2QCH	2QRH
TMR containing 3QCL	3QRL
TMR containing 3QCH	3QRH
TMR containing 4QCL	4QRL
TMR containing 4QCH	4QRH
TMR containing 1TCL	1TRL
TMR containing 1TCH	1TRH
TMR containing 2TCL	2TRL
TMR containing 2TCH	2TRH
TMR containing 3TCL	3TRL
TMR containing 3TCH	3TRH
TMR containing 4TCL	4TRL
TMR containing 4TCH	4TRH

On the basis of replacement level seventeen Total Mixed Rations were divided in to three categories same as concentrate mixture. In Treatment – L, 25% SBM is replaced with treated SBM whereas, in Treatment – H, 50% SBM is replaced with treated SBM. Final ingredient composition of the formulated TMR for *in vitro* analysis is mentioned in Table 5.

Table 5: Ingredient composition of the TMR prepared

Ingredients	**Control**	**Treatment- L**	**Treatment- H**
Maize	14	14	14
Wheat Bran	4.8	4.8	4.8
De-oiled rice polish	5.6	5.6	5.6
Rice polish	4.8	4.8	4.8
SBM	10	7.5	5
Treated SBM	0	2.5	5
Mineral mixture	0.8	0.8	0.8
Salt	0.4	0.4	0.4
Urea	0.4	0.4	0.4
Non leguminous silage	50	50	50
Wheat straw	10	10	10

The finely ground complete feeds were analyzed for proximate and cell wall constituents as mentioned below.

3.3 Proximate principles

3.3.1 Dry matter

A known quantity of the well-mixed sample was taken in an aluminium tray and dried in an oven at 100°C for 24 h. The loss in weight of the sample was taken as the dry matter content of the sample.

DM% = 100- loss in weight

3.3.2 Total ash

Finely ground sample (2 gm) was taken in duplicate and charred in a silica crucible over a hot plate and then ignited in a muffle furnace for 3 hours at 700°C. The crucibles were cooled in a desiccator and weighed. The difference between the

initial weight of empty crucible and final weight of crucible with ash gave the total ash content in the sample and was expressed as percent of DM (AOAC 2000). The loss in weight after ignition in muffle furnace was taken as organic matter.

Total ash% = Final weight (crucible+ ash) – Empty weight of crucible

3.3.3 Organic matter

It was calculated by subtracting the total ash content from DM of samples (AOAC 2000).

OM% = DM of sample – total ash

3.3.4 Crude protein

The N content was estimated by Macro-Kjeldahl method (AOAC 2000). Finely ground sample (0.25 g) or 5 g of fresh feaces was digested with 12 ml (25 ml for feaces) of concentrated sulphuric acid and (5-6 g of digestion mixture Potassium sulphate and copper sulphate, 9:1). The material after digestion was distilled in the presence of 60 ml of 40% sodium hydroxide. The ammonia liberated was collected in 20 ml of 4% of boric acid-mixed indicator solution. The mixed indicator was prepared by taking Bromocresol green and methyl red in 5:1 ratio in 95% ethanol. Ammonium borate thus formed was titrated against standard (0.1 N) sulphuric acid. The reading for blank was also recorded.

$$N\ (\%) = \frac{(\text{ml } H_2SO_4 \text{for sample} - \text{ml } H_2SO_4 \text{ for blank}) \times \text{Normality of acid} \times 0.014 \times 100}{\text{Wt. of sample (gm)}}$$

The crude protein of the sample was calculated as:

$$CP\ (\%) = N\ (\%) \times 6.25$$

3.3.5 Ether Extract

Ether Extract was estimated according to (AOAC 2000). About 3 g of the ground sample was weighed and quantitatively transferred into a thimble made of Whatman filter paper No.1. The sample with thimble was placed in the extraction beaker of SOCS PLUS® (M/S Pelican Equipments) six place automatic solvent extraction systems. The extraction was carried out for 2 hours with 80 ml petroleum ether (b.p

60- 80°C). After completion of the extraction process, the beaker was dried in the hot air oven at 100°C.

$$\text{EE (\%)} = \frac{\text{Wt. of beaker with ether extract — Wt. of empty beaker}}{\text{Wt. of sample (gm)}} \times 100$$

3.3.6 Cell wall constituents

3.3.6.1 Neutral detergent fibre (NDF)

The NDF solution was prepared by dissolving 37.22g disodium salt of EDTA and 13.62 g sodium borate in 700 ml of distilled water. In another beaker 9.58g, disodium hydrogen orthophosphate was dissolved in 100ml distilled water. In another beaker, 60 g sodium lauryl sulphate and 20 ml of ethylene glycol or 2-ethoxy ethanol were dissolved in 850 ml distilled water. The content of all the beakers was mixed and finally, the volume was made to two litres.

Finely ground 0.5 g sample was taken in a spoutless beaker and 50 ml of neutral detergent solution (NDS) was added. The sample with NDS was brought to boiling and refluxed for one hour. The contents were filtered through pre-weighed sintered glass crucible (G-1) and washed with hot distilled water, till free from NDS, followed by two washing with acetone. The residue was dried at 90°C in a hot air oven overnight. The NDF was calculated using the formula (Van Soest *et al* 1991)-

$$\text{NDF (\%)} = \frac{\text{(Wt. of crucible + cell wall constituent) - (Wt. of empty crucible)}}{\text{Wt. of the samples on DM basis}} \times 100$$

3.3.6.2 Acid detergent fiber (ADF)

The sample (1.0 g) was transferred into a spoutless beaker and 100 ml of acid detergent solution was added (20 gm CTAB dissolved in one litre of 1N H_2SO_4). The contents were refluxed for 1 hr. The contents were filtered through previously weighed sintered glass crucibles (G-1) and washed with hot water till free from ADS followed by one washing of acetone. The residue was dried at 80°C in hot air over for overnight. The difference in the initial (empty crucible) and final (crucible + residue) weight of crucible gave ADF content. It was expressed as percent on a DM basis (Van Soest *et al* 1991).

$$\text{ADF (\%)} = \frac{\text{(Wt. of crucible +cell wall constituent) — (Wt. of empty crucible)}}{\text{Wt. of the sample on DM basis}} \times 100$$

3.3.6.3 Acid detergent lignin (ADL)

Sulphuric acid (72%, w/v) was added to the sintered crucibles containing ADF and kept at room temperature for 3 h. After draining the acid, the residue was washed with water till it became acid free, dried in a hot air oven for overnight and then weighed. It was then ignited in a muffle furnace at 500°C for 3 hrs. The loss in weight upon ignition represented ADL, which was expressed as % on DM basis (Van Soest *et al* 1991).

3.3.6.4 Hemicellulose

Hemicellulose was soluble in ADS and thereby calculated by subtraction of ADF from NDF as follows:

Hemicellulose (%) = NDF (%) – ADF (%)

3.3.6.5 Cellulose

Cellulose is soluble in 72% and thereby calculated by subtraction of ADL from ADF as follows:

Cellulose (%) = ADF (%) – ADL (%)

3.3.6.6 Total carbohydrates (TCHO)

It was done according to Sniffen *et al* (1992). Total carbohydrates in the feedstuff are estimated by difference:

TCHO (%) = 100 – (CP% + Ash% + EE%)

3.4 *In vitro* gas production study

The *in vitro* gas production was assessed according to Menke and Steingass (1988). For this, 375 ± 5mg of the sample was weighed in a weighing boat (with removable stem) and the sample was put at the bottom of the 100 ml calibrated glass syringe taking caution that it should not stick to the walls of the syringe. Then the piston, greased with petroleum jelly (Vaseline) was pushed into the cylinder. The syringes containing the sample in triplicate were kept in a water bath at 39°C for 24h.

3.4.1 Collection of rumen liquor

Rumen fistulated male buffaloes (maintained on 2 kg conventional concentrate mixture, 2 kg green and *ad-lib* wheat straw) were used as a donor for rumen liquor. The rumen contents were collected at 0 h in double-walled (Thermos) flask flushed

with CO_2 and maintained at 39°C. The rumen contents were blended for 2-3 min. in a blender, maintained at 39°C and then strained through 4 layered muslin cloth.

3.4.2 Preparation of Solutions

Following solutions were prepared well in advance**:-**

i. Micro mineral solution

$CaCl_2.2H_2O$ – 13.2 g

$MnCl_2.4H_2O$ – 10.2 g

$CoCl_2.6H_2O$ – 1 g

$FeCl_3.6H_2O$ – 8 g

Dissolved in distilled water and made the volume 100 ml.

ii. Macro-mineral solution

Na_2HPO_4 – 5.7 g

KH_2PO_4 – 6.2 g

$MgSO_4.7H_2O$ – 0.6 g

Dissolved in distilled water and made the volume 1000 ml.

iii. Buffer solution

$NaHCO_3$ – 35.0 g

NH_4HCO_3 – 4.0 g

Dissolved in distilled water and made volume 1000 ml.

iv. Resazurin solution: Dissolved 100 mg of resazurin in distilled water and made volume 100 ml and kept in a refrigerator.

v. Reducing solution (This solution was prepared fresh before each incubation).

$Na_2S.H_2O$ – 373.0 mg

1N NaOH – 2.6 ml

Distilled water – 62.0 ml

3.4.3 Procedure

The above solutions were mixed in the following ratio in a Woulff flask (3-litre capacity) mixed with a magnetic stirrer in a water bath at 39°C.

i. Distilled water – 960 ml

ii. Micro mineral solution – 0.16 ml

iii. Buffer – 660 ml

iv. Macro mineral solution – 330 ml

v. Resazurin – 1.6 ml

vi. Reducing solution – 50 ml

Reducing solution was prepared fresh and all solutions were mixed in above-mentioned quantity and sequence. While the reducing solution was added, CO_2 was flushed through a submerged tube. The slightly bluish colour first turned pinkish then became colourless. The rumen fluid was added to the buffer media in a 1:2 ratio only when the solution was colourless. The flushing of CO_2 was continued till the last syringe was filled.

For filling up of syringes, the tube on the capillary attachment to the syringe was firmly fixed on to the bottle top dispenser. Thirty ml of SRL (strained rumen liquor): buffer solution from the flask kept in a water bath was pumped in each syringe. The contents in syringe were mixed by gentle shaking. Air bubbles were brought to the surface and removed through the capillary by careful upward movement of the piston. The clip was closed immediately and the exact volume of the contents in the syringe was noted and kept in a water bath maintained at 39°C. The contents in all the syringes were swirled at 1 h interval for first few h. If at 8h the gas exceeded 70 ml, the volume of gas was recorded and gas was removed. After 24 h volume of gas produced in each syringe was recorded. Blanks and standard hay in triplicate were also run with each set of incubation. After the stipulated time, the contents were taken out and centrifuged. The amount of gas produced was used to calculate the ME value. After 24 h the NH_3-N, OM and NDF of residue were determined. The partitioning factor (PF) defined as the ratio of substrate truly degraded *in vitro* (mg) to the volume of gas (ml) produced by it. (France *et al* 1993).

The incubations were conducted in triplicate for each sample on 2 successive days and these incubations were repeated three times, with an interval of 1 wk. Incubations without sample served as the blanks with every set. The difference in the composition and activity of the rumen inoculum among incubations was controlled by parallel incubation of reference standard feedstuffs as suggested by Menke *et al* (1979). The metabolizable energy (ME) was calculated using the equation suggested by Menke *et al* (1979).

3.4.4 *In vitro* kinetics

For the 375 ± 5mg of the sample was weighed in a weighing boat (with removable stem) and the sample was put at the bottom of the 100 ml calibrated glass syringe taking caution that it should not stick to the walls of a syringe. Then the piston, greased with petroleum jelly (Vaseline) was pushed into the cylinder. The syringes containing the sample in triplicate for each incubation time were kept in a water bath at 39°C. Sample preparation, rumen liquor Collection and solution preparation remained the same as mentioned in 3.5.1, 3.5.2 and 3.5.3, respectively. Each sample was incubated in the 24 syringes and that incubated for 0, 2, 6, 8, 10, 12 and 24 hours. At the desired time of terminating incubations, readings of gas were recorded and the contents were used for the estimation of NH_3-N, OM and NDF of residue. (Raab *et al* (1983))

Kinetics of gas production gas (Y) at a time (t) was fitted to the exponential model of Orskov and McDonald (1979) as follows:

$$\text{Gas (Y)} = a + b\,(1\text{-}\exp^{-ct})$$

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.

3.4.5 *In vitro* true OM digestibility: True OM digestibility (TOMD %)

The content of syringes was transferred to spoutless beaker by repeated washing with 40 ml neutral detergent solution. The flask content was refluxed for one h and filtered through pre-weighed sintered crucibles (grade G1). The dry matter content of the residue was weighed and *in vitro* true digestibility of feeds was calculated (Van Soest and Robertson 1988).

$$\text{TOMD\%} = \frac{\text{Initial OM of feed taken for incubation} - \text{OM residue}}{\text{Initial OM of feed taken for incubation}} \times 100$$

3.4.6 ME Availability

The ME value of the sole ingredient and of TMR was calculated by using the

following equation developed by (Menke *et al* 1979).

ME (MJ/kg DM) = 1.24 + 0.146 G (ml/200 mg DM) + 0.007 CP + 0.0244 EE

Where,

ME = Metabolisable energy

G =Net gas production, ml/200mg DM

CP = Crude protein of sample, g/kg DM

EE = Ether extract of sample, g/kg DM

The ME value of the sole ingredient and of TMR was calculated by using the following equation developed by (Menke *et al* 1979)

ME (MJ/kg DM) = 1.06 + 0.157 G (ml/200 mg DM) + 0.0084 CP + 0.022 EE – 0.0081 Ash

Where,

ME = Metabolisable energy

G = Net gas production, ml/200mg DM

CP = Crude protein of sample, g/kg DM

EE = Ether extract of sample, g/kg DM

Ash = Ash of sample, g/kg DM

3.4.7 Ammonia nitrogen

The centrifuged content (5 ml) from syringes (after 24 hrs of incubation) was taken in one litre boiling flask. Added 250 ml of water and distilled in the presence of 2 ml of 1N NaOH. The ammonia liberated was collected in 20 ml of 4 % boric acid solution containing mixed indicator (same as in case of nitrogen estimation). Ammonia borate thus formed was titrated against standard (0.1 N) sulphuric acid. The % ammonia nitrogen was calculated as follows after subtracting the blank (AOAC 2000).

NH_3-N (%) = ((Vol. of acid used × Normality of acid × 0.014)/SRL, ml) × 100

3.5 Tannins

3.5.1 Extraction of tannins

Tannins were estimated according to (IAEA 2000). The fat and pigments were removed by extracting the ground samples with petroleum ether containing 1% acetic acid using Soxhlet apparatus (AOAC 1995). Tannins were extracted, from fat-free

samples, using 70 per cent aqueous acetone. The contents were centrifuged (3,000 g at 4°C for 10 min) and the supernatant was taken for determination of tannins.

3.5.2 Total phenols

Total phenols were estimated using Folin-Ciocalteu reagent **(**Makkar *et al* 1993) using tannic acid as a standard.

3.5.3 Simple phenols

Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry. The reaction forms a blue chromophore constituted by a phosphotungstic phosphomolybdenum complex, where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds.

Reagents

i. *Folin Ciocalteu reagent (1 N):* Dilute commercially available Folin-Ciocalteu reagent (2 N) with an equal volume of distilled water. Transfer it in a brown bottle and store in a refrigerator (4°C). It should be golden in colour. Do not use it if it turns olive green.
ii. *Sodium carbonate (20%):* Weigh 40 g sodium carbonate (x10 H2O), dissolve it in about 150 ml distilled water and makeup to 200 ml with distilled water.
iii. *Insoluble polyvinyl pyrrolidone (polyvinyl polypyrrolidone, PVPP):* This is commercially available from Sigma (P 6755).
iv. *Standard tannic acid solution (0.1 mg/ml):* Dissolve 25 mg tannic acid (TA) obtained from Merck in 25 ml distilled water and then dilute 1:10 in distilled water (always use a freshly prepared solution).

Procedure: Suitable aliquots of the tannin-containing extract was taken (initially try 0.02, 0.05 and 0.1 ml) in test tubes and the volume was made up to 0.5 ml with distilled water then 0.25 ml of the Folin- Ciocalteu reagent was added and then 1.25 ml of the sodium carbonate solution. Vortex the tubes and absorbance was recorded at 725 nm after 40 min. The amount of total phenols as tannic acid equivalent was calculated from the calibration curve. The total phenolic content was expressed on a dry matter basis (x%).

3.5.4 Determination of condensed tannins

The butanol/ HCl hydrolysis method depolymerizes condensed tannin (proanthocyanidins; a subset of total phenols) to yield a red anthocyanidin product.

Proanthocyanidins depolymerize when treated in hot mineral acid to produce coloured anthocyanidins with an absorbance maximum around 550 nm. Iron sulfate was added to the reagent to accelerate the autoxidation reaction that increases the color intensity and consistency of anthocyanidin yield. (Porter *et al* 1986)

Reagents

i. *Butanol-HCl reagent* (butanol-HCI 95:5 v/v): Mix 950 ml n-butanol with 50 ml concentrated HCl (37%).

ii. *Ferric reagent* (2% ferric ammonium sulfate in 2N HCl): Make 16.6 ml of concentrated HCl up to 100 ml with distilled water to make 2N HCl. Dissolve 2.0 g ferric ammonium sulfate in this volume of 2N HCl. This reagent should be stored in a dark bottle.

Procedure: In a 100 mm x 12 mm glass test tube, pipette 0.50 ml of the tannin extract diluted with 70% acetone. The quantity of acetone should be large enough to prevent the absorbance (550 nm) in the assay from exceeding 0.6. It will depend on the quantity of condensed tannin expected in the sample and occasionally will need to be determined by trial and error. To the tubes add 3.0 ml of the butanol-HCl reagent and 0.1 ml of the ferric reagent. Vortex the tubes. Cover the mouth of each tube with a glass marble and put the tubes in a heating block adjusted at 97 to 100°C (or in a boiling water bath) for 60 min. Cool the tubes and record absorbance at 550 nm. Subtract a suitable blank, which is usually the absorbance of the unheated mixture. If the extract has flavan-4-ols, a pink colour develops without heating. If this happens, use one heated blank for each sample, comprising 0.5 ml of the extract, 3 ml of butanol and 0.1 ml of the ferric reagent. Condensed tannins (% in dry matter) as leucocyanidin equivalent was calculated by the formula:

$$(A\ 550\ nm \times 78.26 \times \text{Dilution factor*}) / (\%\ \text{dry matter})$$

This formula assumes that the effective El%, 1 cm, 550 nm of leucocyanidin is 460.

3.6 Criteria to select tannin source and level of tannin for the in vivo evaluation:

Results of ammonical nitrogen, gas production, PF and EMMP was taken as selection criteria from SBM and TMR *in vitro* degradation. Degradation rate and half–time from TMR *in vitro* kinetics was also used to select level and source of tannin for in vivo evaluation.

3.7 Growth trial on buffalo heifers

A growth trial of 90 days was conducted on 7-13 month old growing buffalo heifers (Average live weight 169- 175 kg) to see the effect of tannin protected protein on weight gain, nutrient digestibility and feed conversion ratio. Ingredient composition of concentrate mixture and of TMR for both groups is given in Table 3 and Table 5, respectively. The growing buffaloes were fed total mixed ration with dry roughage, non-leguminous silage and concentrate in the proportion of 10:50:40.

3.7.1 Selection, distribution and maintenance of animals

Sixteen buffalo heifers were randomly distributed into two groups of eight animals each. Animals were kept at a dairy farm, Guru Angad Dev Veterinary and Animal Sciences University. The animals were provided with *ad lib* clean drinking water along with feed as per the requirement during an experiment. The animals were kept healthy and disease-free by regular vaccination and deworming as per dairy farm schedule. The animals and shed were cleaned regularly and thoroughly washed with water to maintain hygiene.

The animals in control group were fed with basal diet and animals in experimental groups were fed 50% tannin treated soybean meal with 1% tannic acid replaced in the basal diet (selected from *in vitro* evaluation). The daily record of feed intake and residue were maintained. The bodyweight of the animals was recorded at monthly interval. Weight of animals was taken in the morning hours before feeding and watering on error-free weighing balance. The recorded body weight was further used for calculation of different growth indices like average daily gain (ADG) and

Feed conversion ratio (FCR) as per the following formulas:

$$\text{ADG (g)} = \frac{\text{Bodyweight gain (kg)}}{\text{Interval between two BW recordings (days)}} \times 1000$$

$$\text{FCR} = \frac{\text{ADG}}{\text{Daily DMI}}$$

3.7.2 Digestion trial

After 30 days of growth experiment, a digestion trial of 6 days was conducted. Five average weighed heifers from each group were selected and thoroughly washed. They were tied in well ventilated shed by keeping enough distance for ensuring individual feeding during digestion trial and faeces was collected in separate

containers. The faeces and residue were weighed daily at 9.00 a.m. before feeding.

3.7.3 Sampling of feed, residue, faeces and urine

Samples of feedstuffs and residue were collected at 24 h interval and dried in duplicate at 70^{o} C in a hot air oven. The samples were pooled for 7 days, finely ground to pass through 1mm sieve and analyzed for proximate and cell wall constituents.

The feces excreted by each animal were weighed after 24 hours, the thoroughly mixed and exact amount of feces was weighed in an aluminium tray and dried at 70°C in a hot air oven for 48 hours. The six days dried feces of each animal were pooled and finely ground to pass through 1 mm sieve and preserved in an airtight plastic bag.

For nitrogen estimation 20g of fresh 24 hours feces was preserved in wide-mouth plastic bottles containing 25 ml of 20% sulphuric acid. The next day's collection was added to the same bottle and mixed to prevent ammonia loss and possible infestation.

Samples of feed, faeces and residue were analyzed for DM, total ash, CP, EE (AOAC 2000) and cell wall constituents (Van Soest *et al* 1991).

3.8 Blood biochemical profile

To study the effect of tannin treated SBM feeding on nutrient (carbohydrates, fat and protein) metabolism, monitoring of blood parameters was carried out. Blood samples were drawn from buffaloes (4 hrs post-feeding) by puncturing jugular vein at the starting as well at the end of experimental feeding. A blood sample was collected in heparinized tubes and plasma was separated by the centrifugation. Centrifugation was done at 3000 rpm for 15 minutes and plasma was stored in different tubes.

3.8.1 Collection of blood, plasma separation and preservation

Plasma was preserved at -20^{o}C to analyze the following parameters

1. Glucose
2. Blood urea nitrogen
3. Total protein
4. Albumin
5. Triglycerides

6. Cholesterol
7. Alanine transaminase (ALT) / Serum glutamic pyruvic transaminase (SGPT)
8. Creatinine
9. Gamma Glutamyl Transferase (GGT)
10. Alkaline Phosphatase (ALP)

3.8.1.1 Blood glucose

It was estimated by glucose oxidase /peroxidase method Trinder (1969) by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd. To 10µl distilled water, standard and plasma, 1ml of reagent was added. Mixed and incubated for 10 minutes at 37°C. Concentration was noted by using Global 240 analyzer at 520 nm.

3.8.1.2 Blood urea nitrogen

It was estimated by urease method Talke and Schubert (1965) by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd. To 10µl distilled water, standard and plasma, 1ml of reagent was added. The contents were mixed thoroughly and concentration was noted by Global 240 analyzer at 340 nm.

3.8.1.3 Total protein

It was estimated by the Biuret method (Henry *et al* 1974) by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd. To 50µl of distilled water, standard and plasma, 1ml of reagent were added. The contents were mixed and incubated at 37°C for 10 minutes. Concentration was noted by using Global 240 analyzer at 555 nm.

3.8.1.4 Albumin

It was estimated by Bromocresol green (BCG) dye-binding method (Doumas *et al* 1971) by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd. To 10µl distilled water, standard and plasma, 1ml of reagent was added. The contents were mixed at 37°C. Concentration was noted by using Global 240 analyzer at 630 nm.

3.8.1.5 Triglycerides

It was estimated by enzymatic colourimetric method Bucolo and David (1973) by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd. To 10µl distilled water, standard and plasma, 1ml of reagent was added. The contents were

mixed thoroughly and kept for 10 minutes at 37°C. Concentration was noted by using Global 240 analyzer at 500 nm.

3.8.1.6 Cholesterol

It was estimated by (Roeschlau *et al* 1974) using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd. To 10µl distilled water, standard and plasma, 1ml of reagent was added. The contents were mixed thoroughly and kept for 10 minutes at 37°C. Concentration was noted by using Global 240 analyzer at 505 nm.

3.8.1.7 ALT

It was estimated by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd based on the International Federation of Clinical Chemistry (IFCC). To 100 µl of plasma 1ml of reagent was added and mixed thoroughly and allowed to stand for 60 sec at 37°C. Concentration was noted by using Global 240 analyzer at 340 nm.

3.8.1.8 Creatinine

It was estimated by the picrate method (Hawk *et al* 1976) with the diagnostic kit supplied by Transasia-Biomedicals Ltd. To 100 µl sample (urine/plasma) and standard, 1ml of reagent (alkaline picrate prepared as per protocol supplied) was added. The contents were mixed thoroughly and read immediately, using distilled water as blank. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 500 nm.

3.8.1.9 Gamma-Glutamyl Transferase (GGT)

GGT catalyzes the transfer of the gamma-glutamyl group from the donor substrate (L-gamma-glutamyl-3-carboxy-4-nitroanilide) to the glycylglycine acceptor to yield 3-carboxy-4-nitroaniline. It was estimated by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd based on the International Federation of Clinical Chemistry (IFCC). To 100 µl of plasma 1000 µl of reagent was added and mixed thoroughly and allowed to stand for 30 sec at 37°C. Concentration was noted by using Global 240 analyzer at 405 nm.

3.8.1.10 Alkaline Phosphatase (ALP)

It was estimated by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd based on the International Federation of Clinical Chemistry (IFCC). To 20 µl of plasma 1000 µl of reagent was added and mixed thoroughly and

allowed to stand for 60 sec at 37°C. Concentration was noted by using Global 240 analyzer at 405 nm.

3.9 Statistical analysis

Data were analysed by simple ANOVA, as described by Snedecor and Cochran (1994), by using SPSS (2012) version 21. The differences in means were tested by Duncan's multiple range test.

CHAPTER-IV

RESULTS AND DISCUSSION

4.1 Chemical composition

4.1.1 Chemical composition of tannin treated SBM

The chemical composition of quebracho treated SBMs used in the experiment is given in Table 6. The OM was varying between 90.20 to 91.28%. The CP content of SBM was 44.81% whereas, SBM-W, 1QSBM, 2QSBM, 3QSBM and 4QSBM had 46.72, 47.44, 47.56, 46.10 and 45.52% CP, respectively. The ether extract content of SBM was 1.88% and it was varying in between 1.68 to 2.15%. The NDF content in SBM was 18.3% which was lower than the quebracho treated SBMs and it was 19.45, 19.10, 19.10 20.40 and 25.50% in SBM-W, 1QSBM, 2QSBM, 3QSBM and 4QSBM respectively. So, lower total DMI can be predicted of quebracho treated SBM compared to untreated SBM. The hemicellulose in SBM was 5.95% and it was lower than the quebracho treated SBM. The cellulose content was varying in between 8.55 to 10.95.

Table 6. Chemical composition of Quebracho treated SBM (% DM basis)

Parameters	SBM	SBM-W	1QSBM	2QSBM	3QSBM	4QSBM
OM	90.20	90.82	90.43	90.80	91.28	91.10
CP	44.81	46.72	47.44	47.56	46.10	45.52
EE	1.88	2.15	2.15	2.05	1.83	1.68
Total ash	9.8	9.18	9.57	9.20	8.72	8.90
NDF	18.3	19.45	19.10	19.10	20.40	25.50
ADF	12.35	11.15	10.60	10.05	11.65	11.05
Hemicellulose	5.95	8.30	8.50	9.05	8.75	14.45
ADL	1.40	2.40	1.35	1.50	1.35	1.80
Cellulose	10.95	8.75	9.25	8.55	10.30	9.25
TCHO	30.22	35.61	26.68	27.85	31.33	33.57
NFC	11.92	16.16	7.58	8.75	10.93	8.07

The chemical composition of tannic acid treated SBMs used in the experiment is given in Table 7. The OM was varying between 90.20 to 92.05%. The CP content of SBM was 44.81% whereas SBM-W, 1TSBM, 2TSBM, 3TSBM and 4TSBM had 46.72, 43.67, 44.15, 44.18 and 43.13% CP, respectively. The ether extract content was varying between 1.33 and 2.15. The NDF content in SBM was 18.3% which was lower than the tannic acid treated SBMs and it was 19.45, 24.40, 26.10, 24.20 and 26.40% in SBM-W, 1TSBM, 2TSBM, 3TSBM and 4TSBM respectively. The total carbohydrates in SBM were 30.22% and it was lower than the treated SBMs except for 1TSBM. The non-fiber carbohydrates (NFC) content was higher in SBM (11.92%) as compared to tannic acid treated SBM.

Table 7. Chemical composition of tannic acid treated SBM, (% DM basis)

Parameters	SBM	SBM-W	1TSBM	2TSBM	3TSBM	4TSBM
OM	90.20	90.82	91.83	92.05	91.48	90.13
CP	44.81	46.72	43.67	44.15	44.18	43.13
EE	1.88	2.15	1.40	1.35	1.33	1.38
Total ash	9.8	9.18	8.17	7.95	8.52	9.87
NDF	18.3	19.45	24.40	26.10	24.20	26.40
ADF	12.35	11.15	10.95	10.25	11.75	12.55
Hemicellulose	5.95	8.30	13.45	15.85	12.45	13.85
ADL	1.40	2.40	2.00	1.85	2.00	1.15
Cellulose	10.95	8.75	8.95	8.40	9.75	11.40
TCHO	30.22	35.61	29.92	34.85	35.27	34.85
NFC	11.92	16.16	5.52	8.75	11.07	8.45

4.1.2 Chemical composition of concentrate mixtures containing different levels and source of tannins

The chemical composition of various concentrates with graded levels of quebracho and replacement level of treated SBM is given in Table 8. The OM of concentrates varied from 92.02 to 92.70%. The CP content of concentrates varied from 21.13 to 21.94 %. All the concentrate mixtures prepared were isonitrogenous.

Table 8. Chemical composition of concentrate mixtures containing quebracho treated SBM (CM, % DM basis)

Parameters	CONC	1QCL	1QCH	2QCL	2QCH	3QCL	3QCH	4QCL	4QCH
OM	92.21	92.70	92.31	92.68	92.42	92.02	92.64	92.06	92.44
CP	21.69	21.68	21.13	21.65	21.18	21.29	21.41	21.94	21.66
EE	3.68	3.66	3.52	3.43	3.54	3.78	3.57	3.78	3.90
Total ash	7.79	7.30	7.69	7.32	7.58	7.98	7.36	7.94	7.56
NDF	30.17	30.28	31.68	31.07	30.46	30.88	31.72	30.30	31.87
ADF	11.37	11.46	11.60	11.38	11.54	12.17	11.62	11.56	11.18
Hemicellulose	18.80	18.82	20.08	19.69	18.92	18.71	20.10	18.74	20.69
ADL	2.34	2.44	2.14	2.31	2.33	2.30	2.39	2.45	2.20
Cellulose	9.02	9.02	9.46	9.07	9.21	9.87	9.23	9.11	8.99
TCHO	66.84	67.36	67.66	67.60	67.70	66.95	67.66	66.35	66.87
NFC	36.67	37.08	35.98	36.53	37.24	36.07	35.94	36.05	35.00

Table 9. Chemical composition of concentrate mixtures containing tannic acid treated SBM (% DM basis)

Parameters	CONC	1TCL	1TCH	2TCL	2TCH	3TCL	3TCH	4TCL	4TCH
OM	92.21	92.09	92.72	92.70	92.68	92.32	92.45	92.57	92.29
CP	21.69	21.94	21.11	21.60	21.68	21.76	21.26	21.76	21.92
EE	3.68	3.86	3.86	3.48	3.28	3.56	3.73	3.54	3.45
Total ash	7.79	7.91	7.28	7.30	7.32	7.68	7.55	7.43	7.71
NDF	30.17	31.18	30.82	31.20	31.52	30.36	30.82	31.68	31.88
ADF	11.37	11.25	11.58	11.70	12.04	11.16	11.90	11.21	11.26
Hemicellulose	18.80	19.93	19.24	19.50	19.48	19.20	18.92	20.47	20.62
ADL	2.34	2.45	2.40	2.43	2.36	2.28	2.30	2.36	2.21
Cellulose	9.02	8.80	9.18	9.27	9.68	8.88	9.60	8.85	9.05
TCHO	66.84	66.29	67.75	67.62	67.72	67.00	67.46	67.27	66.92
NFC	36.67	35.11	36.93	36.42	36.20	36.64	36.64	35.59	35.04

The ether extract content of the concentrate mixtures was in the range of 3.43 to 3.90%. The total ash content of concentrates was in the range of 7.30 to 7.98%. The NDF content varied from 30.17 to 31.87%. The ADF content varied from 11.37 to 12.17%. The hemicellulose varied in the range from 18.71 to 20.69%. The ADL varied in the range from 2.14 to 2.45%. The cellulose varied in the range from 8.99 to 9.87%. The total carbohydrates (TCHO) in concentrate mixtures varied from 66.84 to 67.70%. The non-fiber carbohydrates (NFC) of the concentrate mixtures varied from 35.00 to 37.24%.

The chemical composition of various concentrates with graded levels of tannic acid and replacement level of treated SBM is given in Table 9. The OM of concentrates varied from 92.09 to 92.72%. The CP content of concentrates varied from 21.11 to 21.94 %. All the concentrate mixtures prepared were isonitrogenous. The ether extract content of the concentrate mixtures was in the range of 3.28 to 3.86%. The total ash content of concentrates was in the range of 7.28 to 7.91%. The NDF content varied from 30.17 to 31.88%. The ADF content varied from 11.21 to 12.04%. The hemicellulose varied in the range from 18.80 to 20.62%. The ADL varied in the range from 2.21 to 2.45%. The cellulose varied in the range from 8.80 to 9.68%. The total carbohydrates (TCHO) in concentrate mixtures varied from 66.29 to 67.75%. The non-fiber carbohydrates (NFC) of the concentrate mixtures varied from 35.04 to 36.93%.

4.1.3 Chemical composition of TMRs containing graded levels and source of tannins

The chemical composition of various TMRs with graded levels of quebracho and replacement level of treated SBM in its concentrate mixture is given in Table 10. The OM of TMR varied from 90.74 to 91.42%. The CP content of TMRs varied from 14.18 to 15.19%. The ether extract content of the TMR was in the range of 2.46 to 2.87%. The total ash content of TMRs was in the range of 8.58 to 9.26%. The NDF content varied from 51.0 to 55.6%. The ADF content varied from 29.06 to 32.52%. The hemicellulose varied in the range from 20.04 to 24.51%. The ADL varied in the range from 4.04 to 5.26%. The cellulose varied in the range from 25.02 to 28.06%. The total carbohydrates (TCHO) in TMR mixtures varied from 72.76 to 74.72%. The non-fiber carbohydrates (NFC) of the TMR mixtures varied from 18.84 to 22.89%.

Table 10. Chemical composition of TMRs containing quebracho treated SBM (% DM basis)

Parameters	COT	1QRL	1QRH	2QRL	2QRH	3QRL	3QRH	4QRL	4QRH
OM	91.20	91.35	91.41	91.42	91.33	90.83	90.98	90.74	91.11
CP	14.18	15.00	15.14	15.01	15.10	15.19	14.69	14.77	14.83
EE	2.53	2.46	2.81	2.67	2.50	2.87	2.73	2.53	2.56
Total ash	8.80	8.65	8.59	8.58	8.67	9.17	9.02	9.26	8.89
NDF	55.10	51.00	54.08	52.68	52.59	51.30	53.29	55.60	53.60
ADF	29.06	30.28	31.64	32.52	32.48	31.26	30.92	31.09	30.44
Hemicellulose	26.04	20.72	22.44	20.16	20.11	20.04	22.37	24.51	23.16
ADL	4.04	5.02	4.70	4.46	5.26	4.60	5.03	4.88	4.24
Cellulose	25.02	25.26	26.94	28.06	27.22	26.66	25.89	26.21	26.20
TCHO	74.48	73.89	73.46	73.74	73.73	72.76	73.55	74.44	74.72
NFC	19.38	22.89	19.38	21.06	21.14	21.46	20.26	18.84	21.12

Table 11. Chemical composition of TMRs containing tannic acid treated SBM (% DM basis)

Parameters	COT	1TRL	1TRH	2TRL	2TRH	3TRL	3TRH	4TRL	4TRH
OM	91.20	90.66	91.07	90.52	90.77	90.37	90.12	90.06	89.79
CP	14.18	14.63	14.50	14.95	14.48	14.76	14.56	14.68	14.64
EE	2.53	2.43	2.45	2.80	2.90	2.46	2.26	2.77	2.43
Total ash	8.80	9.34	8.93	9.48	9.23	9.63	9.88	9.94	10.21
NDF	55.10	53.90	56.60	52.78	55.38	53.37	52.59	52.89	54.37
ADF	29.06	31.74	32.10	31.54	31.46	32.28	30.88	31.72	31.68
Hemicellulose	26.04	22.16	24.50	21.24	23.92	21.09	21.71	21.17	22.69
ADL	4.04	4.81	5.05	4.59	4.72	4.38	5.08	4.56	4.70
Cellulose	25.02	26.93	27.05	26.95	26.74	27.90	25.80	27.16	26.98
TCHO	74.48	74.60	75.12	72.77	73.38	73.14	73.30	72.61	72.73
NFC	19.38	20.70	18.52	20.00	18.00	19.77	20.71	19.71	18.36

The chemical composition of various TMRs with graded levels of tannic acid and replacement level of treated SBM in its concentrate mixture is given in Table 11. The OM of TMR varied from 89.79 to 91.07%. The CP content of TMRs varied from 14.18 to 14.95%. The ether extract content of the TMR was in the range of 2.26 to 2.90%. The total ash content of TMRs was in the range of 8.93 to 10.21%. The NDF content varied from 52.59 to 56.6%. The ADF content varied from 30.88 to 32.28%. The hemicellulose varied in the range from 21.09 to 24.50%. The ADL varied in the range from 4.38 to 5.08%. The cellulose varied in the range from 25.02 to 27.90%. The total carbohydrates (TCHO) in TMR mixtures varied from 72.61 to 75.12%. The non-fiber carbohydrates (NFC) of the TMR mixtures varied from 18.0 to 20.71%.

The active composition of quebracho and tannic acid as well SBM used in the experiment is given in Table 12. Total phenol content of quebracho was found 20.92% whereas, it was 34.65% in tannic acid but was much lower in SBM that was 0.16. Condensed tannins were 45.70, 0.43 and 0.23% in quebracho, tannic acid and SBM, respectively.

Table 12. Tannin of tannic acid, Quebracho and SBM

Parameters	Quebracho	Tannic acid	SBM
Total ash	4.83	0.11	9.8
Total phenols	20.92	34.65	0.16
Condensed tannins	45.70	0.43	0.23

4.2 *In vitro* evaluation of Tannin treated SBM

4.2.1 *In vitro* gas production and digestibility of Quebracho treated SBM

Results of *in vitro* gas production and digestibility of Quebracho treated SBM is given in the Table 13. The NGP/200mg DM in the current study did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM. Changes in ruminal fibre digestion and gas production usually occur as a result of tannin-protein binding (Bueno *et al* 2015).

Table 13. *In vitro* gas production and digestibility of Quebracho treated SBM

Variable	SBM-W	1QSBM	2QSBM	3QSBM	4QSBM
NGP/200mg DM	38.00±0.15	37.73±0.0	37.91±0.08	37.56±1.18	37.91±0.24
NH_3-N, mg/dl	102.23±6.33	99.71±1.02	99.26±0.36	96.92±0.21	95.40±0.67
PF, mg/ml	4.64±0.03	4.649±0.0	4.65±0.01	4.69±0.0	4.63±0.02
NDFD, %	83.75^{a}±0.96	80.13^{ab}±1.05	76.96^{ab}±2.09	75.16^{ab}±5.23	72.77^{b}±0.69
MMP, mg	173.63^{b}±0.64	173.27^{b}±0.0	173.90^{b}±0.36	177.10^{a}±0.73	174.99^{b}±0.97
EMMP, %	52.55±0.33	52.68±0.0	52.71±0.16	53.12±0.0	52.53±0.17
DMD, %	95.47±0.27	94.80±0.13	95.60±0.4	94.93±1.07	94.93±0.27
SCFA, mmole	1.54±0.01	1.53±0.0	1.54±0.0	1.52±0.01	1.54±0.01
ME, MJ/kg DM	10.54^{a}±0.02	10.55^{a}±0.00	10.56^{a}±0.01	10.36^{b}±0.03	10.34^{b}±0.03

Figures with different superscripts in a row differ significantly, $P<0.05$

The results are in agreement with El-Waziry *et al* (2007) who reported cumulative gas production 43.41, 40.98, 36.89 and 39.62 at 72 h was for the SBM, SBM treated with 1% QT, SBM treated with 2% QT and SBM treated with 3% QT respectively. The result of Mohammadabadi and Chaji (2012) experiment showed that the tannin of oak leaves and pistachio hull did not reduce the net gas production but, tannin of oak fruit tannin and pistachio leaves reduced these parameters ($P<0.05$) which was 149.8, 124.2, 108.2, 126.2 and 119.2 for untreated SBM, SBM treated with 30 g/kg DM oak leaves tannin, oak fruit tannin, pistachio hull tannin and pistachio leaves tannin respectively. Getachew *et al* (2008b) reported that quebracho decreased gas production ($P<0.001$) in alfalfa hay at the rate of 5, 10, and 15%. That may be due to the higher inclusion level of quebracho than levels in the current study. Alipour and Rouzbehan (2010) reported decreased gas production due to the incorporation of tannin extract from grape pomace in SBM. ($P<0.01$).

The ammonical nitrogen did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM but it was numerically lower in treated SBM samples. El-Waziry *et al* (2007) reported the mean values of NH_3-N concentrations 10.17, 8.76, 7.68 and 7.47 mM for untreated SBM, SBM treated with 3% QT, SBM treated with 1% QT, SBM treated with 2% QT and autoclaving SBM, respectively. Gupta *et al* (2011) reported a decrease in NH_3 -N production from oil cake-Acacia catechu leaves pellets treated with different levels of tannins. Mohammadabadi and Chaji (2012) reported the mean values of NH_3-N concentrations of 31.6, 19.2, 15.3, 19.5 and 18.2 mg/dL for untreated SBM, SBM treated with 30 g/kg DM oak fruit tannin, oak leaves tannin, pistachio hull tannin and pistachio leaves tannin respectively. Alipour and Rouzbehan (2010) found that the ammonia concentration at 24 h of incubation decreased with the increased addition of tannin extract from grape pomace in SBM. ($P<0.01$). Getachew *et al* (2008b) reported that the addition of QT in alfalfa hay at the rate of 50, 100, and 150 g/kg resulted in a reduction of NH_4^+-N by 12%, 31%, and 51%, respectively.

The partition factor (PF) did not vary significantly ($P<0.05$) with different levels of quebracho treatment. The PF is the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (ml) produced. A higher partitioning factor means that proportionally more of the degraded matter is incorporated into microbial mass i.e. the

efficiency of microbial protein synthesis is higher. The partitioning factor provides useful information for predicting the dry matter intake, microbial mass production in the rumen and the methane emission of the ruminant animal (Blummel *et al* 1997).

Neutral detergent fibre degradability (NDFD) was significantly ($P<0.05$) higher in control as compared to treated SBM. MMP was higher significantly ($P<0.05$) in 3QSBM and did not vary in other samples. Whereas, there was no significant ($P<0.05$) difference in EMMP. DMD in the current study did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM.

Short-chain fatty acids in this study did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM. Mohammadabadi and Chaji (2012) reported that effect of tannins on VFA production varies with source of tannin and they reported the mean values of SCFA concentrations of 0.82, 0.73, 0.48, 0.74 and 0.64 µmol/L for untreated SBM, SBM treated with 30 g/kg DM oak leaves tannin, oak fruit tannin, pistachio hull tannin and pistachio leaves tannin, respectively. Getachew *et al* (2008b) reported QT decreased total SCFA ($P<0.001$) at the rate of 50, 100, and 150 g/kg ($P<0.001$). The difference may be due to levels of tannin used were much higher than the present study.

ME was higher significantly ($P<0.05$) in control 1QSBM, and 2QSBM as compared to 3QSBM and 4QSBM. This showed that Quebracho treatment higher than 2% decreased the ME value of SBM. Mohammadabadi and Chaji (2012) reported that the values of ME were 12.3, 11.2, 8.1, 10.9 and 9.2 MJ/kg DM for untreated SBM, SBM treated with 30 g/kg DM oak leaves tannin, oak fruit tannin, pistachio hull tannin and pistachio leaves tannin, respectively. The increasing ME values can be due to a lower level of tannins in the source of tannin used in their study. Which was 53, 79, 48, and 65 g/kg DM for oak leaves, oak fruit, pistachio hull and pistachio leaves, respectively.

4.2.2 *In vitro* gas production and digestibility of Tannic acid treated SBM

Results of *in vitro* gas production and digestibility of Tannic acid treated SBM is given in Table 14. The NGP/200mg DM in the current study did not vary significantly ($P<0.05$) with different levels of tannic acid treatment of SBM. Singh *et al* (2001) concluded that the tannic acid caused an increase in the gas volume.

Getachew *et al* (2008b) reported that the TA decreased gas production ($P<0.001$), at an inclusion level of 50, 100, and 150 g/kg. El-Waziry *et al* (2005) reported that the highest mean values of cumulative gas production during 72 h was obtained by SBM followed by SBM treated with 1% TA, SBM treated with 2%TA and SBM treated with 3% TA.

The ammonical nitrogen in the current study reduced significantly ($P<0.05$) in the 4TSBM compared to SBM-W. Singh *et al* (2001) also concluded that the tannic acid caused a decreased in the ammonical nitrogen. Getachew *et al* (2008b) reported that the addition of TA at the rate of 50, 100, and 150 g/kg resulted in a reduction of NH_4^+-N by 13.8%, 25.9% and 46.5%. El-Waziry *et al* (2005) reported that the concentrations of NH_3-N were significantly ($P<0.05$) decreased when SBM treated by with TA. The mean values of NH_3 -N concentrations were 10.17, 8.99, 8.89 and 7.69 mM for SBM, SBM treated with 1% TA, SBM treated with 2% TA and SBM treated with 3% TA, respectively.

The partition factor did not vary significantly ($P<0.05$) with different levels of tannic acid treated SBM. Neutral detergent fiber degradability was highest in SBM-W and was lowest in 1TSBM and rest treatments fall in between and did not vary significantly ($P<0.05$) either from control or 1TSBM. MMP was lower significantly ($P<0.05$) in control and 4TSBM and was higher ($P<0.05$) in other tannic acid treated SBM samples. Whereas, EMMP was higher non-significantly ($P<0.05$) in all tannic acid treated SBM than control and was highest in 1% tannic acid treated SBM.

DMD in the current study did not vary significantly ($P<0.05$) with different levels of tannic acid treatment of SBM but, it was lower in all tannin treated SBM compared to SBM-W non-significantly ($P<0.05$). Hervas *et al* (2000) reported that the tannic acid treatment of SBMs (9% of the as-fed weight of SBM) had a negative effect on DM disappearances from bags, this effect being clearly dependent on the incubation time and the dose of tannic acid used to treat the meals. Martínez *et al* (2005) reported disappearances of DM from TA-treated barley were lower ($P<0.05$) than in the controls, and the response became more pronounced as TA treatment level increased.

Table 14. *In vitro* gas production and digestibility of tannic acid treated SBM

Variable	SBM-W	1TSBM	2TSBM	3TSBM	4TSBM
NGP/200mg DM	38.00±0.15	38.18±0.24	38.18±0.18	37.82±0.24	37.64±0.24
NH_3-N, mg/dl	102.23^{a}±6.33	97.11^{ab}±0.55	95.09^{ab}±0.85	94.43^{ab}±1.18	91.00^{b}±0.66
PF, mg/ml	4.64±0.03	4.69±0.03	4.67±0.03	4.67±0.03	4.67±0.03
NDFD, %	83.75^{a}±0.96	78.14^{b}±0.0	82.12^{ab}±0.51	82.97^{ab}±2.08	83.16^{ab}±1.96
MMP, mg	173.63^{b}±0.64	176.53^{a}±0.97	177.35^{a}±0.73	176.72^{a}±0.97	172.50^{b}±0.97
EMMP, %	52.55±0.33	53.07±0.33	52.86±0.33	52.89±0.33	52.85±0.34
DMD, %	95.47±0.27	94.67±0.0	95.33±0.13	94.53±0.67	94.27±0.67
SCFA, mmole	1.54±0.01	1.55±0.01	1.55±0.01	1.53±0.01	1.52±0.01
ME, MJ/kg DM	10.54^{a}±0.02	10.18^{b}±0.03	10.21^{b}±0.03	10.15^{bc}±0.03	10.07^{c}±0.03

Figures with different superscripts in a row differ significantly, $P<0.05$

Short-chain fatty acids in the current study did not vary significantly (P<0.05) with different levels of tannic acid treatment of SBM. It increased in 1TSBM and 2TSBM but decreased thereafter in 3 TSBM and 4 TSBM compared to SBM-W non-significantly (P<0.05). Getachew *et al* (2008b) concluded that the TA decreased total SCFA (P<0.001) at the rate of 50, 100, and 150 g/kg which were much higher levels than taken in the current study.

ME was significantly lower (P<0.05) in all tannic acid treated SBMs. The results are in agreement with El-Waziry *et al* (2005) who reported that the predicted ME calculated from gas production after 24 h incubation were reduced when SBM was treated by heating or TA.

4.2.3 *In vitro* kinetic parameters of tannin treated SBM

The results of tannin treated SBMs for *in vitro* Kinetics of NGP/200mg DM is given in Table 15. Comparison of gas production in between quebracho treated SBMs was represented in Fig 1 and comparison in between tannic acid treated SBMs were represented in Fig 2. Results showed that there was a reduction in the gas production rate as well in the total gas production in the treated SBMs with both the tannin sources. Results were more prominent in the 4% tannin treated SBMs in both quebracho and tannic acid. At the end of 24 hours, all the treated SBMs were having significantly (P<0.05) lower gas production compared to SBM-W and lowest results were found for the 4QSBM and 4TSBM which was 30.27 and 30.53 ml respectively.

Results of tannin treatment on *in vitro* Kinetics of ammonical Nitrogen is given in Table 16. Comparison of ammonical nitrogen in between quebracho treated SBMs was represented in Fig 3 and comparison of ammonical nitrogen in between tannic acid treated SBMs were represented in Fig 4. Results clearly showed that there was significant (P<0.05) reduction in all the treatments from 8 hours onwards however there was more prominent effect on the ammonical nitrogen in tannic acid treated SBM compared to quebracho treatment. There was significant (P<0.05) reduction in the ammonical nitrogen into the tannic acid treated SBMs from 6 hours onwards which indicates tannic acid was more efficient in protein protection. At the end of 24 hours and throughout the path as well results of 1TSBM was also comparable to the higher level of quebracho treatments that is 3QSBM and 4QSBM.

Table 15. Effect of tannin treatment on *in vitro* Kinetics of NGP/200mg DM

Incubation hour	SBM-W	1QSBM	2QSBM	3QSBM	4QSBM	1TSBM	2TSBM	3TSBM	4TSBM
2	3.69^{b}±0.09	2.13^{c}±0.00	4.27^{a}±0.15	2.27^{c}±0.27	1.20^{d}±0.00	2.09^{c}±0.18	2.00^{c}±0.15	3.60^{b}±0.27	3.24^{b}±0.09
4	9.91^{a}±0.09	9.16^{b}±0.39	10.49^{a}±0.44	7.78^{c}±0.09	4.40^{f}±0.00	5.38^{e}±0.32	6.98^{d}±0.24	7.96^{c}±0.09	5.73^{e}±0.15
6	16.84^{b}±0.09	16.27^{b}±0.31	17.87^{a}±0.56	14.18^{c}±0.24	9.73^{f}±0.31	10.71^{d}±0.24	11.51^{d}±0.09	13.47^{c}±0.00	10.89^{d}±0.09
8	23.42^{ab}±0.32	22.76^{b}±0.09	23.82^{a}±0.09	19.42^{c}±0.18	15.60^{f}±0.56	15.69^{f}±0.32	17.02^{e}±0.36	18.18^{d}±0.24	15.60^{f}±0.15
10	28.04^{a}±0.09	27.02^{b}±0.18	28.00^{a}±0.27	24.13^{c}±0.00	19.78^{f}±0.64	20.40^{f}±0.15	21.38^{e}±0.24	22.89^{d}±0.09	20.13^{f}±0.15
12	30.53^{ab}±0.41	29.51^{b}±0.09	31.47^{a}±0.15	26.62^{c}±0.18	21.38^{f}±0.24	23.51^{e}±0.32	25.56^{cd}±0.94	24.93^{d}±0.15	22.09^{f}±0.09
24	42.53^{a}±0.15	37.87^{cd}±0.00	39.29^{bc}±0.39	35.96^{d}±0.32	30.27^{e}±1.67	37.38^{cd}±0.36	40.40^{b}±0.15	37.47^{cd}±0.31	30.53^{e}±0.15

Figures with different superscripts in a row differ significantly, $P<0.05$

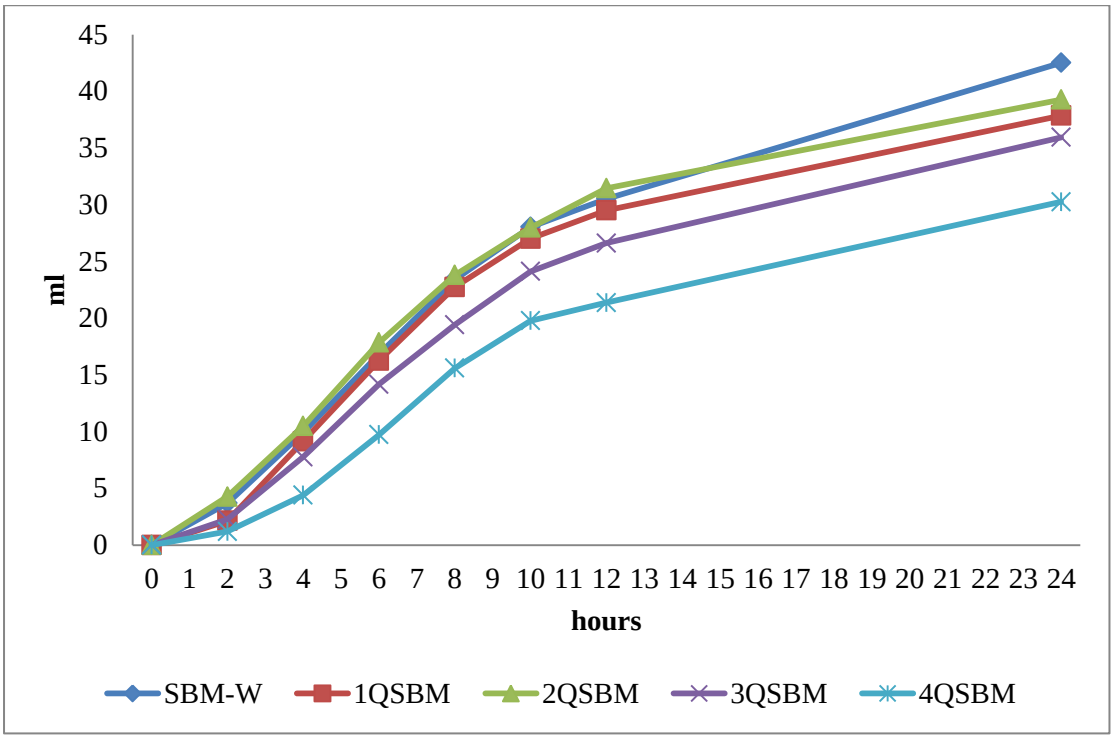

Fig 1. Effect of Quebracho treatment on *in vitro* Kinetics of NGP/200mg DM

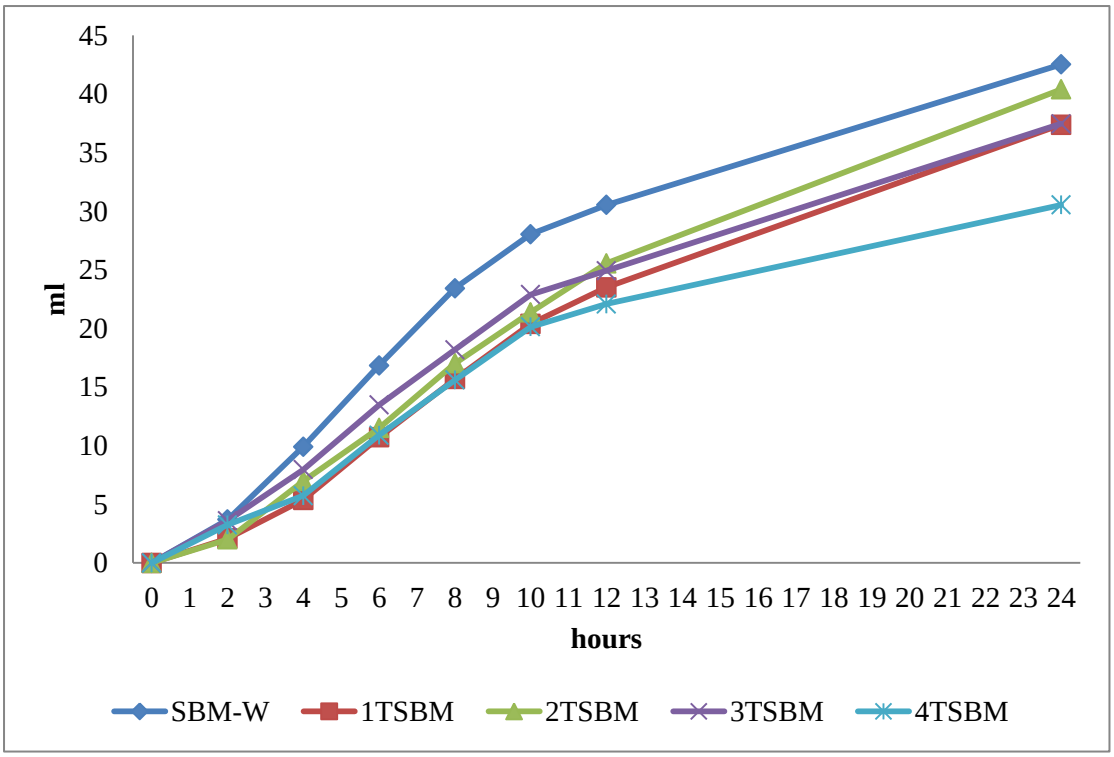

Fig 2. Effect of Tannic acid treatment on *in vitro* Kinetics of NGP/200mg DM

Table 16. Effect of tannin treatment on *in vitro* Kinetics of ammonical nitrogen, mg/dL

Incubation hour	SBM-W	1QSBM	2QSBM	3QSBM	4QSBM	1TSBM	2TSBM	3TSBM	4TSBM
0	$33.68^{a}\pm2.64$	$37.33^{a}\pm1.13$	$36.20^{a}\pm1.13$	$37.05^{a}\pm0.28$	$37.90^{a}\pm1.13$	$37.90^{a}\pm1.13$	$35.35^{a}\pm0.28$	$36.48^{a}\pm0.28$	$36.50^{a}\pm1.50$
2	$47.23^{a}\pm2.55$	$46.38^{a}\pm0.57$	$44.12^{ab}\pm0.00$	$42.42^{bc}\pm0.57$	$41.57^{bc}\pm0.85$	$42.42^{bc}\pm0.57$	$41.85^{bc}\pm1.70$	$41.85^{bc}\pm0.00$	$40.00^{c}\pm0.00$
4	$50.67^{a}\pm2.05$	$48.64^{ab}\pm0.00$	$47.23^{ab}\pm0.28$	$46.10^{b}\pm0.85$	$47.23^{ab}\pm1.41$	$49.49^{ab}\pm0.28$	$46.94^{b}\pm1.13$	$47.51^{ab}\pm0.00$	$46.00^{b}\pm1.00$
6	$65.33^{a}\pm5.94$	$61.65^{ab}\pm1.13$	$62.78^{a}\pm0.00$	$58.82^{abc}\pm1.13$	$54.86^{bcd}\pm0.00$	$54.30^{bcd}\pm0.57$	$51.75^{cd}\pm0.85$	$50.62^{d}\pm1.98$	$50.50^{d}\pm0.50$
8	$68.83^{a}\pm1.46$	$63.91^{b}\pm0.00$	$62.78^{b}\pm1.13$	$61.37^{b}\pm0.28$	$56.84^{c}\pm0.28$	$57.13^{c}\pm1.13$	$53.17^{d}\pm0.57$	$50.62^{d}\pm1.98$	$52.00^{d}\pm1.00$
10	$78.20^{a}\pm0.88$	$71.27^{b}\pm2.26$	$68.15^{bc}\pm1.98$	$66.46^{c}\pm0.28$	$62.22^{d}\pm0.57$	$60.24^{d}\pm0.28$	$56.56^{e}\pm0.00$	$53.45^{ef}\pm0.28$	$52.50^{f}\pm0.50$
12	$84.64^{a}\pm1.46$	$75.79^{b}\pm1.70$	$72.68^{c}\pm0.28$	$72.40^{c}\pm0.00$	$65.61^{d}\pm1.13$	$62.78^{e}\pm0.00$	$61.37^{e}\pm0.28$	$54.86^{f}\pm0.00$	$53.50^{f}\pm0.50$
24	$110.29^{a}\pm0.57$	$98.41^{b}\pm2.26$	$95.59^{b}\pm0.00$	$92.76^{b}\pm1.13$	$90.21^{b}\pm0.85$	$93.61^{b}\pm0.85$	$92.19^{b}\pm2.83$	$82.01^{c}\pm5.66$	$76.50^{c}\pm1.50$

Figures with different superscripts in a row differ significantly, $P<0.05$

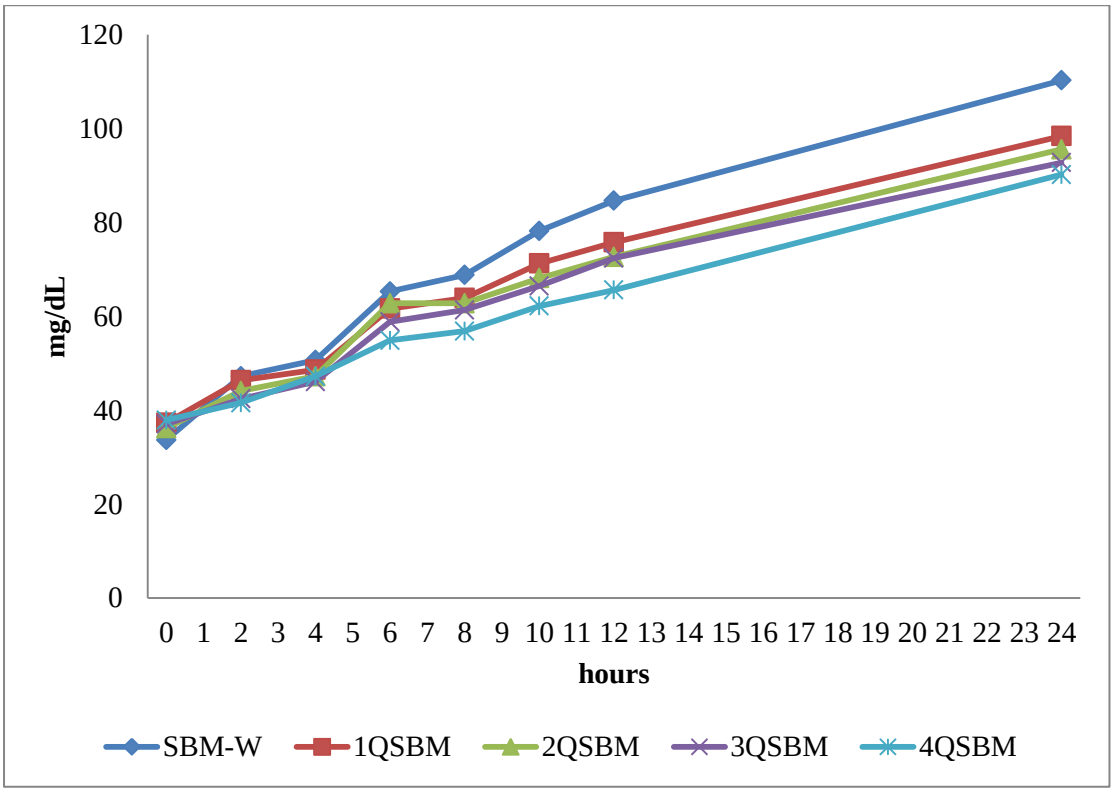

Fig 3. Effect of Quebracho treatment on *in vitro* Kinetics of ammonical nitrogen mg/dL

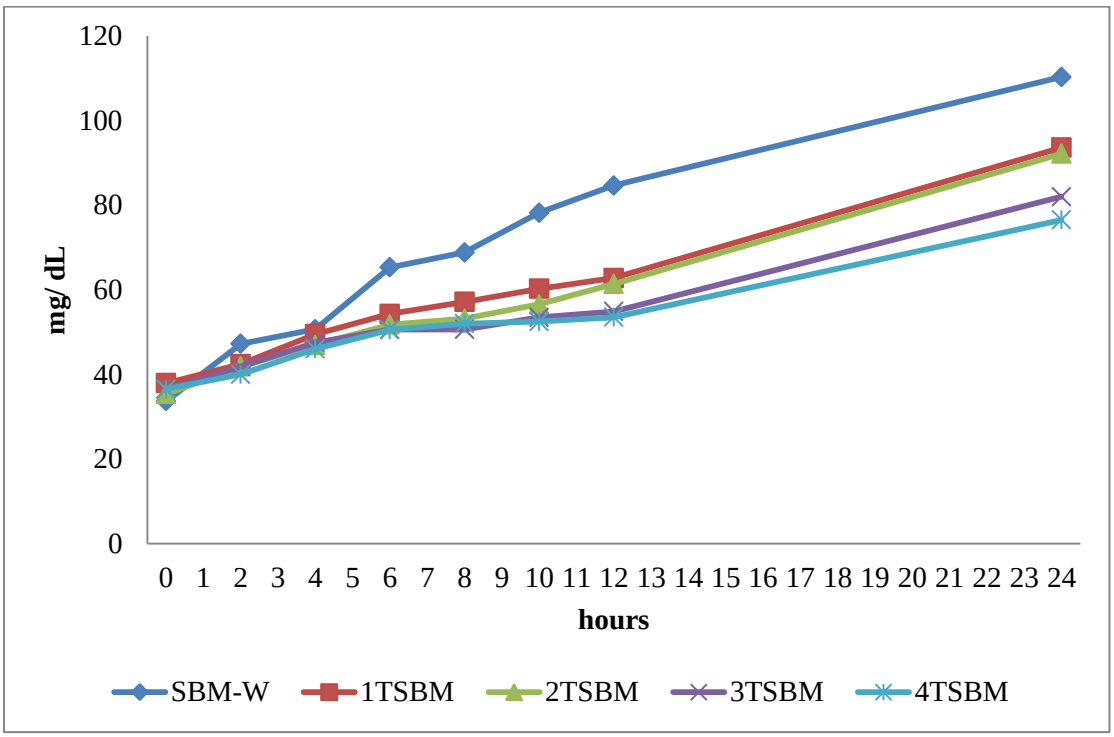

Fig 4. Effect of tannic acid treatment on *in vitro* Kinetics of ammonical nitrogen mg/dL

Results of tannin treatment on *in vitro* Kinetics of NDFD, % is given in Table 17. Results showed that there was a slight reduction in the NDFD but the trend was not consistent for the treatments and the values were numerically similar. There was a reduction in the NDFD at the end of 24 hours in 3QSBM and 2TSBM significantly ($P<0.05$) compared to control. During the 24 hours there was slower degradation in the 1TSBM and 2TSBM.

Results of tannin treatment on *in vitro* Kinetics of SCFA, mmole was significantly ($P<0.05$) reduced in the all treatments compared to tannin treated SBMs at the end of incubation (Table 18). In the initial hours' effect of tannic acid was more prominent and all the tannic acid treated SBMs showed significant ($P<0.05$) difference from the lower level of quebracho treated SBMs. The lower level of tannic acid treatment of SBM was similar to the higher level of quebracho treated SBMs.

Results of tannin treatment on *in vitro* Kinetics of ME, MJ/kg DM is given in Table 19. Till the 12 hours of incubation, 2QSBM was having highest values but at the end of the 24 hours, it had significantly ($P<0.05$) lower value than the SBM-W. The results showed that tannic acid was reducing ME significantly ($P<0.05$) at all the levels as compared to SBM-W.

Results of tannin treated SBM on the *in vitro* kinetic parameters are given in Table 20. The results of the study revealed that tannic acid was more effective in protecting protein degradation. There was no significant ($P<0.05$) reduction of the rate of degradation in the Quebracho treated SBM. 1TSBM had significantly lower ($P<0.05$) degradation rate and significantly high ($P<0.05$) t-half as compared to control.

Potentially degradable fraction (a+b) was lowest ($P<0.05$) in the 4QSBM and effects were more prominent in quebracho treatment compared to tannic acid. Results of all quebracho treatment as well higher level of tannic acid affected potential degradability of the SBM. Tannic acid was found more effective for protecting ruminal degradation. Significant ($P<0.05$) reduction of degradation rate was not found for the quebracho treated SBM. Even at the highest level of Quebracho difference was non-significant ($P<0.05$) from the control.

Table 17. Effect of tannin treatment on *in vitro* Kinetics of %NDFD

Incubation hour	SBM-W	1QSBM	2QSBM	3QSBM	4QSBM	1TSBM	2TSBM	3TSBM	4TSBM
0	$28.79^{b}\pm0.48$	$13.44^{e}\pm1.40$	$13.66^{e}\pm0.00$	$20.26^{cd}\pm0.00$	$39.87^{a}\pm2.61$	$15.03^{de}\pm3.28$	$23.37^{c}\pm1.02$	$29.80^{b}\pm2.08$	$15.66^{de}\pm0.51$
2	$25.93^{bc}\pm0.48$	$14.83^{d}\pm1.40$	$22.51^{c}\pm2.09$	$20.26^{cd}\pm0.00$	$30.98^{ab}\pm0.00$	$16.56^{d}\pm3.83$	$22.35^{c}\pm2.04$	$35.20^{a}\pm2.49$	$20.20^{cd}\pm2.02$
4	$31.18^{abc}\pm0.00$	$30.89^{abc}\pm0.70$	$28.10^{bc}\pm0.70$	$18.95^{d}\pm0.00$	$32.03^{ab}\pm0.00$	$20.22^{d}\pm1.09$	$19.80^{d}\pm4.60$	$35.62^{a}\pm3.74$	$24.24^{cd}\pm2.02$
6	$36.92^{abc}\pm1.91$	$42.06^{ab}\pm2.09$	$39.27^{abc}\pm0.70$	$52.29^{a}\pm20.26$	$37.25^{abc}\pm0.00$	$20.49^{c}\pm0.55$	$28.48^{abc}\pm1.02$	$50.99^{a}\pm4.15$	$26.26^{bc}\pm0.00$
8	$48.39^{ab}\pm0.00$	$49.04^{ab}\pm0.70$	$53.93^{a}\pm0.00$	$39.87^{c}\pm1.31$	$46.67^{b}\pm0.00$	$37.04^{c}\pm2.73$	$37.16^{c}\pm3.58$	$50.57^{ab}\pm1.25$	$47.98^{b}\pm1.52$
10	$57.47^{ab}\pm0.48$	$58.81^{a}\pm4.89$	$58.81^{a}\pm2.09$	$50.98^{ab}\pm0.65$	$56.60^{ab}\pm1.57$	$36.60^{c}\pm3.83$	$48.91^{b}\pm3.07$	$56.80^{ab}\pm0.83$	$52.53^{ab}\pm2.02$
12	$64.64^{a}\pm5.73$	$63.70^{a}\pm4.19$	$58.81^{ab}\pm7.68$	$56.86^{ab}\pm0.00$	$64.44^{a}\pm0.00$	$47.54^{b}\pm0.00$	$52.49^{ab}\pm3.58$	$55.14^{ab}\pm0.00$	$59.09^{ab}\pm3.54$
24	$83.27^{ab}\pm1.43$	$87.43^{a}\pm0.00$	$81.85^{ab}\pm2.79$	$69.93^{c}\pm6.54$	$83.27^{ab}\pm2.09$	$75.41^{bc}\pm0.55$	$67.82^{c}\pm1.53$	$83.80^{ab}\pm1.25$	$85.86^{a}\pm3.03$

Figures with different superscripts in a row differ significantly, $P<0.05$

Table 18. Effect of tannin treatment on *in vitro* Kinetics of SCFA, mmole

Incubation hour	SBM-W	1QSBM	2QSBM	3QSBM	4QSBM	1TSBM	2TSBM	3TSBM	4TSBM
2	0.11^{b}±0.00	0.05^{c}±0.00	0.14^{a}±0.01	0.05^{c}±0.01	0.01^{d}±0.00	0.04^{c}±0.01	0.04^{c}±0.01	0.11^{b}±0.01	0.09^{b}±0.00
4	0.37^{a}±0.00	0.34^{b}±0.02	0.39^{a}±0.02	0.28^{c}±0.00	0.14^{f}±0.00	0.18^{e}±0.01	0.25^{d}±0.01	0.29^{c}±0.00	0.20^{e}±0.01
6	0.66^{b}±0.00	0.63^{b}±0.01	0.70^{a}±0.02	0.55^{c}±0.01	0.36^{e}±0.01	0.40^{d}±0.01	0.44^{d}±0.00	0.52^{c}±0.00	$0.41\pm^{d}0.00$
8	0.93^{ab}±0.01	0.90^{b}±0.00	0.95^{a}±0.00	0.77^{c}±0.01	0.61^{f}±0.02	0.61^{f}±0.01	0.67^{e}±0.01	0.71^{d}±0.01	0.61^{f}±0.01
10	1.12^{a}±0.00	1.08^{b}±0.01	1.12^{a}±0.01	0.96^{c}±0.00	0.78^{f}±0.03	0.81^{f}±0.01	0.85^{e}±0.01	0.91^{d}±0.00	0.80^{f}±0.01
12	1.23^{ab}±0.02	1.19^{b}±0.00	1.27^{a}±0.01	1.07^{c}±0.01	0.85^{f}±0.01	0.94^{e}±0.01	1.02^{cd}±0.04	1.00^{d}±0.01	0.88^{f}±0.00
24	1.73^{a}±0.01	1.53^{cd}±0.00	1.59^{bc}±0.02	1.45^{d}±0.01	1.22^{e}±0.07	1.51^{cd}±0.01	1.64^{b}±0.01	1.52^{cd}±0.01	1.23^{e}±0.01

Figures with different superscripts in a row differ significantly, P<0.05

Table 19. Effect of tannin treatment on *in vitro* Kinetics of ME, MJ/kg DM

Incubation hour	SBM-W	1QSBM	2QSBM	3QSBM	4QSBM	1TSBM	2TSBM	3TSBM	4TSBM
2	5.53^{b}±0.01	5.35^{c}±0.00	5.65^{a}±0.02	5.21^{d}±0.04	4.98^{ef}±0.00	4.92^{f}±0.03	4.92^{f}±0.02	5.16^{d}±0.04	5.04^{f}±0.01
4	6.44^{b}±0.01	6.38^{b}±0.06	6.56^{a}±0.06	6.01^{c}±0.01	5.45^{f}±0.00	5.40^{f}±0.05	5.65^{e}±0.03	5.79^{d}±0.01	5.41^{f}±0.02
6	7.45^{b}±0.01	7.42^{a}±0.04	7.64^{a}±0.08	6.95^{c}±0.03	6.22^{ef}±0.04	6.17^{f}±0.03	6.31^{e}±0.01	6.60^{d}±0.00	6.16^{f}±0.01
8	8.41^{ab}±0.05	8.36^{b}±0.01	8.51^{a}±0.01	7.71^{c}±0.03	7.08^{e}±0.08	6.90^{f}±0.05	7.12^{e}±0.05	7.29^{d}±0.03	6.85^{f}±0.02
10	9.09^{ab}±0.01	8.99^{b}±0.03	9.12^{a}±0.04	8.40^{c}±0.00	7.69^{ef}±0.09	7.59^{fg}±0.02	7.75^{e}±0.03	7.97^{d}±0.01	7.51^{g}±0.02
12	9.45^{b}±0.06	9.35^{b}±0.01	9.62^{a}±0.02	8.76^{c}±0.03	7.92^{ef}±0.03	8.04^{e}±0.05	8.36^{d}±0.14	8.27^{d}±0.02	7.79^{f}±0.01
24	11.20^{a}±0.02	10.57^{b}±0.00	10.76^{b}±0.06	10.13^{c}±0.05	9.22d±0.24	10.07c±0.05	10.53^{b}±0.02	10.10^{c}±0.04	9.03^{d}±0.02

Figures with different superscripts in a row differ significantly, P<0.05

Table 20. Effect of tannin treated SBM on the *in vitro* kinetics parameters by exponential model

Parameters	SBM-W	1QSBM	2QSBM	3QSBM	4QSBM	1TSBM	2TSBM	3TSBM	4TSBM
Rapidly degradable fraction (a)	-6.597^{b}± 0.7113	-9.828^{a}± 0.07726	-7.351^{b}± 0.9274	-7.377^{b}± 0.6754	-7.298^{b}± 1.42	-5.01^{c}± 0.6961	-4.947^{c}± 0.6372	-3.672^{d}± 0.5567	-4.465^{cd}± 0.9707
Insoluble fraction (b)	54.61^{b}± 0.8264	50.38^{c}± 0.728	49.73^{c}± 0.8906	47.5^{c}± 0.7266	42.34^{d}± 1.711	57.15^{b}± 2.288	62.64^{a}± 2.243	50.39^{c}± 1.082	40.54^{d}± 1.331
Potentially degradable fraction (a+b)	48.01^{c}± 0.9686	40.55^{ef}± 0.6737	42.38^{de}± 0.862	40.12^{efg}± 0.8133	35.04^{g}± 2.035	52.74^{b}± 2.697	52.69^{a}± 2.626	46.72^{cd}± 1.337	36.07^{fg}± 1.633
Degradation rate	0.09671^{bc}± 0.004393	0.1264^{a}± 0.005091	0.1215^{a}± 0.006184	0.1039^{b}± 0.004749	0.09396^{bc}± 0.01137	0.05572^{e}± 0.004918	0.05404^{e}± 0.004217	0.071^{d}± 0.004049	0.08589^{c}± 0.008272
t- half	7.167^{cd}± 0.12	5.483^{d}± 0.05	5.706^{d}± 0.20	6.674^{cd}± 0.17	7.377^{c}± 1.04	12.44^{a}± 1.03	12.83^{a}± 0.68	9.762^{b}± 0.22	8.07^{c}± 0.07

Figures with different superscripts in a row differ significantly, P<0.05

Half time was significantly (P<0.05) increased in the tannic acid treated SBM. And highest half time was found for the 1TSBM and 2TSBM. There was no significant (P<0.05) improvement in quebracho treated SBM compared to control.

El-Waziry *et al* (2007) reported similar results. They reported that the soluble fractions (a) of SBM were significantly (P<0.05) decreased when SBM was treated by autoclaving or QT. El-Waziry *et al* (2005) reported that the values of (a) on average were 3.41, -1.96, -0.90 and - 0.81 ml for SBM, SBM treated with 1%TA, SBM treated with 2%TA and SBM treated with 3% TA, respectively. The insoluble fraction (b) was significantly (P<0.05) decreased when SBM was treated by TA. The lowest value of insoluble fraction was obtained when SBM treated with 3% of TA (41.36 ml). The results are in agreement with Hervas *et al* (2000) who reported that the treatment of SBM with tannic acid significantly (P<0.001) decreased the rapidly degradable fraction (a). That effect was observed in all the doses studied. They found potential degradability values (a+b) significantly (P<0.05) lower (quadratic effect; P<0.001) in comparison to the control. Tannic acid treatment significantly (P<0.05) decreased the rate of degradation (P<0.001) but only the three soya bean meals treated with the highest doses of tannic acid (10, 15 and 25%) showed significant (P<0.05) lower rates of degradation (c) than the control meal.

4.2.4 Carbohydrate fermentation and protein degradation kinetics in tannin treated SBM

Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Makkar 2002), whereas ammonical nitrogen relates to protein degradation in the rumen inoculum. A graph in the Fig.5 shows that there was a higher difference between carbohydrate fermentation and protein degradation into the 2QSBM compared to control as there was more gap. Higher level of quebracho that is 4QSBM decreases more gas production as well decreases ammonical nitrogen more prominently at the end of 24 hours but it clearly indicates that there was more effective on the carbohydrate protection as line of 4QSBM raises above the control which means there is higher degradation of protein compared to carbohydrate in to the 4QSBM in contrast to control.

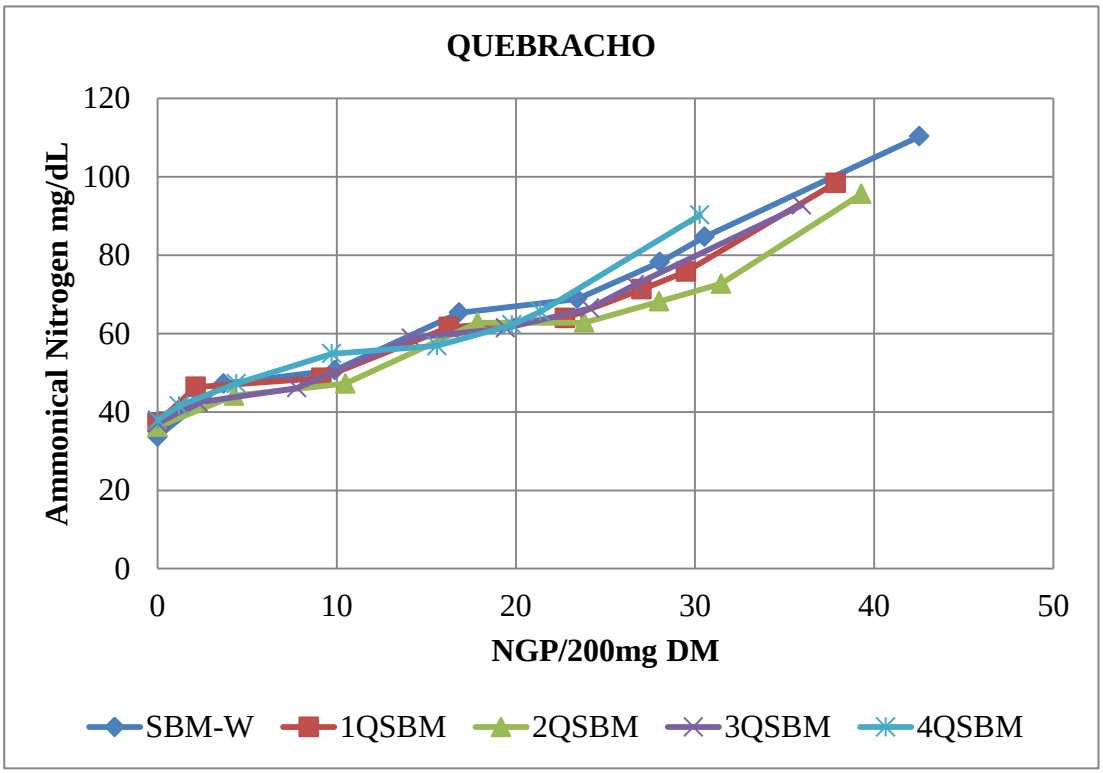

Fig. 5: Relationship between *in vitro* NGP and ammonical nitrogen in quebracho treated SBM

Fig. 6 shows that tannic acid was more effective in the protein protection compared to carbohydrate protection. The gas and ammonical nitrogen can see that 4TSBM was decreasing both parameters but the line of the 4TSBM ends near to 1TSBM. The lowest line was found for the 3 TSBM but even 1TSBM was reducing protein degradability more prominently than the carbohydrate degradability. It is well known that fermentable carbohydrates are needed for the ruminal microbial mass production. Rumen microbes incorporate available nitrogen with carbon skeleton to synthesize microbial protein but a higher amount of ruminal ammonical nitrogen hampers the growth of rumen microbes (National Research Council 2001). From the study, it was observed that tannic acid protected protein preferentially over fermentable carbohydrates. So it can be useful for higher microbial protein production.

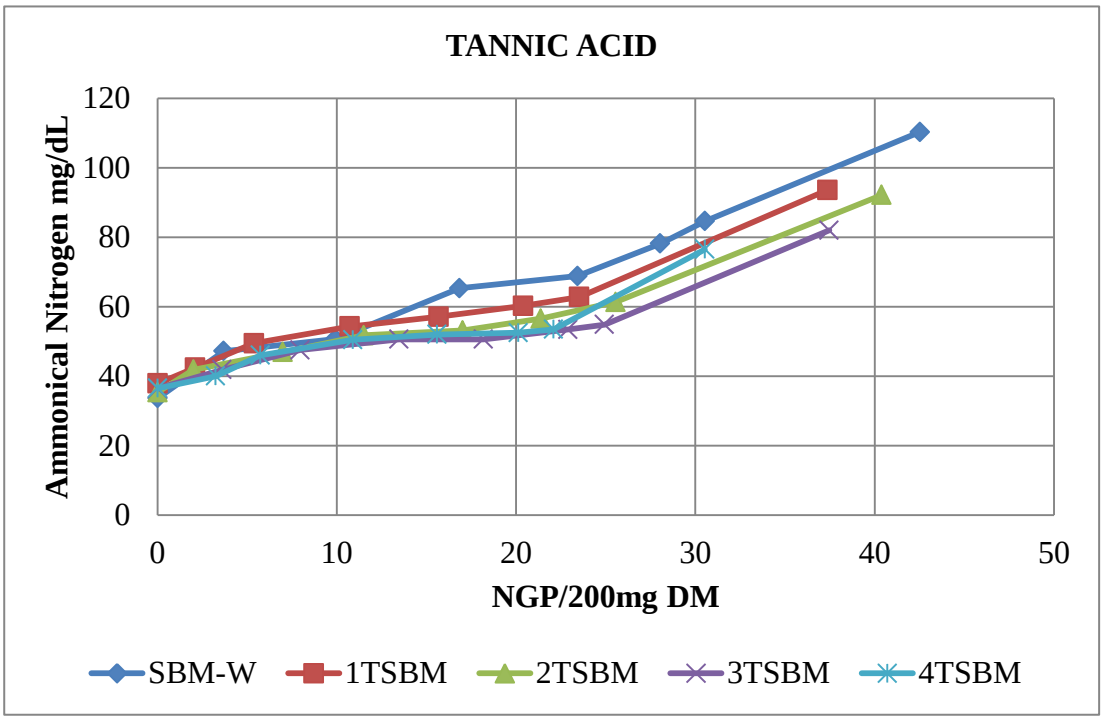

Fig. 6: Relationship between *in vitro* NGP and ammonical nitrogen in tannic acid treated SBM

4.3 *In vitro* evaluation of TMRs containing graded levels of tannins

4.3.1 *In vitro* gas production and digestibility of TMRs with quebracho treated SBM

Results of *in vitro* gas production and digestibility of TMRs containing quebracho treated SBM is given in Table 21. The NGP/200 mg DM in the current study was significantly (P<0.05) lower in 4QRH as compared to other treatments and control. The NGP/200mg DM was varying from 43.41 to 46.36 ml and it was highest in COT. Results were in agreement with Getachew *et al* (2008b) who found that quebracho tannin decreased gas production. Gerlach *et al* (2018) found that the *in vitro* gas production of rations with 1, 3 and 5% CT supplementation was reduced by 3.0, 9.2 and 18.2% in the ration. Jayanegara *et al* (2015b) reported mimosa and quebracho (purified condensed tannin) decreased total gas production in hay: concentrate substrate (70:30 w/w) ($P \leq 0.05$). Anantasook *et al* (2013) found that cumulative gas production was higher (P<0.01) with *T. chebula* supplementation at 12, 16 and 20mg than other treatments. Frutos *et al* (2002) found that the CT negatively correlated (P<0:05) with cumulative gas production.

Table 21. *In vitro* gas production and digestibility of TMRs containing quebracho treated SBM

Variable	COT	1QRL	1QRH	2QRL	2QRH	3QRL	3QRH	4QRL	4QRH
NGP/200mg DM	$46.36^{a}\pm0.47$	$45.05^{bc}\pm0.32$	$45.21^{bc}\pm0.50$	$45.32^{b}\pm0.31$	$44.31^{bcd}\pm0.38$	$44.15^{cd}\pm0.40$	$44.36^{bcd}\pm0.25$	$44.10^{cd}\pm0.15$	$43.41^{d}\pm0.13$
NH_3-N, mg/dl	$40.00^{ab}\pm0.56$	$41.41^{a}\pm0.28$	$39.44^{ab}\pm1.18$	$39.34^{ab}\pm0.28$	$37.56^{bcd}\pm0.50$	$37.75^{bc}\pm1.13$	$36.68^{cde}\pm0.75$	$35.24^{de}\pm0.44$	$34.98^{e}\pm0.56$
PF, mg/ml	2.80±0.04	2.87±0.07	2.86±0.04	2.79±0.03	2.91±0.04	2.90±0.05	2.92±0.02	2.84±0.024	2.88±0.015
OMD,%	$73.58^{a}\pm0.04$	$73.20^{ab}\pm0.89$	$72.44^{abc}\pm0.18$	$71.81^{cde}\pm0.45$	$72.19^{bcd}\pm0.26$	$72.87^{abc}\pm0.12$	$72.92^{abc}\pm0.12$	$70.91^{de}\pm0.46$	$70.81^{e}\pm0.00$
NDFD, %	$57.58^{a}\pm0.06$	$53.44^{bcd}\pm1.55$	$54.81^{bc}\pm0.30$	$52.55^{cd}\pm0.75$	$53.16^{bcd}\pm0.44$	$53.40^{bcd}\pm0.21$	$55.16^{b}\pm0.19$	$53.95^{bcd}\pm0.73$	$51.86^{d}\pm0.01$
MMP, mg	$140.87^{a}\pm1.78$	$147.36^{b}\pm1.79$	$146.42^{b}\pm1.72$	$146.75^{b}\pm1.27$	$149.54^{ab}\pm1.88$	$148.89^{ab}\pm1.18$	$148.20^{ab}\pm0.58$	$148.44^{ab}\pm0.77$	$152.21^{a}\pm1.04$
EMMP, %	$57.22^{e}\pm1.27$	$59.98^{d}\pm0.13$	$61.04^{bcd}\pm1.46$	$60.51^{cd}\pm0.08$	$62.79^{abc}\pm0.53$	$61.32^{bcd}\pm0.99$	$61.68^{bcd}\pm0.26$	$63.56^{ab}\pm0.23$	$64.94^{a}\pm0.40$
DMD, %	$75.03^{a}\pm0.23$	$74.27^{abcd}\pm0.67$	$73.44^{bcde}\pm0.09$	$73.28^{cde}\pm0.53$	$73.63^{bcde}\pm0.64$	$74.50^{abc}\pm0.10$	$74.63^{ab}\pm0.23$	$72.80^{e}\pm0.15$	$73.00^{de}\pm0.12$
SCFA, mmole	$1.89^{a}\pm0.02$	$1.84^{abc}\pm0.01$	$1.84^{abc}\pm0.03$	$1.86^{ab}\pm0.01$	$1.80^{cd}\pm0.01$	$1.80^{cd}\pm0.02$	$1.81^{bcd}\pm0.02$	$1.80^{cd}\pm0.01$	$1.76^{d}\pm0.00$
ME, MJ/kg DM	$9.57^{a}\pm0.07$	$9.43^{abc}\pm0.05$	$9.51^{ab}\pm0.07$	$9.51^{ab}\pm0.04$	$9.33^{cd}\pm0.06$	$9.39^{bc}\pm0.06$	$9.36^{bcd}\pm0.04$	$9.21^{de}\pm0.02$	$9.12^{e}\pm0.02$

Figures with different superscripts in a row differ significantly, $P<0.05$

Ammonical nitrogen was significantly (P<0.05) highest in 1QRL whereas, it was significantly (P<0.05) lower in 4QRH. Salem *et al* (2005) also reported that the Acacia feeding decreased NH_3-N with a maximum at 4 h post-feeding. Aguerre *et al* (2016) found that the tannin supplementation was effective in reducing ruminal NH_3-N concentration with increasing level of tannins. Kai *et al* (2016) found similar results with the rumen fluid collected from lambs fed purple prairie clover hay. Cieslak *et al* (2012) reported a reduction in the ammonia concentration by 46% with the supplementation of tannin-containing *Vaccinium vitis idaea* (VVI) at the rate of 140 g. Dschaak *et al* (2011) added quebracho condensed tannin extract (CTE) and reported a reduction in the concentration of ammonical-N. Koenig *et al* (2018), Liu *et al* (2011) and Niderkorn *et al* (2012) also found a reduction in the concentration of ammonical-N with supplementation of tannins.

The % OMD was significantly (P<0.05) lower in TMRs containing quebracho tannin treated SBM. The TMR containing 4% quebracho treated SBM had significantly lower (P<0.05) OMD as compared to control and other treatments except 2% quebracho treatment. Mohammadabadi and Chaji (2012) also reported the value of OMD of sunflower meal were decreased by tannin of oak fruit and pistachio leaves, but oak leaves and pistachio hull did not influence these parameters (P<0.05). Frutos *et al* (2002) found that the CT negatively correlated (P<0:05) with OM degradation.

A similar trend as OMD% was seen for NDFD%. The difference in percent NDFD was found non-significant (P<0.05) and it was lower in treatments than control numerically.

PF varied non-significantly (P<0.05) among treatments and control. Results were in agreement with Jayanegara *et al* (2015b) who reported non-significant (P<0.05) difference in PF with the addition of quebracho.

MMP and EMMP were found significantly (P<0.05) higher in 4QRH. Results were in agreement with Getachew *et al* (2008b) who found that quebracho tannin increased the efficiency of microbial protein synthesis when 50 and 100 g QT/kg DM was added compared with the control. Makkar *et al* (1995) reported that the efficiency of microbial protein synthesis, expressed as the ratio of N incorporation per unit of SCFA production, was higher with the tannins.

DMD was lowest (P<0.05) in 4QRL (72.80%) and was highest (P<0.05) in COT (75.03%). Getachew *et al* (2008b) found that QT decreased *in vitro* true

degradability of dry matter. Makkar *et al* (1995) reported reduction in the *in vitro* true digestibility of dry matter by 3, 6 and 7% for *Q incana*, *D cinerea* and *A barteri*, respectively, at a tannin concentration of 0.47 mg/ml, and 17, 21 and 27%, respectively, at 0.93 mg/ml.

The values of short-chain fatty acid showed that these were decreasing with the inclusion of the quebracho and were lowest (P<0.05) in the 4QRH and was highest (P<0.05) in COT. Jayanegara *et al* (2015b) reported that the mimosa and quebracho (purified condensed tannin) decreased total short-chain fatty acid (P<0.05). Getachew *et al* (2008b) found that QT decreased total short-chain fatty acid. Makkar *et al* (1995) reported that tannins decreased the production of SCFA.

The ME concentration decreased significantly (P<0.05) in TMRs with quebracho treated SBM. The results were in agreement with Gerlach *et al* (2018) who found the ME concentration of the concentrates decreased markedly (−25%) from 12.9 to 9.7 MJ/kg DM (P<0.05), for control and 5% CT, respectively. Rivera-Mendez *et al* (2016) reported tannin supplementation decreased estimated dietary NE (linear effect, P < 0.01).

4.3.2 *In vitro* gas production and digestibility of TMRs with tannic acid treated SBM

Results of *in vitro* gas production and digestibility of tannic acid TMRs with tannic acid treated SBM are given in Table 22. The NGP/200 mg DM in the current study was significantly (P<0.05) reduced in TMR containing tannic acid treated SBM and was lowest in 4TRH. It was ranging from 42.07 to 46.36 in 4TRH and COT, respectively. Bhatta *et al* (2014) reported 24-h gas production linearly decreased at more than 5% of tannin level. Jayanegara *et al* (2015b) reported chestnut and sumach (purified hydrolysable tannin) decreased total gas production (P≤0.05). Getachew *et al* (2008b) reported TA decreased gas production. El-Waziry (2005) found that the highest Mean values of cumulative gas production during 72 h was obtained by SBM followed by roasted SBM, SBM treated with 1%TA, SBM treated with 2%TA and SBM treated with 3% TA. Getachew *et al* (2008a) reported that the addition of TA had no influence on the extent and rate of gas production at 30, 60 and 90 g/kg DM. Jayanegara *et al* (2015a) reported that the all purified tannins decreased total gas production. Gunun *et al* (2017) found that rambutan peel powder supplementation did not affect gas production kinetics (p>0.05).

Table 22. *In vitro* gas production and digestibility of TMRs containing tannic acid treated SBM

Variable	COT	1TRL	1TRH	2TRL	2TRH	3TRL	3TRH	4TRL	4TRH
NGP/200 mg DM	46.36^{a}±0.47	44.81^{b}±0.16	43.48^{cd}±0.10	44.25^{bc}±0.12	43.36^{cd}±0.26	43.14^{d}±0.24	42.75^{de}±0.47	43.22^{d}±0.24	42.07^{e}±0.44
NH_3-N, mg/dl	40.00^{a}±0.56	37.92^{b}±0.50	35.76^{bc}±0.28	36.49^{bc}±0.13	34.90^{cd}±0.15	35.77^{bc}±1.41	33.40^{de}±0.78	32.12^{ef}±0.50	30.21^{f}±0.28
PF, mg/ml	2.80±0.04	2.86±0.04	2.92±0.00	2.91±0.04	2.93±0.03	2.90±0.00	2.74±0.22	2.90±0.05	3.02±0.08
OMD,%	73.58±0.04	73.20±0.86	71.78±0.08	73.18±0.97	71.83±0.38	71.52±0.49	67.66±5.59	71.91±0.53	72.40±0.80
NDFD, %	57.58±0.06	56.27±1.41	55.95±0.12	55.38±1.62	55.22±0.60	53.22±0.80	46.24±9.29	53.61±0.88	55.78±1.28
MMP, mg	140.87^{d}±1.78	145.21^{c}±0.83	151.24^{ab}±0.32	147.64^{bc}±0.79	151.02^{ab}±1.41	151.56^{ab}±0.70	151.46^{ab}±2.29	149.82^{ab}±1.15	153.63^{a}±1.97
EMMP, %	57.22^{d}±1.27	59.80^{bc}±0.63	64.06^{abc}±0.10	61.11^{abc}±0.84	64.16^{abc}±0.09	63.89^{abc}±1.03	67.92^{a}±5.90	63.20^{abc}±0.20	65.23^{ab}±0.30
DMD, %	75.03±0.23	74.70±0.37	73.86±0.20	74.87±0.86	73.47±0.20	73.18±0.70	70.66±4.61	74.14±0.20	74.24±0.83
SCFA, mmole	1.89^{a}±0.02	1.83^{b}±0.01	1.76^{c}±0.01	1.81^{b}±0.00	1.76^{c}±0.01	1.76^{c}±0.02	1.74^{cd}±0.02	1.76^{c}±0.01	1.71^{d}±0.02
ME, MJ/kg DM	9.57^{a}±0.07	9.28^{bc}±0.02	9.08^{efg}±0.01	9.37^{b}±0.02	9.23^{bcd}±0.04	9.12^{def}±0.03	9.01^{fg}±0.07	9.20^{cde}±0.04	8.95^{g}±0.06

Figures with different superscripts in a row differ significantly, P<0.05

Ammonical nitrogen was significantly (P<0.05) higher in COT and was lowest in 4TRH. Zhou *et al* (2019) reported that the TA at the rate of 16.9 g/kg decreased the ruminal concentration of NH_3-N (P<0.001). Bhatta *et al* (2014) reported at the end of 24-h there was a significant (P<0.05) reduction in the NH_3-N (mg/dl) concentration at the inclusion of tannin level more than 5%. Getachew *et al* (2008b) reported that the addition of TA reduced NH_4^+-N concentration by 14%, 31% and 67% at the TA levels of 30, 60 and 90 g/kg DM, respectively. El-Waziry (2005) found that the concentrations of NH_3 -N were significantly decreased when SBM treated by heating or TA. Singh *et al* (2001) found that the ammonia concentration decreased in the presence of tannic acid. Getachew *et al* (2008a) reported that the addition of TA had significantly decreased NH_4-N concentration at 30 (P<0.05), 60 and 90 (P<0.0001) g/kg DM. Gunun *et al* (2017) found that the concentration of NH_3-N decreased linearly with the increasing levels of rambutan peel powder supplementation (P<0.05). Junior *et al* (2017) reported that there was no effect of *Acacia mearnsii* tannins on the concentrations of ruminal NH_3-N. Orlandi *et al* (2015) found that the concentration of ammonia N in ruminal fluid linearly decreased (P<0.05) with tannin extract inclusion.

Partitioning factor did not vary significantly (P<0.05). Similar results were found by Jayanegara *et al* (2015b) who reported a non-significant difference in PF with the addition of chestnut and sumach (purified hydrolysable tannin).

OMD%, as well as NDFD%, was not varying significantly (P<0.05). OMD% was in the range of 67.66% to 73.58%., whereas, NDFD% was varying from 46.24% to 57.58%. El-Waziry *et al* (2005) found that the OMD was higher in SBM and roasting SBM and lowest in SBM treated with 2 or 3% TA. Gemeda and Hassen (2015) revealed that tanniferous browse plants significantly (P<0.05) decreased IVOMD. Orlandi *et al* (2015) found that the ruminal OM digestibility of feed linearly decreased (P<0.05) with increasing tannin extract levels.

MMP was significantly (P<0.05) higher in the 4TRH and it was lowest (P<0.05) in the COT. EMMP was varying significantly (P<0.05) in the TMRs containing tannic acid treated SBM compare to COT. It was found significantly (P<0.05) higher in the 3TRH and was found lowest in COT. Getachew *et al* (2008b) also found similar results

and reported that the efficiency of microbial protein synthesis increased when 50 and 100 g TA/kg DM was added compared with the control.

DMD was lower non-significantly (P<0.05) in TMRs containing tannic acid treated SBM. Results were in agreement with Salawu *et al* (1999) who found the silage containing tannins had lower (P<0.05) DM disappearance in the rumen than the control silage. Results were in agreement with Getachew *et al* (2008b) who reported that the TA did not reduced *in vitro* true degradability of dry matter (P<0.05) at 30 g/kg DM compared with the control. Gunun *et al* (2017) found that rambutan peel powder supplementation did not affect *in vitro* digestibility (p>0.05).

Short-chain fatty acid was lowest significantly (P<0.05) in 4TRH and was found highest in COT. Results were in agreement with Getachew *et al* (2008b) who reported TA decreased Short-chain fatty acid. Getachew *et al* (2008a) reported that Spraying of alfalfa with TA did not affect (P<0.05) the total and individual SCFA acid production. Jayanegara *et al* (2015b) reported chestnut tannins decreased total SCFA (P $\leq$ 0.05).

ME values were ranging from the 8.95 to 9.57 MJ/kg DM which decreased significantly (P<0.05) in TMR containing tannic acid treated SBM and was found lowest in 4TRH and was highest in COT. El-Waziry *et al* (2005) found that the predicted ME and NE which calculated from gas production after 24 h incubation were reduced when SBM was treated by heating or TA. Gemeda and Hassen (2015) revealed that the tanniferous browse plants significantly (P<0.05) decreased ME content.

4.3.3 Comparison between TMRs containing tannic acid treated SBM and Quebracho treated SBM on the basis of *in vitro*

Figure 7 shows that quebracho was effectively reducing the ammonical nitrogen at higher level. Comparison between treatments showed that there was more reduction of ammonical nitrogen in TMRs with tannic acid treated SBM. Tannic acid reduced ammonical nitrogen and ultimately protected protein more efficiently than the TMRs with quebracho treated SBM. Figure 8 and 9 shows DMD and OMD, respectively. There was a perceptive reduction of DMD as well as OMD at the 3TRH and at higher levels.

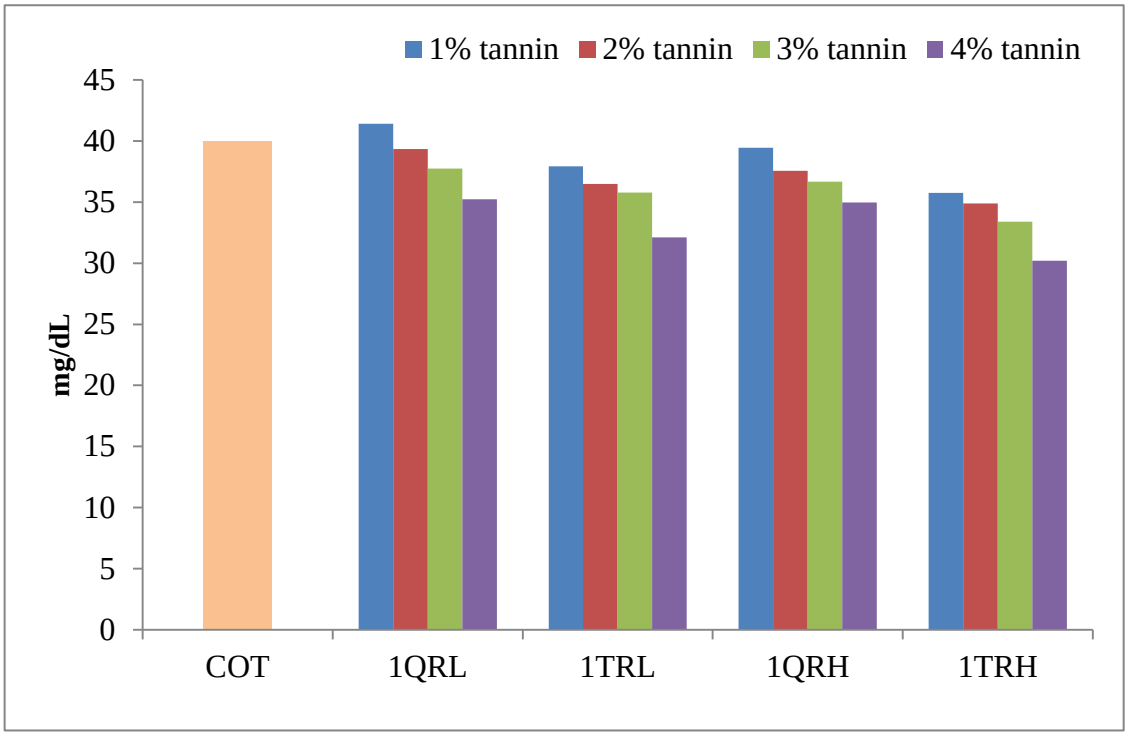

Fig 7. ***In vitro*** **Ammonical Nitrogen of TMRs containing tannic acid treated SBM and quebracho treated SBM, mg/dL**

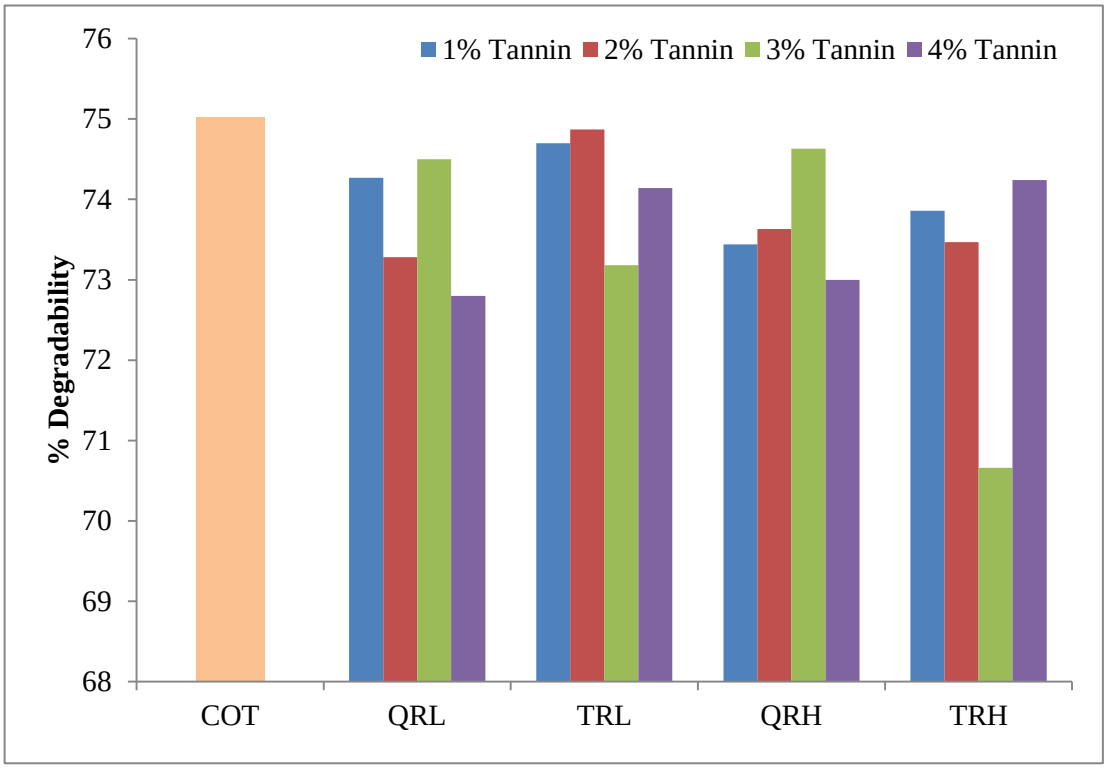

Fig 8. ***In vitro*** **Dry matter degradability of TMRs containing tannic acid treated SBM and quebracho treated SBM, %**

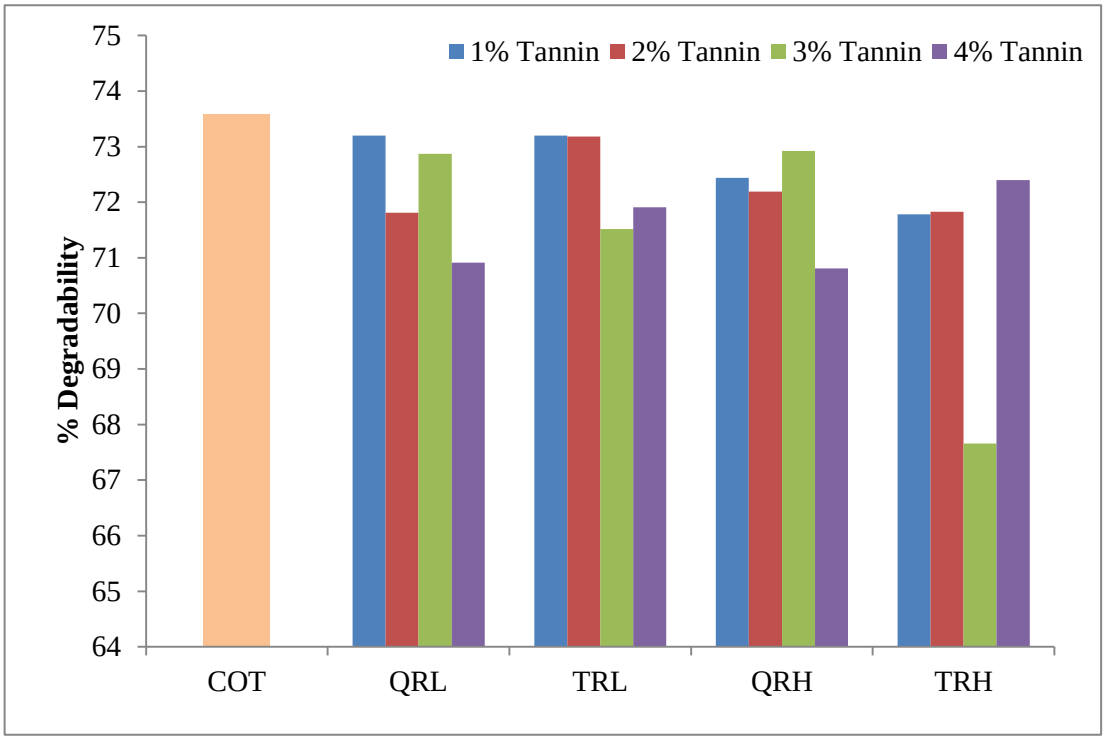

Fig 9. *In vitro* Organic matter degradability of TMRs containing tannic acid treated SBM and quebracho treated SBM, %

4.3.4 *In vitro* kinetic parameters TMRs with tannin treated SBM

Results of TMRs with tannin treated SBM on *in vitro* kinetics parameters are given in Table 23. Soluble fraction (a) was significantly (P<0.05) lower in 3QRH but the difference between other all treatments was significant (P<0.05) from the control. Potentially degradable fraction (a+b) was significantly differing in 2QRH and a higher level of TMRs with quebracho treated SBM. There was no significant (P<0.05) difference in the degradation time as well as t-half with the different level of quebracho treatment. Frutos *et al* (2002) noted that the CT was positively correlated with lag time.

Soluble fraction (a) was significantly (P<0.05) lower in TMRs with tannic acid treated SBM compared to control (Table 24). Potentially degradable fraction (a+b) was lowest (P<0.05) in the 4TRH but effect was significant (P<0.05) in more than 2% tannic acid TMRs with tannic acid treated SBM. Values of a+b were lower in all of the TMRs with tannic acid treated SBM but in 1% TMRs with tannic acid treated SBM, values were not differing significantly (P<0.05) as compared to control. The degradation rate was lowest (P<0.05) in 4TRH and 3TRH but also in the 1TRH degradation rate reduced significantly (P<0.05) compared to control.

Table 23. Effect of Quebracho treatment on the *in vitro* kinetics parameters by exponential model of TMRs

Parameters	COT	1QRL	1QRH	2QRL	2QRH	3QRL	3QRH	4QRL	4QRH
a	1.5447^{ab}± 0.28	1.5490^{ab}± 0.08	1.7180^{a}± 0.11	1.2527^{abc}± 0.16	1.3887^{ab}± 0.29	1.0544^{bc}± 0.29	0.6246^{c}± 0.20	0.9415^{bc}± 0.03	1.1266^{abc}± 0.14
(a+b)	50.3500^{a}± 0.57	49.0333^{ab}± 0.41	49.4700^{ab}± 0.47	49.4833^{ab}± 0.45	48.6300^{bc}± 0.54	48.4567^{bc}± 0.47	48.3733^{bc}± 0.32	47.3233^{cd}± 0.27	46.6333^{d}± 0.66
Degradation rate	0.1038± 0.00	0.1035± 0.00	0.1008± 0.00	0.1025± 0.00	0.1000± 0.00	0.1001± 0.00	0.1035± 0.00	0.1027± 0.00	0.1004± 0.00
Half-Time	6.6827± 0.08	6.6983± 0.04	6.8730± 0.04	6.7623± 0.09	6.9357± 0.07	6.9283± 0.16	6.7000± 0.09	6.7500± 0.11	6.9073± 0.06
b	48.8067^{a}± 0.78	47.4867^{ab}± 0.34	47.7533^{ab}± 0.38	48.2267^{a}± 0.35	47.2433^{ab}± 0.83	47.4033^{ab}± 0.28	47.7500^{ab}± 0.20	46.3767^{bc}± 0.25	45.5033^{c}± 0.78

Figures with different superscripts in a row differ significantly, P<0.05

Table 24. Effect of Tannic acid treatment on the *in vitro* kinetics parameters by an exponential model of TMRs

Parameters	COT	1TRL	1TRH	2TRL	2TRH	3TRL	3TRH	4TRL	4TRH
a	$1.5447^{a}\pm$ 0.28	$0.1484^{b}\pm$ 0.50	$-0.0861^{b}\pm$ 0.29	$0.2321^{b}\pm$ 0.39	$-0.3613^{b}\pm$ 0.04	$-0.1135^{b}\pm$ 0.16	$-0.3465^{b}\pm$ 0.30	$-0.0916^{b}\pm$ 0.12	$-0.6300^{b}\pm$ 0.29
(a+b)	$50.3500^{a}\pm$ 0.57	$49.4367^{a}\pm$ 0.62	$49.5033^{a}\pm$ 0.27	$47.9233^{b}\pm$ 0.15	$47.3767^{b}\pm$ 0.26	$47.6500^{b}\pm$ 0.35	$47.3667^{b}\pm$ 0.78	$47.7300^{b}\pm$ 0.35	$46.6833^{b}\pm$ 0.61
Degradation rate	$0.1038^{ab}\pm$ 0.00	$0.1039^{ab}\pm$ 0.00	$0.0998^{bc}\pm$ 0.00	$0.1065^{a}\pm$ 0.00	$0.1039^{ab}\pm$ 0.00	$0.0988^{bc}\pm$ 0.00	$0.0980^{c}\pm$ 0.00	$0.0985^{bc}\pm$ 0.00	$0.0972^{c}\pm$ 0.00
Half-Time	$6.6827^{bc}\pm$ 0.08	$6.6717^{bc}\pm$ 0.08	$6.9443^{ab}\pm$ 0.06	$6.5147^{c}\pm$ 0.18	$6.6717^{bc}\pm$ 0.04	$7.0153^{ab}\pm$ 0.07	$7.0793^{a}\pm$ 0.19	$7.0387^{ab}\pm$ 0.09	$7.1350^{a}\pm$ 0.12
b	$48.8067^{ab}\pm$ 0.78	$49.2900^{a}\pm$ 1.05	$49.5900^{a}\pm$ 0.31	$47.6933^{ab}\pm$ 0.26	$47.7400^{ab}\pm$ 0.25	$47.7600^{ab}\pm$ 0.29	$47.7133^{ab}\pm$ 0.54	$47.8200^{ab}\pm$ 0.35	$48.8067^{b}\pm$ 0.78

Figures with different superscripts in a row differ significantly, $P<0.05$

4.3.5 Effect of tannins on *in vitro* fermentation of TMRs irrespective of level and source of tannins

NGP/200 mg DM was significantly (P<0.05) lower than control (Table 25). It was lowest (P<0.05) in TRH and was highest (P<0.05) in the COT. NH_3 – N was lowest (P<0.05) in TRH and was highest (P<0.05) in COT. It was varying from 33.57 to 40.0 mg/dL. Partitioning factor was improved in the treatment but statistical (P<0.05) difference was not found. There was no statistical (P<0.05) difference in %DMD, OMD% and NDFD%. MMP was significantly (P<0.05) higher in the TRH and it was lowest in COT. EMMP was ranging from 65.34 to 57.22% and there was significant (P<0.05) difference. It was found higher in TRH. Short-chain fatty acid as well ME values were lower for the treatments significantly (P<0.05) as compared to control. significant (P<0.05) difference in the NGP/200mg DM was found in quebracho as well tannic acid treated samples but there was no significant (P<0.05) difference in ammonical nitrogen in quebracho but tannic acid caused significant (P<0.05) reduction in the ammonical nitrogen. MMP in all the treated samples were higher than the COT but TRH had highest. So it was concluded that TRH was having better effects on protein protection and improving ruminal parameters.

Results of the study revealed that the Replacement of SBM with tannic acid treated SBM in TMR reduced NGP/200mg DM significantly (P<0.05) from the control as well quebracho treated SBM contain TMRs irrespective of the level of treatment of SBM and level of replacement (Table 26). Quebracho reduced NGP/200mg DM significantly (P<0.05) from the control but it was higher than the TMR with tannic acid treated SBM. The same trend NGP/200mg DM was found for the NH_3-N it was lowest significantly (P<0.05) in the TMRs with tannic acid treated SBM but the TMRs with quebracho treated SBM was not having significant (P<0.05) effect from the control. There was no significant (P<0.05) difference in PF, DMD, OMD and NDFD %. MMP and EMMP were significantly (P<0.05) improved in quebracho as well as tannic acid treated SBM. There was significant (P<0.05) reduction in short-chain fatty acids and ME in both the treatments and the results showed there was more reduction in tannic acid compared to quebracho and result between both treatments were also significant(P<0.05). From the results, it was concluded that tannic acid reduces NGP as well as ammonical nitrogen which means tannic acid is more efficient for protecting the protein from ruminal degradation.

Table 25. Effect of replacement level of SBM with treated soybean meal in TMR on *in vitro* gas production parameters, irrespective of level of tannin

Variable	COT	QRL	QRH	TRL	TRH
NGP/200mg DM	46.36^{a}±0.47	44.66^{b}±0.21	44.32^{b}±0.24	43.86^{b}±0.23	42.92^{c}±0.23
NH_3-N, mg/dL	40.00^{a}±0.56	38.43^{ab}±0.89	37.16^{ab}±0.68	35.58^{bc}±0.86	33.57^{c}±0.82
PF, mg/ml	2.80±0.04	2.85±0.02	2.89±0.01	2.89±0.01	2.90±0.06
OMD,%	73.58±0.04	72.20±0.40	72.09±0.30	72.45±0.40	70.92±1.29
NDFD, %	57.58±0.06	53.34±0.40	53.75±0.51	54.62±0.66	53.30±2.35
MMP, mg	140.87^{c}±1.78	147.86^{b}±0.61	149.09^{ab}±0.88	148.56^{b}±0.81	151.84^{a}±0.78
EMMP, %	57.22^{c}±1.27	61.34^{b}±0.55	62.61^{ab}±0.64	62.00^{b}±0.68	65.34^{a}±1.26
DMD, %	75.03±0.23	73.71±0.31	73.68±0.26	74.22±0.33	73.05±1.03
SCFA, mmole	1.89^{a}±0.02	1.83^{b}±0.01	1.80^{b}±0.01	1.79^{b}±0.01	1.74^{c}±0.01
ME, MJ/kg DM	9.57^{a}±0.07	9.38^{b}±0.04	9.33^{b}±0.05	9.24^{b}±0.03	9.07^{c}±0.04

Figures with different superscripts in a row differ significantly, P<0.05

QRL- TMR containing concentrate mixture that have 25% treated SBM independent of the level of quebracho.

QRH- TMR containing concentrate mixture that have 50% treated SBM independent of the level of quebracho.

TRL- TMR containing concentrate mixture that have 25% treated SBM independent of the level of tannic acid.

TRH- TMR containing concentrate mixture that have 50% treated SBM independent of the level of tannic acid.

Table 26. Effect of source of tannins in TMR on *in vitro* gas production parameters

Variable	Control	Quebracho	Tannic acid
NGP/200mg DM	46.36^{a}±0.47	44.49^{b}±0.16	43.39^{c}±0.18
NH_3-N, mg/dL	40.00^{a}±0.56	37.80^{a}±0.56	34.57^{b}±0.63
PF, mg/ml	2.80±0.04	2.87±0.01	2.90±0.03
OMD,%	73.58±0.04	72.14±0.24	71.68±0.68
NDFD, %	57.58±0.06	53.54±0.32	53.96±1.19
MMP, mg	140.87^{b}±1.78	148.48^{a}±0.54	150.20^{a}±0.65
EMMP, %	57.22^{b}±1.27	61.98^{a}±0.44	63.67^{a}±0.82
DMD, %	75.03±0.23	73.69±0.20	73.64±0.55
SCFA, mmole	1.89^{a}±0.02	1.82^{b}±0.01	1.77^{c}±0.01
ME, MJ/kg DM	9.57^{a}±0.07	9.36^{b}±0.03	9.16^{c}±0.03

Figures with different superscripts in a row differ significantly, P<0.05

Results of the level of tannins on *in vitro* gas production and digestibility of TMRs irrespective of the source of replacement level of treated soybean meal is given in Table 27. There is a linear effect of tannins on NGP/200mg DM and difference was significant (P<0.05) as compared to control, the highest being in control. Ammonical nitrogen was significantly (P<0.05) lower in 4R and highest in COT. COT was not varying significantly (P<0.05) with 1R. Partitioning factor improved for the tannin treated samples compared to COT. There was no significant (P<0.05) difference in partitioning factor but it was numerically highest in 4R. There was no significant (P<0.05) difference in % OMD, NDFD and DMD but all these parameters in treatments with various concentrations decreased numerically as a level of tannins was increased. There was significant (P<0.05) improvement in MMP and highest values were found for the 4R. COT had differing significantly (P<0.05) lower MMP from all of the treatments. EMMP was not varying (P<0.05) in between treatments but all the treatments were significantly (P<0.05) higher than the COT. Short-chain fatty acid and ME was reduced significantly (P<0.05) from the COT and trend for both parameters were same (P<0.05) and it was lowest (P<0.05) in the 4R and was highest (P<0.05) in COT. Significant (P<0.05) effect compared to COT on the gas production

Table 27. Effect of level of tannins on i*n vitro* gas production and digestibility of TMRs, irrespective of the source of tannins replacement level of treated soybean meal

Variable	COT	1R	2R	3R	4R
NGP/200 mg DM	46.36^{a}±0.47	44.64^{b}±0.24	44.31^{bc}±0.24	43.60^{cd}±0.25	43.20^{d}±0.25
NH_3-N, mg/dL	40.00^{a}±0.56	38.63^{ab}±0.82	37.07^{b}±0.62	35.90^{b}±0.72	33.14^{c}±0.81
PF, mg/ml	2.80±0.04	2.88±0.02	2.89±0.02	2.86±0.05	2.91±0.03
OMD,%	73.58±0.04	72.65±0.33	72.25±0.31	71.24±1.33	71.51±0.32
NDFD, %	57.58^{a}±0.06	55.12^{ab}±0.58	54.08^{ab}±0.60	52.01^{b}±2.18	53.80^{ab}±0.62
MMP, mg	140.87^{c}±1.78	147.56^{b}±0.88	148.74^{ab}±0.78	150.03^{ab}±0.74	151.03^{a}±0.83
EMMP, %	57.22^{b}±1.27	61.22^{a}±0.71	62.14^{a}±0.57	63.70^{a}±1.52	64.23^{a}±0.35
DMD, %	75.03±0.23	74.07±0.23	73.81±0.33	73.24±1.07	73.54±0.30
SCFA, mmole	1.89^{a}±0.02	1.82^{b}±0.01	1.81^{b}±0.01	1.78^{bc}±0.01	1.76^{c}±0.01
ME, MJ/kg DM	9.57^{a}±0.07	9.33^{b}±0.05	9.36^{b}±0.03	9.22^{bc}±0.05	9.12^{c}±0.04

Figures with different superscripts in a row differ significantly, P<0.05

1R- 1% tannin treated SBM containing TMR irrespective of the source of tannin

2R- 2% tannin treated SBM containing TMR irrespective of the source of tannin

3R- 3% tannin treated SBM containing TMR irrespective of the source of tannin

4R- 4% tannin treated SBM containing TMR irrespective of the source of tannin

was seen even at 1% tannin level whereas significant (P<0.05) effect on ammonical nitrogen was seen at 2% and higher levels of the tannins. At 1% tannin level there was a significant (P<0.05) effect on MMP compared to COT.

TMRs with tannin treated SBM made by replacing soybean meal 25% or 50% with different concentration of tannin treated soybean meal with either quebracho or tannic acid. Comparison of different level of replacement irrespective of source and concentration of tannin used to treat SBM in TMR is given in Table 28. NGP/200 mg DM was reduced significantly (P<0.05) in the 25R and 50R. Ammonical nitrogen reduced significantly (P<0.05) in the 50R but not in 25R as compared to COT and it was in between of those two. Partitioning factor was improved non-significantly (P<0.05) in both of the treatments compared to COT. There was no significant (P<0.05) difference in % OMD, NDFD and DMD but all these parameters in 25 R and 50R decreased numerically.

Table 28. Effect of replacement level of treated SBM on *in vitro* gas production and digestibility of TMRs, irrespective of the source of tannins and treatment level of soybean meal

Variable	COT	25R	50R
NGP/200 ml/200mg	46.36^{a}±0.47	44.26^{b}±0.17	43.62^{b}±0.22
NH_3-N, mg/dL	40.00^{a}±0.56	37.00^{ab}±0.70	35.37^{b}±0.69
PF, mg/ml	2.80±0.04	2.87±0.01	2.90±0.03
OMD,%	73.58±0.04	72.32±0.28	71.50±0.66
NDFD, %	57.58±0.06	53.98±0.41	53.52±1.16
MMP, mg	140.87^{b}±1.78	148.21^{a}±0.50	150.47^{a}±0.64
EMMP, %	57.22^{b}±1.27	61.67^{a}±0.43	63.98^{a}±0.77
DMD, %	75.03±0.23	73.97±0.23	73.37±0.52
SCFA, mmole	1.89^{a}±0.02	1.81^{b}±0.01	1.77^{b}±0.01
ME, MJ/kg DM	9.57^{a}±0.07	9.31^{b}±0.03	9.20^{b}±0.04

Figures with different superscripts in a row differ significantly, P<0.05
25R- TMR containing 2.5% of treated SBM
50R - TMR containing 5.0% of treated SBM

There was significant (P<0.05) improvement in MMP at both levels and highest values were found for the 50R. EMMP was not varying (P<0.05) in between treatments but both the treatments were significantly (P<0.05) higher than the COT. Short-chain fatty acid and ME was reduced significantly (P<0.05) from the COT and

it was lowest (P<0.05) in the 50R but there was no significant (P<0.05) difference between the levels of replacement. 25R was having a significant (P<0.05) difference for the NGP but was not varying significantly (P<0.05) for the NH_3-N compared to COT which indicates lower protein protecting effects than the 50R.

Results of *in vitro* gas production and digestibility of TMRs containing quebracho treated SBM irrespective of the level of tannins is given In Table 29. NGP/200 mg DM was found significantly (P<0.05) in the TMR containing quebracho treated SBM it was lowest (P<0.05) in the 4QR whereas it was highest in COT. It showed a decreasing trend as the level of quebracho tannin increased in TMR. Ammonical nitrogen and OMD was highest in COT and was lowest in 4QR with 1QR, 2QR and 3QR being similar. NDFD and MMP were significantly higher (P<0.05) in COT as compared to treatment where 1QR was similar across the level of quebracho used. EMMP was improved significantly (P<0.05) in the TMR containing quebracho treated SBM and was lowest (P<0.05) in COT. DMD % was significantly (P<0.05) reduced on the addition of quebracho treated SBM into the TMR and it was found lowest in the 4QR. Short-chain fatty acid as well ME was decreasing in the TMRs containing quebracho treated SBM and it was lowest (P<0.05) in 4QR. The effect of quebracho on parameters like NGP/200 mg DM, ammonical nitrogen, NDFD, MMP and SCFA was observed significant (P<0.05) at all the levels of Quebracho treatment.

Results of *in vitro* gas production and digestibility of TMRs containing tannic acid treated SBM irrespective of the level of tannins is given In Table 30. NGP/200 mg DM and NH_3-N were significantly (P<0.05) reduced in all the levels of Tannic acid as compared to COT, 4TR being the lowest. Ammonical nitrogen was significantly (P<0.05) lower in the 4TR from all of the treatments as well as COT. Ammonical nitrogen in other treatments was also lower (P<0.05) in the treated samples than control. PF was numerically higher in the treatments compared to COT but there was no significant (P<0.05) difference in the PF values. OMD, NDFD and DMD were not varying significantly (P<0.05) but it was lower numerically in the TMRs containing tannic acid treated SBM. MMP and EMMP were significantly (P<0.05) improved in the treated samples. Short-chain fatty acid was significantly (P<0.05) lower in all treatments and was lowest in the 4TR. ME was significantly (P<0.05) lower in the treated samples compared to COT. The effect of tannic acid on parameters like NGP/200 mg DM, ammonical nitrogen, MMP was observed significant (P<0.05) even at 1% tannic acid level.

Table 29. *In vitro* gas production parameters of TMRs containing quebracho treated SBM irrespective of level of replacement of SBM

Variable	COT	1QR	2QR	3QR	4QR
NGP/200mg DM	46.36^{a}±0.47	45.13^{b}±0.27	44.82^{bc}±0.31	44.26^{cd}±0.21	43.75^{d}±0.18
NH_3-N, mg/dL	40.00^{a}±0.56	36.84^{b}±0.67	35.70^{b}±0.47	34.59^{b}±0.95	31.16^{c}±0.60
PF, mg/ml	2.80^{b}±0.04	2.86^{ab}±0.03	2.85^{ab}±0.04	2.91^{a}±0.02	2.86^{ab}±0.02
OMD,%	73.58^{a}±0.04	72.82^{ab}±0.43	72.00^{b}±0.24	72.89^{ab}±0.07	70.86^{c}±0.19
NDFD, %	57.58^{a}±0.06	54.12^{b}±0.76	52.85^{b}±0.40	54.28^{b}±0.52	52.91^{b}±0.67
MMP, mg	140.87^{b}±1.78	146.89^{a}±1.13	148.14^{a}±1.19	148.55^{a}±0.61	150.33^{a}±1.02
EMMP, %	57.22^{c}±1.27	60.51^{b}±0.67	61.65^{b}±0.69	61.50^{b}±0.43	64.25^{a}±0.44
DMD, %	75.03^{a}±0.23	73.85^{bc}±0.36	73.46^{cd}±0.35	74.57^{ab}±0.11	72.90^{d}±0.10
SCFA, mmole	1.89^{a}±0.02	1.84^{b}±0.01	1.83^{b}±0.01	1.81^{bc}±0.01	1.78^{c}±0.01
ME, MJ/kg DM	9.57^{a}±0.07	9.47^{ab}±0.04	9.42^{b}±0.05	9.38^{b}±0.03	9.16^{c}±0.02

Figures with different superscripts in a row differ significantly, $p<0.05$

1QR- TMR containing 1% quebracho treated SBM irrespective of the level of replacement.

2QR- TMR containing 2% quebracho treated SBM irrespective of the level of replacement.

3QR- TMR containing 3% quebracho treated SBM irrespective of the level of replacement.

4QR- TMR containing 4% quebracho treated SBM irrespective of the level of replacement.

Table 30. *In vitro* gas production parameters of TMRs containing tannic acid treated SBM irrespective of the level of replacement of SBM

Variable	COT	1TR	2TR	3TR	4TR
NGP/200mg DM	46.36^{a}±0.47	44.14^{b}±0.31	43.81^{bc}±0.24	42.95^{cd}±0.25	42.65^{d}±0.34
NH_3-N, mg/dL	40.00^{a}±0.56	36.84^{b}±0.67	35.70^{b}±0.47	34.59^{b}±0.95	31.16^{c}±0.60
PF, mg/ml	2.80±0.04	2.89±0.02	2.92±0.02	2.82±0.10	2.96±0.05
OMD,%	73.58±0.04	72.49±0.54	72.51±0.58	69.59±2.55	72.16±0.42
NDFD, %	57.58±0.06	56.11±0.58	55.30±0.71	49.73±4.31	54.70±0.89
MMP, mg	140.87^{b}±1.78	148.23^{a}±1.41	149.33^{a}±1.05	151.51^{a}±1.07	151.73^{a}±1.33
EMMP, %	57.22^{b}±1.27	61.93^{ab}±1.26	62.63^{ab}±0.95	65.91^{a}±2.71	64.21^{a}±0.60
DMD, %	75.03±0.23	74.28±0.30	74.17±0.54	71.92±2.04	74.19±0.35
SCFA, mmole	1.89^{a}±0.02	1.79^{b}±0.02	1.79^{b}±0.01	1.75^{bc}±0.01	1.74^{c}±0.01
ME, MJ/kg DM	9.57^{a}±0.07	9.18^{bc}±0.05	9.30^{b}±0.04	9.07^{c}±0.04	9.07^{c}±0.06

Figures with different superscripts in a row differ significantly, $p<0.05$

1TR- TMR containing 1% tannic acid treated SBM irrespective of the level of replacement.

2TR- TMR containing 2% tannic acid treated SBM irrespective of the level of replacement.

3TR- TMR containing 3% tannic acid treated SBM irrespective of the level of replacement.

4TR- TMR containing 4% tannic acid treated SBM irrespective of the level of replacement.

SBM treated with 1% tannic acid significantly (P<0.05) lowered NGP/200mg DM, ammonical nitrogen compared to control and no further effect of higher levels of quebracho and tannic acid was observed indicating that this level of tannic acid is effective in *in vitro* protein protection of SBM. 1TSBM improved PF significantly (P<0.05) as compared to control and was comparable with 2TSBM and 3TSBM. TMR containing quebracho treated SBM did not reduce degradation rate significantly (P<0.05) compared to control but tannic acid reduced degradation rate significantly (P<0.05). Half time was also reduced significantly (P<0.05) in TMRs containing tannic acid treated SBM whereas there was non-significant (P<0.05) difference in case of TMRs containing Quebracho treated SBM. The TMR prepared by replacing 50% SBM with 1% tannic acid treated SBM was selected for *in vivo* trial on the basis of *in vitro* because it reduced ammonical nitrogen compared to control significantly (P<0.05), there was higher EMMP than control and PF was numerically higher.

4.4 Growth trial in buffalo heifers

4.4.1 Chemical evaluation of concentrate mixtures used in *in vivo* trial

Chemical composition of both concentrate mixtures was similar and it is given in Table 31. CP was varying from 21.46 to 22.31. Ash content was 7.77 and 8.01 in treatment and control concentrate mixtures, respectively. EE was 3.77 and 3.56 in treatment and control concentrate mixtures, respectively.

Table 31. Chemical composition of tannic acid treated concentrate mixture (CM) used in *in vivo* trial % DM basis

Parameter	Control	Treatment
DM	91	90
CP	22.31	21.46
Ash	8.01	7.77
OM	91.99	92.23
EE	3.56	3.77
NDF	31.78	32.25
ADF	11.41	11.39
ADL	2.25	2.58
Cellulose	9.16	8.81
Hemicellulose	20.37	20.86

NDF content was 32.25 and 31.78 in treatment and control concentrate mixtures, respectively. ADF content was varying in between 11.39 and 11.41 whereas, ADL was varying between 2.25 to 2.58. Cellulose content was 8.81 in treatment concentrate mixture and it was 9.16 in control concentrate mixture. Hemicellulose content was in between 20.37 to 20.86.

4.4.2 Effect of dietary tannic acid on nutrient intake during growth trial.

Nutrient intake in buffalo heifers by the individual animals throughout the trial was estimated and it is given in Table 32. Intake of different nutrients during feeding trial was estimated and only significant ($P<0.05$) difference was found in CP intake which was significantly ($P<0.05$) lower in the treatment group. There was no significant ($P<0.05$) difference between the control and treatment group in the rest of the parameters. Results observed were in agreement with Jolazadeh *et al* (2015) reported that the treatment of dietary SBM with pistachio extract concentrate did not affect DMI relative to SBM-0. Ebert *et al* (2017) dry matter intake did not differ ($P=0.57$) with condensed tannin extract supplementation as well they didn't find differences ($P\geq0.13$) in OM intake and starch intake among treatments. Nitrogen intake did not differ ($P=0.16$) among treatments. Brown *et al* (2018) found that daily dry matter intakes were similar in *Acacia karroo* leaf. Dschaak *et al* (2011) reported that supplementing CTE decreased intakes of DM and other nutrients. Gunun *et al* (2016) revealed that supplementation with MOSM did not affect feed intake ($P<0.05$). Molina-Botero *et al* (2019) found no significant ($P=0.272$) difference in dry matter intake (DMI) of ground pods of *Enterolobium cyclocarpum* blended with foliage of *Gliricidia sepium*. Koenig *et al* (2018) reported DMI was not affected at 1.2% and 2.4% but tended to decrease at 3.5% CT extract. Aguerre *et al* (2016) reported that the intakes of DM, OM, and CP were similar among dietary tannin levels. However, NDF intake decreased linearly with increasing level of tannin in the diet from 7.2 kg/d at 0 % tannin till 6.5 kg/ d at 1.8% tannin of DM. Raju *et al* (2015) who found that the DM intake through roughage and total DM intake in oak leaves fed groups were higher ($P<0.05$) than the control group. The nutrient intake (g/kg $W^{0.75}$) through DM, OM, CP and TDN was also higher ($P<0.01$) in oak leaves fed a group than grass-fed control. Kai *et al* (2016) reported lower dry matter intake of lambs fed purple prairie clover hay diet ($P<0.01$)than those of lambs fed purple prairie clover hay diet supplemented with polyethylene glycol (PH-p) diet. Rivera-Mendez *et al* (2016) found that the tannin supplementation increased (linear effect, $P <0.01$) dry matter

intake (DMI). Rivera-Mendez *et al* (2017) DMI likewise tended to increase (linear effect, P= 0.14) with level of supplementation.

Table 32. Effect of tannic acid on nutrient intake in buffalo heifers in individual animals throughout the trial

Parameter	Control	Treatment
DM	5.153±0.252	5.034±0.273
CP	0.837^{a}±0.098	0.796^{b}±0.089
Ash	0.482±0.026	0.461±0.028
EE	0.154±0.016	0.160±0.019
NDF	2.444±0.058	2.352±0.056
ADF	1.332±0.041	1.279±0.035
ADL	0.205±0.006	0.207±0.005
Cellulose	1.127±0.035	1.072±0.031
Hemicellulose	1.747±0.049	1.705±0.049

Figures with different superscripts in a row differ significantly, p<0.05

Nutrient intake in buffalo heifers per 100kg of bodyweight of the animals (%B. Wt.) is given in Table 33. Intake of different nutrients during the trial was estimated and there was no significant (P<0.05) difference found between control and treatment group. DM intake per 100kg of bodyweight of the animals (%B.Wt.) per week is given in Fig 10. The graph clearly indicates intake as % of B.Wt. was similar in both groups.

Table 33. Effect of tannic acid on nutrient intake in buffalo heifers per 100kg of bodyweight of the animals

Parameter	Control	Treatment
DM	2.681±0.072	2.672±0.078
CP	0.436±0.028	0.420±0.025
Ash	0.258±0.011	0.251±0.012
EE	0.083±0.005	0.085±0.006
NDF	1.245±0.043	1.243±0.040
ADF	0.665±0.031	0.654±0.030
ADL	0.098±0.006	0.100±0.005
Cellulose	0.567±0.026	0.554±0.025
Hemicellulose	0.939±0.033	0.975±0.044

Figures with different superscripts in a row differ significantly, p<0.05

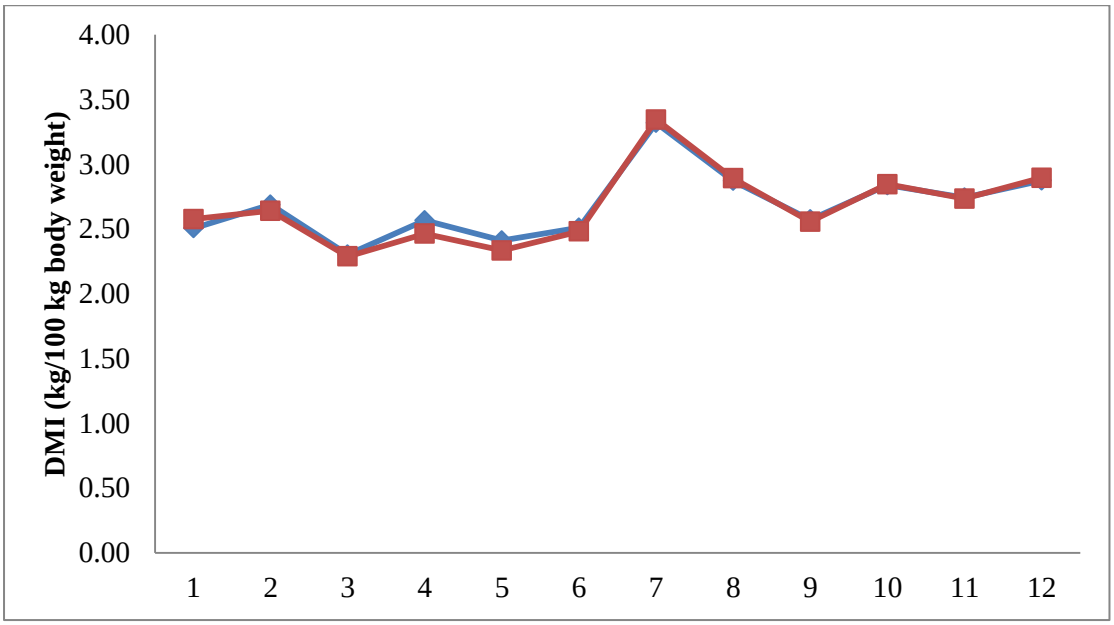

Fig 10. Dry matter Intake on %B.Wt. (weekly)

Nutrient intake in buffalo heifers per metabolic of body weight ($W^{0.75}$) of the animals is given in Table 34. Intake of different nutrients during the trial was estimated and there was no significant ($P<0.05$) difference found between control and treatment group but total DM, CP, ASH, NDF, ADF and cellulose were higher in control group non-significantly ($P<0.05$) whereas EE, ADL and hemicellulose were higher in the treatment group and difference between the two groups was non-significant ($P<0.05$).

Table 34. Effect of tannic acid on nutrient intake (kg) in buffalo heifers per metabolic of body weight ($W^{0.75}$) of the animals

Variable	Control	Treatment
DM	100.90±2.99	99.95±3.23
CP	16.44±1.13	15.77±1.02
Ash	9.71±0.42	9.38±0.47
EE	3.14±0.20	3.21±0.23
NDF	34.64±2.54	28.78±1.63
ADF	24.92±1.04	24.38±0.98
ADL	3.66±0.20	3.74±0.18
Cellulose	21.26±0.86	20.64±0.84
Hemicellulose	35.26±1.17	36.42±1.63

4.4.3 Effect of tannic acid during digestibility trial

Animals were tied for 90 days and faeces outgo was measured. The intakes of DM, CP, DCP, Ash, OM, EE, NDF, ADF, ADL and CELLULOSE were lower significantly (P<0.05) in the treatment group compared to control group animals (Table 35). The difference between hemicellulose intake was found non-significant (P<0.05). Results were in agreement with Kai *et al* (2016) who reported lower dry matter intake of lambs fed purple prairie clover hay diet (P<0.01)than those of lambs fed purple prairie clover hay diet supplemented with polyethylene glycol (PH-p) diet.

Table 35. Intake of nutrients by an individual animal during digestibility trial (kg/animal)

Parameter	Control	Treatment
DM	4.88^{a}±0.15	4.46^{b}±0.16
CP	0.69^{a}±0.02	0.60^{b}±0.02
DCP	0.47^{a}±0.01	0.40^{b}±0.01
Ash	0.36^{a}±0.02	0.33^{b}±0.01
OM	4.52^{a}±0.13	4.13^{b}±0.15
EE	0.15^{a}±0.00	0.13^{b}±0.00
NDF	2.60^{a}±0.10	2.42^{b}±0.10
ADF	1.56^{a}±0.06	1.37^{b}±0.06
ADL	0.26^{a}±0.01	0.23^{b}±0.01
Cellulose	1.30^{a}±0.05	1.14^{b}±0.05
Hemicellulose	1.05±0.04	1.05±0.04

Figures with different superscripts in a row differ significantly, p<0.05

Results of tannic acid on nutrient digestibility (%) in buffalo heifer is given in Table 36. The DM digestibility (%) in control and treatment groups was 58.06 and 56.66%, respectively. There was no significant (P<0.05) difference in DM digestibility between control and treatment groups but it was lower non-significantly (P<0.05) in the treatment group. Yang *et al* (2016) reported that the Supplementing TA at 26.0 g/kg DM decreased the DM digestibility (P<0.05) results in the current study were differing maybe because of a much lower dose of tannic acid in the current study as compared to study of Yang *et al* (2016). Ahnert *et al* (2015) reported that the apparent total tract

digestibility of DM (P≤0.045) was lower at 4 and 6% QTE compared to control and 1 and 2% QTE. Zhou *et al* (2019) reported that the TA at the level of 16.9 g/kg DM decreased the digestibility of the dietary DM (P<0.05). Raju *et al* (2015) reported that the feeding of oak leaves containing a higher level of CT showed a depressing effect on DM digestibility. Jolazadeh *et al* (2015) Treatment of SBM with pistachio extract concentrate did not affect total tract apparent digestibility of DM in bulls fed TMR diets. Mezzomo *et al* (2011) didn't find the effect of condensed tannins on ruminal digestibility of DM. Salem *et al* (2005) found that the 100g acacia feeding had non-significant (P<0.05) DM digestibility. Aguerre *et al* (2016) reported that the DM apparent digestibility% decreased with increasing level of tannins as 66.2, 65.6, 64.2 and 61.5% at 0, 0.45, 0.90 and 1.80 % tannin of DM. feeding tannin extracts linearly increased both wet and dry fecal (kg/d) output which varied from 7.8 DM kg/d at 0% tannin, 8.1 kg/d at 0.45% tannin, 8.5 kg/d at 0.90% tannin and 8.5 kg/d at 1.80% tannin. Dschaak *et al* (2011) reported that there was no significant effect on digestibility of DM and nutrients with CTE supplementation. Ishlak *et al* (2015) didn't find any effect (P<0.05) of feed additives on apparent dry matter (DM) digestibility with the quebracho condensed tannin extract and cinnamaldehyde diets. Supapong *et al* (2017) that the DM digestibility was decreased when *Delonix regia* seed meal levels increased (P<0.05).

Table 36. Effect of tannic acid on nutrient digestibility (%) in buffalo heifers

Parameter	Control	Treatment
DM	58.06±1.18	56.66±1.00
CP	68.64^{a}±0.81	66.39^{b}±0.73
OM	62.02±1.08	60.61±0.88
EE	78.67^{a}±1.21	76.41^{b}±0.82
NDF	45.34±1.73	43.99±1.56
ADF	43.15^{a}±1.78	38.06^{b}±1.76
Cellulose	51.76^{a}±1.49	46.52^{b}±1.63
Hemicellulose	48.59±1.74	51.55±1.41

Figures with different superscripts in a row differ significantly, p<0.05

The CP digestibility (%) in control and treatment groups was 68.04 and 66.39%, respectively (Table 36). There was significant (P<0.05) difference in CP

digestibility in control as compared to the treatment group. Yang *et al* (2016) reported that the Supplementing TA at 6.5, 13.0 or 26.0 g/kg DM decreased the CP digestibility (P<0.01). Gerlach *et al* (2018) found that the *in vivo* digestibility of crude protein of the concentrates was unaffected by CT 1% and decreased significantly with CT 3% (−21%) and CT 5% (-28%; P < .05). Ebert *et al* (2017) reported that the Fecal N linearly increased (P=0.01) with CT inclusion in the diet. Salem *et al* (2005) found that the control and with 100g acacia feeding had similar (P<0.05) CP digestibility. Zhou *et al* (2019) reported that the TA at the level of 16.9 g/kg DM decreased the digestibility of the dietary CP (P<0.001). Kai *et al* (2016) found that the Lambs consuming purple prairie clover hay exhibited lower digestibility of CP (P<0.001) than those fed purple prairie clover hay diet supplemented with polyethylene glycol diet. Raju *et al* (2015) found that the feeding of oak leaves showed a depressing effect on CP digestibility. Aguerre *et al* (2016) reported that the CP apparent digestibility% decreased with increasing level of tannins. Ishlak *et al* (2015) reported that the apparent protein digestibility decreased (P<0.01) with the quebracho condensed tannin extract and cinnamaldehyde diets. Orlandi *et al* (2015) reported that the tannin extract linearly reduced (P<0.05) the total-tract digestibility of N compounds.

The OM digestibility (%) in control and treatment groups was 62.02 and 60.61%, respectively (Table 36) and the difference being non-significant (P<0.05). Jolazadeh *et al* (2015) also found similar effects. They found a treatment of SBM with pistachio extract concentrate did not affect total tract apparent digestibility of OM in bulls fed TMR diets. Mezzomo *et al* (2011) didn't find the effect of condensed tannins on ruminal digestibility of OM. Yang *et al* (2016) reported that the Supplementing TA at 26.0 g/kg DM decreased the OM digestibility (P<0.05). Zhou *et al* (2019) reported that TA at the level of 16.9 g/kg DM decreased the digestibility of the dietary OM (P<0.01)but effect was not found in current study may be due to level of tannic acid was lower compared to Yang *et al* (2016)as well as Zhou *et al* (2019). Salem *et al* (2005) found that the 100g acacia feeding had non-significant (P<0.05) OM digestibility (0.749 and 0.700 in control and treatment, respectively). Aguerre *et al* (2016) reported that the OM apparent digestibility% decreased with increasing level of tannins as 68.1, 67.6, 66.0 and 63.2% at 0, 0.45, 0.90 and 1.80 % tannin of DM. Ishlak *et al* (2015) didn't find any effect (P<0.05) of feed additives on apparent organic matter (OM) digestibility with the quebracho condensed tannin extract and

cinnamaldehyde diets. Orlandi *et al* (2015) reported apparent total-tract organic matter (OM) digestibility was not affected with the inclusion of tannin extract. Supapong *et al* (2017) reported that the OM digestibility was decreased when *Delonix regia* seed meal levels increased from 0 to 270 g/d ($P<0.05$). Tiemann *et al* (2008) reported that the apparent total tract digestibility of organic matter was reduced when the tannin-rich plants were incorporated.

The EE digestibility (%) in control and treatment groups was 78.67 and 76.41%, respectively (Table 36). In the treatment group, digestibility was significantly ($P<0.05$) low as compared to control. These results are in contradictory to Jolazadeh *et al* (2015) found that the treatment of SBM with pistachio extract concentrate did not affect total tract apparent digestibility of EE in bulls fed TMR diets. Zhou *et al* (2019) found that the TA at the level of 16.9 g/kg DM did not affect the digestibility of EE.

The NDF digestibility (%) in control and treatment groups was 45.34 and 43.99%, respectively (Table 36). Mezzomo *et al* (2011), Zhou *et al* (2019) and Jolazadeh *et al* (2015) didn't find the effect of condensed tannins on ruminal digestibility of NDF. Salem *et al* (2005) found that the 100g acacia feeding had a non-significant difference in ($P<0.05$) NDFom (0.712 and 0.680 for control and treatment, respectively) digestibilities. The results maybe because of the difference in tannin source and level of tannin in the study of Salem *et al* (2005). Ishlak *et al* (2015) didn't find any effect ($P<0.05$) of feed additives on apparent neutral detergent fiber (NDF) digestibility with the quebracho condensed tannin extract and cinnamaldehyde diets.

The ADF digestibility (%) in control and treatment groups was 43.15 and 38.06%, respectively (Table 36). In the treatment group, digestibility was significantly ($P<0.05$) low as compared to control. Raju *et al* (2015) also found that the feeding of oak leaves showed a depressing effect on ADF digestibility. Kai *et al* (2016) found that the Lambs consuming purple prairie clover hay exhibited lower digestibility of ADF ($P<0.05$) than those fed purple prairie clover hay diet supplemented with polyethylene glycol diet. Tiemann *et al* (2008) reported that the apparent total tract digestibility of ADF was reduced when the tannin-rich plants were incorporated.

The cellulose digestibility (%) in control and treatment groups was 51.76 and 46.52%, respectively (Table 36). In the treatment group digestibility was lowered with a significant ($P<0.05$) difference compared to control.

The hemicellulose digestibility (%) in control and treatment groups was 48.59 and 51.55%, respectively (Table 36). In the treatment group digestibility was increased with non-significant (P<0.05) difference compared to control.

4.4.4 Effect of dietary tannic acid on growth performance.

4.4.4.1 Effect of tannic acid on body weight gain

Results of tannic acid on body weight gain (kg) in buffalo heifers and average daily weight gain in different groups of the animals is given in Table 37. Difference between body weight control and treatment was non-significant (P<0.05) at starting of the trial but treatment group animals were having lower body weight and weight during the whole trial varied non-significantly (P<0.05). Average weight gain during the first and third month was higher non-significantly (P<0.05) higher in the treatment group than the control group. The results in the present study did not show significant (P<0.05) effect on weight gain. This may be due to higher variation within the group and less number of animals per group as compared to other studies. Average weight gain was higher throughout the trial in the treatment group compared to control but the difference was non-significant (P<0.05). Ahnert *et al* (2015) reported that the mean body weight increased linearly with incremental QTE infusion (P<0.001) from 475 kg at CON 1 to 504 kg at 6% QTE (P≤0.045). Jolazadeh *et al* (2015) reported that the treatment of dietary SBM with pistachio extract concentrate did not affect final BW relative to SBM-0 but linearly increased ADG (L, P=0.001). Raju *et al* (2015) reported an average daily gain (g/d) was higher (P<0.01) in oak leaves fed group (32.5) compared to control (13.5). Aguerre *et al* (2016) reported BW change kg/d was higher with the inclusion of tannin it was 0.13, 0.62, 0.48 and 0.54 for the 0, 0.45, 0.90 and 1.80 % tannin of DM. Rivera-Mendez *et al* (2016) reported tannin supplementation did not affect (P = 0.97) daily weight gain. Rivera-Mendez *et al* (2017) reported supplemental tannin increased (6.5%, P = 0.05) average daily gain (ADG), with a tendency (linear effect, P= 0.15) for ADG to increase with the level of supplementation. Ebert *et al* (2017) found no differences (P ≥ 0.86) were observed in final BW and ADG with condensed tannin extract supplementation. Brown *et al* (2018) reported there were no effects of dietary treatments on final live weights of goats. However, live weight gains were higher in goats fed a diet containing 50% *Acacia karroo* leaf meal inclusion level. Koenig *et al* (2018) noted no effect (P ≥

0.12) of increasing CT extract on ADG. Pathak *et al* (2017) found that the total body weight gain (kg) and average daily gain (g) were significantly higher in CT-1.5 followed by CT-1 and CT-0, respectively.

Table 37. Effect of tannic acid on body weight gain (kg) and average daily

Parameter	Control	Treatment
Initial Weight	175.00±13.48	169.00±12.40
1st-month weight	201.50±16.32	198.00±15.03
2nd-month weight	210.13±16.87	207.50±15.23
3rd-month weight	226.25±16.14	225.38±15.07
Av wt. g/d	0.69±0.04	0.76±0.06

4.4.4.2 Effect of tannic acid on feed efficiency (gain in grams/kg feed intake)

Results of tannic acid on feed efficiency in buffalo heifers in individual animals throughout the trial is given in Table 38. It was non-significant (P<0.05) in case of total DM intake. Except for total DM, all the nutrients showed a significant (P<0.05) difference in weight gain per kg nutrient intake and was higher in the treatment group. Rivera-Mendez *et al* (2017) reported tannin supplementation increased gain efficiency (5.5%, P = 0.04). Jolazadeh *et al* (2015) reported the inclusion of PEC increased feed efficiency (L, P=0.001) relative to SBM-0. Dschaak *et al* (2011) also reported increased feed efficiency

Table 38. Effect of tannic acid on feed efficiency (gain in grams/kg feed intake)

Parameter	Control	Treatment
DM	135.103±15.372	153.157±17.931
CP	961.223^{a}±177.126	1094.850^{b}±193.778
EE	4999.800^{a}±839.376	5573.590^{b}±1034.090
NDF	330.094±8.157	343.059±8.673
ADF	499.808^{a}±28.728	574.135^{b}±35.127
Cellulose	591.023^{a}±34.365	684.298^{b}±40.300
Hemicellulose	384.881^{a}±30.154	434.716^{b}±33.834

Figures with different superscripts in a row differ significantly, p<0.05

4.4.4.3 Effect of tannic acid on FCR.

FCR in buffalo heifers throughout the trial is given in Table 39. The feed conversion ratio was significantly (P<0.05) lower in the treatment group compared to the control group for all the nutrients except in the DM in which it was numerically lower in the treatment group. Raju *et al* (2015) also reported feed gain ratio (DMI/unit body weight gain) which was comparatively better in oak leaves fed group (22.9) than control (39.5). Rivera-Mendez *et al* (2017) reported tannin supplementation increased gain efficiency (5.5%, P = 0.04). Pathak *et al* (2017) found that feed conversion ratio (FCR) in lambs was significantly (linear, p<0.01) higher for CT-1.5 than CT-0.

Table 39. Effect of tannic acid on FCR

Parameter	Control	Treatment
DM	8.185±0.864	7.265±0.790
CP	1.375^{a}±0.228	1.180^{b}±0.189
Ash	0.768^{a}±0.084	0.666^{b}±0.076
EE	0.251^{a}±0.038	0.238^{b}±0.039
NDF	3.044±0.072	2.929±0.070
ADF	2.051^{a}±0.111	1.792^{b}±0.104
ADL	0.316^{a}±0.016	0.292^{b}±0.020
Cellulose	1.736^{a}±0.095	1.500^{b}±0.084
Hemicellulose	2.720^{a}±0.196	2.407^{b}±0.173

Figures with different superscripts in a row differ significantly, p<0.05

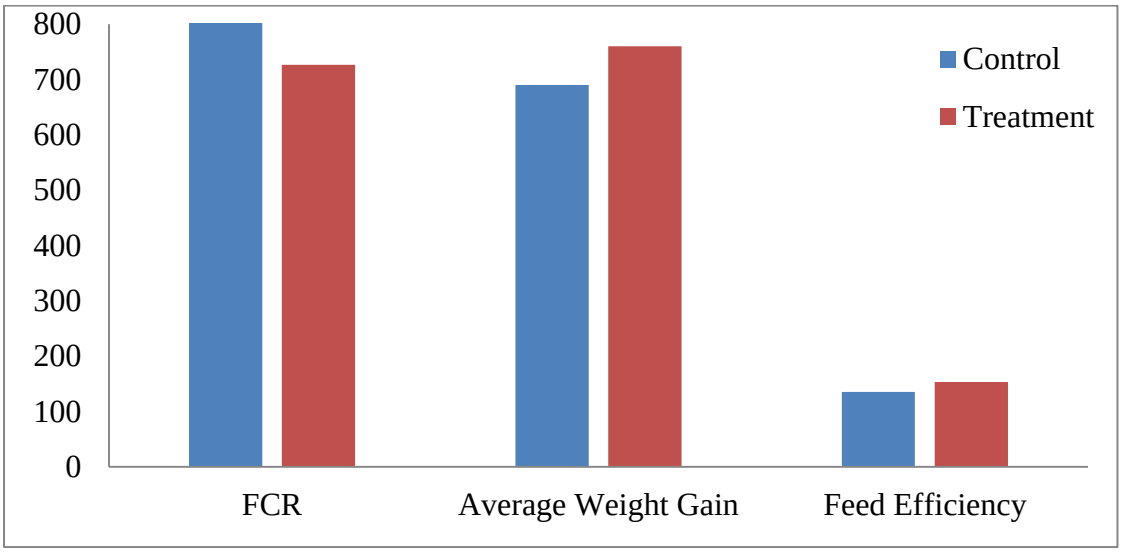

Fig 10. Effect of Tannic acid treatment of SBM on performance parameters

Feed conversion ratio in gms DMI/Kg gain in B.Wt. of the animal was improved and decreased in the treatment group (Fig 11). Average weight gain as weight gain in grams/day was comparatively higher in the treatment group. Feed efficiency as weight gain in grams/kg DMI was higher in the treatment group compared to control.

4.4.5 Effect of dietary tannic acid on blood profile in buffalo heifers.

Blood profile of buffalo heifers at the end of the feeding trial is given in Table 40. Alkaline Phosphatase and Alanine transaminase both were found significantly ($P<0.05$) lower in the treatment group compared to control at the end of the trial of 90 days. Rest all the parameters were varying non-significantly ($P<0.05$). Jolazadeh *et al* (2015) reported albumin and total protein in plasma were both greater (L, P=0.001 and L, P=0.002, respectively) for bulls fed increasing levels of SBM treated with PEC in diets. Concentrations of glucose, urea nitrogen, and triglyceride in plasma were not affected by dietary inclusion of PEC-treated SBM in bulls.

Table 40. Effect of tannic acid on blood profile in buffalo heifers at the end of feeding trial

Parameters	Control	Treatment
Glucose (mg/dL)	87.71±13.81	84.74±2.26
Cholesterol (mg/dL)	84.16±0.48	88.46±5.24
Triglycerides (mg/dL)	24.20±1.78	26.68±1.56
BUN (mg/dL)	18.23±0.98	19.30±0.43
GGT (U/L)	17.13±2.66	11.21±1.48
ALP (U/L)	286.1^{a}±35.76	196.5^{b}±12.93
Total Protein (g/dL)	7.63±0.07	7.65±0.11
Albumin(g/dL)	2.93±0.03	3.02±0.05
ALT (U/L)	52.46^{a}±1.98	41.48^{b}±4.53
Creatinine (mg/dL)	1.43±0.05	1.53±0.06

Figures with different superscripts in a row differ significantly, $p<0.05$

Yang *et al* (2016) supplemented TA at 6.5, 13.0 or 26.0 g/kg DM and noted that it increased plasma concentration of total antioxidant capability (P<0.05), whereas did not affect the plasma concentration of glucose, total cholesterol, triglyceride, total protein, albumin, aspartate aminotransferase, alanine amino-transferase, glutathione peroxidase, superoxide dismutase (P<0.05) Raju *et al* (2015) found that the mean serum urea level (mg/dl) of the control group was at par with oak leaves fed group. Aguerre *et al* (2016) found that the BUN decreased linearly when tannin content in the diet was increased from 0 to 1.80% of DM. BUN, mg/dL 15.4, 15.2, 14.6 and 13.5 for the 0, 0.45, 0.90 and 1.80 % tannin of DM. Kai *et al* (2016) reported that the animals fed purple prairie clover hay diet supplemented with polyethylene glycol diet had greater Glucose (P<0.05) and Blood urea nitrogen (P < 0.001) than those fed purple prairie clover hay. Jolazadeh *et al* (2015) reported Albumin and total protein in plasma were both significantly higher for bulls fed increasing levels of SBM treated with pistachio extract concentrate in diets. Concentrations of glucose, urea nitrogen, and triglyceride in plasma were not affected by dietary inclusion of pistachio extract concentrate -treated SBM in bulls. Getachew *et al* (2008a) reported that Serum metabolites and liver enzymes were not affected by TA (P.0.05). Gunun *et al* (2016) reported a reduction in blood urea nitrogen was in goats supplemented with MOSM at 2.4% of total DM intake.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present study was conducted to investigate the effect of tannin treatment on protein protection of soybean meal and performance of buffalo heifers. The study was conducted in two phases, *viz.* phase I and phase II. In the phase I SBM was treated with various levels of quebracho and tannic acid (1, 2, 3 and 4%), the chemical composition of treated SBM in comparison to control SBM was determined. *In vitro* studies were conducted using treated SBMs, and TMRs (containing treated SBM) as substrates. In Phase II, *in vivo* study was carried out to determine the effect of tannic acid treated SBM (selected from *in vitro* study) on nutrient utilization, the performance of buffalo heifers and blood metabolites. The results obtained during the course of this study have been summarized.

SBM was treated by diluting the desired gm of tannin source in 100 ml of distilled water and then mixing 100 gms of ground SBM with the tannin solution. Then treated SBM was kept overnight by closing lead and then on the next day it was dried at 70^{o} C in a hot air oven and then treated SBM was finely ground.

The OM of SBM was 90.20, CP content was 44.81, EE content was 1.88, Total ash content was 9.8, NDF content was 18.3 and ADF content was 12.35% on DM basis. Values were almost similar in treated SBMs and slightly was higher for the NDF content.

The OM of TMRs containing quebracho treated SBM varied from 90.74 to 91.42%. The CP content varied from 13.77 to 15.19%. The ether extract content of the TMRs containing quebracho treated SBM was in the range of 2.46 to 2.87%. The total ash content was in the range of 8.58 to 9.26%. The NDF content varied from 51.0 to 55.6%. The ADF content varied from 29.06 to 32.52%.

The OM of TMRs containing tannic acid treated SBM varied from 89.79 to 91.07%. The CP content varied from 13.5 to 14.95%. The ether extract content was in the range of 2.26 to 2.90%. The total ash content was in the range of 8.93 to 10.21%. The NDF content varied from 52.59 to 56.6%. The ADF content varied from 30.88 to 32.28%.

Total phenol content of quebracho was found 20.92% and it was 99.18% in tannic acid Condensed tannins were 45.70 and 0.43 quebracho and tannic acid, respectively.

The NGP/200 mg DM did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM. The ammonical nitrogen did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM but it decreased numerically. The partition factor (PF) did not vary significantly ($P<0.05$) but was improving numerically. MMP was higher significantly ($P<0.05$) in 3QSBM and did not vary in other samples. DMD and short-chain fatty acids did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM. ME was higher significantly ($P<0.05$) in 1 and 2% quebracho treated SBM and then it decreased significantly ($P<0.05$) with each percent of quebracho added but the lowest was found in control SBM.

The NGP/200 mg DM in the current study did not vary significantly ($P<0.05$) with the tannic acid treatment of SBM. The ammonical nitrogen reduced significantly ($P<0.05$) in the 4TSBM compared to SBM-W and was also lower in other treatments compare to SBM-W. The partition factor did not vary significantly ($P<0.05$) with different levels of tannic acid treated SBM but was improving numerically in treated SBM. MMP was lower significantly ($P<0.05$) in control and 4TSBM and was higher in other tannic acid treated SBMs. DMD did not vary significantly ($P<0.05$) with different levels of tannic acid treatment of SBM but, it was lower in all tannin treated SBM compared to SBM-W non-significantly ($P<0.05$). Short-chain fatty acids did not vary significantly ($P<0.05$) with different levels of tannic acid treatment of SBM. ME was lower significantly ($P<0.05$) in all tannic acid treated SBMs.

In vitro Kinetics of NGP/200 mg DM showed that there was a reduction in the gas production rate as well in the total gas production in the treated SBMs with both the tannin sources. There was a significant ($P<0.05$) reduction in the ammonical nitrogen with the tannin treatment. NDFD, % was slightly reduced in the TMRs containing tannin treated SBM. *In vitro* Kinetics of SCFA was significantly ($P<0.05$) reduced in all treatments compared to tannin treated SBMs at the end of incubation. Tannic acid was reducing ME more effectively than the quebracho and all the levels of the tannic acid were varying significantly ($P<0.05$) from the SBM-W.

The NGP/200 mg DM was varying from 43.41 to 46.36 ml and it was highest in COT. Ammonical nitrogen was reduced with the inclusion of Quebracho. The difference in percent OMD was found non-significant (P<0.05) and it was lower in treatments than control numerically. PF was varying non-significantly (P<0.05) treatments and control. MMP and EMMP were found highest in 4QRH significantly (P<0.05). DMD was lowest (P ≤ 0.05) in 4QRL (72.80%) and was highest (P ≤ 0.05) in COT (75.03%). Short-chain fatty acid showed it was decreasing with the inclusion of the quebracho and it was lowest (P ≤ 0.05) in the 4QRH and was highest (P ≤ 0.05) in COT. The ME concentration decreased significantly (P ≤ 0.05) in quebracho treated SBM.

The NGP/200 mg DM was significantly (P<0.05) reduced in tannic acid treated SBM and was lowest in 4TRH. It was ranging from 42.07 to 46.36 in 4TRH and COT, respectively. Ammonical nitrogen was significantly (P<0.05) higher in COT and was lowest in 4TRH. Partitioning factor was not varying significantly (P<0.05) but it was improving with the inclusion of tannic acid. OMD%, as well as NDFD%, was not varying significantly (P<0.05). MMP was significantly (P<0.05) higher in the 4TRH and it was lowest (P<0.05) in the COT. EMMP was varying significantly (P<0.05) in the TMR containing tannic acid treated SBM compare to COT. DMD was lower non-significantly (P<0.05) in TMR containing tannic acid treated SBM. Short-chain fatty acid was lowest significantly (P<0.05) in 4TRH and was found highest in COT. ME values were ranging from the 8.95 to 9.57 MJ/kg DM which decreased significantly (P<0.05) in TMR containing tannic acid treated SBM and was found lowest in 4TRH and was highest in COT.

The TMR prepared by replacing 50% SBM with 1% tannic acid treated SBM was selected for *in vivo* trial on the basis of *in vitro* because it reduced ammonical nitrogen compared to control significantly (P<0.05), there was higher EMMP than control and PF was numerically higher.

Intake of nutrients by an individual animal during feeding trial was estimated. CP intake was lower significantly (P<0.05) in the treatment group compared to control group animals. DM, EE, NDF and ADF intake were not showing significant (P<0.05) difference.

The DM digestibility (%) in control and treatment groups was 58.06 and 56.66%, respectively. There was no significant (P<0.05) difference in DM

digestibility between control and treatment groups. The CP, EE, ADF and cellulose digestibility significantly ($P<0.05$) reduced whereas, OM and NDF digestibility reduced non-significantly ($P<0.05$).

Average weight gain was higher throughout the trial in the treatment group compared to control but the difference was non-significant ($P<0.05$). Feed efficiency was not showing a significant ($P<0.05$) difference but it improved numerically Feed conversion ratio was significantly ($P<0.05$) lower in the treatment group compared to the control group for all the nutrients except in the DM in which it was numerically lower in the treatment group. FCR was 8.18 and 7.26 for control and treatment respectively.

Alkaline Phosphatase and Alanine transaminase both were found significantly ($P<0.05$) lower in the treatment group compared to control at the end of the trial of 90 days. Rest all the parameters were varying non-significantly ($P<0.05$).

CONCLUSION

SBM treated with 1% tannic significantly ($P<0.05$) lowered NGP/200mg DM, ammonical nitrogen compared to control and no further effect of higher levels of quebracho and tannic acid. 1TSBM improved PF significantly ($P<0.05$) as compared to control and was comparable with 2TSBM and 3TSBM. The TMR prepared by replacing 50% SBM with 1% tannic acid treated SBM was selected for *in vivo* trial on the basis of *in vitro* because it reduced ammonical nitrogen compared to control significantly ($P<0.05$), there was higher EMMP than control and PF was numerically higher. Tannic acid treatment of SBM was effective in decreasing *in vitro* rumen protein degradation. In *in vivo* digestibility of CP, EE and cellulose decreased significantly ($P<0.05$). The tannic acid treatment of SBM at 12.5% in concentrate gave encouraging results in terms of growth in buffalo heifers. Tannic acid significantly ($P<0.05$) increased weight gain/kg CP intake. It also improved FCR. Tannic acid reduced alkaline phosphatase and alanine transaminase significantly ($P<0.05$).

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