Int.J.Curr.Microbiol.App.Sci (2020) *9*(7): 3991-4000

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 7 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.907.469

Effect of Varying Levels of Tannins Treatment on *in vitro* Degradability of Soybean Meal

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Keywords

Tannin treatment. Protein protection, in vitro fermentation

Article Info

Accepted:
22 June 2020
Available Online:
10 July 2020

A B S T R A C T

Present study was conducted to see the effect of varying levels of tannins on protein protection of soybean meal through in vitro protein degradability. Soybean meal was treated with different levels of tannins from 1, 2, 3 and 4 percent with two different tannin sources i.e. quebracho and tannic acid. The NGP/200 did not vary significantly ($P<0.05$) with different levels of quebracho as well with tannic acid treatment of SBM. The ammonical nitrogen did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM but it decreased significantly ($P<0.05$) in the tannic acid treatment of SBM. DMD and short chain fatty acids did not vary significantly ($P<0.05$) with different levels of tannin treatment of SBM. These results lead to the conclusions that tannic acid treatment of SBM is more effective in the protection of protein from the ruminal degradation.

Introduction

The age at first calving in buffalo heifers is quite high (>42 months) in field impacts the economics of buffalo farming. As protein is one of the limiting factors for weight gain in growing animals, attention must be given for better utilization of it. To decrease the degradation of the protein in the rumen many methods have been tried. These include heat processing, chemical treatments or 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 the nature so, harmful for the handler. Some chemicals like acids, alkalis, ethanol denatures protein structure and makes 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)

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. To nullify this limitation any pure source of tannin should be used to evaluate the effect. So the components like Tannic acid (as a source of hydrolysable tannins) and Quebracho extract (as a source of condensed tannins) fulfils this lacuna.

Although few studies have been conducted with tannic acid and quebracho as the source of tannin but no research has been conducted in the buffalo species for the growth 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 objective to study the effect of varying levels of tannins on protein protection of soybean meal through in vitro protein degradability.

Materials and Methods

Treatment of Soybean meal

Soybean meal was treated with different levels of tannins from 1, 2, 3 and 4 percent 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 grinded SBM with the quebracho solution. Then treated SBM was kept overnight by closing lead and then on the next day it was dried in hot air oven at 70^{o} C in a hot air oven and then treated SBM was finely grinded for in vitro analysis of the sample. Likewise, for each percent source of tannin was weighed and mixed with SBM same as mentioned. 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.

The finally ground samples of complete feeds were analysed for total ash, crude protein, ether extract (AOAC, 2000) and cell wall fractions (Robertson and Van Soest, 1981). Complete feeds were evaluated by *in-vitro* gas production technique (Menke *et al.*, 1988). 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.

Results and Discussion

Chemical composition of treated SBM

The chemical composition of quebracho treated SBMs used in the experiment is given in Table-2. The OM of 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 non-fiber carbohydrates (NFC) content was higher in SBM (11.92%) as compared to quebracho treated SBMs.

The chemical composition of tannic acid treated SBMs used in the experiment is given in Table-3. The OM of 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 to 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. So, lower total DMI can be predicted of tannic acid treated SBM compared to untreated SBM. The total carbohydrates in SBM was 30.22% and it was lower than the treated SBMs except 1TSBM. The non-fiber carbohydrates (NFC) content was higher in SBM (11.92%) as compared to tannic acid treated SBM.

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 4. The NGP/200 in the current study did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM. It was lowered non-significantly ($P<0.05$) in quebracho treated SBM compared to SBM-W (38.0) and was found lowest in 3QSBM. 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.,* (2008) reported quebracho decreased gas production ($P<0.001$) in alfalfa hay at the rate of 5, 10, and 15%. That may be due to higher inclusion level of quebracho than evaluated levels in the current study. Alipour and Rouzbehan (2010) reported decreased gas production due to incorporation of tannin extract from grape pomace in SBM. ($P<0.01$). 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.,* (2015) reported mimosa and quebracho (purified condensed tannin) decreased total gas production in hay: concentrate substrate (70:30 w/w) ($P \leq 0.05$).

The ammonical nitrogen did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM but it decreased numerically. 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) noted decrease in NH_3 -N production from oil cake-Acacia catechu leaves pellets which 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.

Mohammadabadi and Chaji (2012) reported the mean values of NH_3-N concentrations were 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.,* (2008) reported that the addition of QT in alfalfa hay at the rate of 50, 100, and 150 g/kg resulted in reduction of NH_4^+-N by 12%, 31%, and 51%, respectively. Salem *et al.,* (2005) 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 as 11.3, 10.3, 10.1 and 8.8mg/dL at 0, 0.45, 0.90 and 1.80 % tannin of DM. Kai*et al.,* (2016) found similar results that the rumen fluid collected from lambs fed purple prairie clover hay had lower ($P < 0.05$) concentrations of ammonia as compared to lambs fed purple prairie clover hay diet supplemented with polyethylene glycol (PH-p) diet.

The partition factor (PF) did not vary significantly ($P<0.05$) with different levels of quebracho treated SBM. 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 calculated *in vitro* 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). Jayanegara *et al.,* (2015) reported higher levels of mimosa and quebracho (purified condensed tannin) linearly improved PF ($P \leq 0.05$).

Neutral detergent fibre degradability (NDFD) was highest in control and was lowest in 1QSBM and rest treatments fall in between and did not vary significantly ($P<0.05$) either from control or treatment.

MMP was higher significantly ($P<0.05$) in 3QSBM and did not vary in other samples. Whereas, EMMP increases with increasing quebracho percent in SBM till 3% of quebracho treatment non-significantly then it decreased.

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 the current study did not vary significantly ($P<0.05$) with different levels of quebracho treatment of SBM. Mohammadabadi and Chaji (2012) reported the mean values of SCFA concentrations were 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.,* (2008) reported QT decreased total SCFA ($P<0.001$) at the rate of 50, 100, and 150 g/kg.

ME was higher significantly in 1 and 2% quebracho treated SBM and then it decreased significantly ($P<0.05$) with each percent of quebracho added but lowest was found in control 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 lower level of tannins in selected tannin source in their study.

Table.1 Names and abbreviations of Soybean meals with different level and source 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

Table.2 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

Table.3 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

Table.4 *In vitro* gas production and digestibility of Quebracho treated SBM

Variable	SBM-W	1QSBM	2QSBM	3QSBM	4QSBM
NGP/200 ml/200mg	38.00±0.15	37.73±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	4.65±0.01	4.69±0	4.63±0.02
NDFD, %	83.75±0.96^{a}	80.13±1.05^{ab}	76.96±2.09^{ab}	75.16±5.23^{ab}	72.77±.69^{b}
MMP, mg	173.63±0.64^{b}	173.27±0^{b}	173.90±.36^{b}	177.10±0.73^{a}	174.99±0.97^{b}
EMMP, %	52.55±0.33	52.68±0	52.71±0.16	53.12±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	1.54±0	1.52±0.01	1.54±0.01
ME, MJ/kg DM	10.54±0.02^{a}	10.55±0.00^{a}	10.56±0.01^{a}	10.36±0.03^{b}	10.34±0.03^{b}

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

Table.5 *In vitro* gas production and digestibility of tannic acid treated SBM

Variable	SBM-W	1TSBM	2TSBM	3TSBM	4TSBM
NGP/200 ml/200mg	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±6.33^{a}	97.11±0.55^{ab}	95.09±0.85^{ab}	94.43±1.18^{ab}	91.00±0.66^{b}
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±0.96^{a}	78.14±0^{b}	82.12±0.51^{ab}	82.97±2.08^{ab}	83.16±1.96^{ab}
MMP, mg	173.63±0.64^{b}	176.53±0.97 ^{a}	177.35±0.73^{a}	176.72±0.97^{a}	172.50±0.97^{b}
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	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±0.02^{a}	10.18±0.03^{b}	10.21±0.03^{b}	10.15±0.03^{bc}	10.07±0.03^{c}

Figures with different superscripts in a row differ significantly, $p<0$.

Which was 53, 79, 48, and 65 g/kg DM for oak leaves, oak fruit, pistachio hull and pistachio leaves, respectively. Gerlach *et al.,* (2018) found the ME concentration of the concentrates decreased markedly (−25%) from 12.9 to 9.7 MJ/kg DM ($P < .05$), for control and 5% CT, respectively. Rivera-Mendez *et al.,* (2016) reported tannin supplementation decreased estimated dietary NE (linear effect, $P < 0.01$).

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 the Table 14. The NGP/200 in the current study did not vary significantly ($P<0.05$) with different levels of tannic acid treatment of SBM but, it increased in 1TSBM and 2TSBM and then it decreased at higher level i.e. 3TSBM and 4TSBM non-significantly ($P<0.05$). Singh *et al.,* (2001) concluded that the tannic acid caused an increase in the gas volume. Getachew *et al.,* (2008) reported that the TA decreased gas production ($P<0.001$), at inclusion level of 50, 100, and 150 g/kg. Bhatta *et al.,* (2014) reported 24-h gas production linearly decreased at more than 5% of tannin level. Jayanegara *et al.,* (2015) reported chestnut and sumach (purified hydrolysable tannin) decreased total gas production ($P \leq 0.05$). Getachew *et al.,* (2008) 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 roasting SBM, SBM treated with 1%TA, SBM treated with 2%TA and SBM treated with 3% TA. Singh *et al.,* (2001) found that the gas volume increased in the presence of tannic acid.

The ammonical nitrogen in the current study reduced significantly (P<0.05) in the 4TSBM compared to SBM-W. Rest treatments were reducing ammonical nitrogen numerically but there was no significant (P<0.05) difference was found from the SBM-W as well from the 4TSBM. Singh *et al.,* (2001) found that the tannic acid caused a decreased in the ammonical nitrogen. Getachew *et al.,* (2008) reported that the addition of TA at the rate of 50, 100, and 150 g/kg resulted in reduction of NH_4^+-N 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. Zhou *et al.,* (2019) reported that the TA at the rate of 16.9 g/kg decreased 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 inclusion of tannin level more than 5%. Getachew *et al.,* (2008) 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

The partition factor did not vary significantly (P<0.05) with different levels of tannic acid treated SBM. But, increased non-significantly compared to SBM-W in all TA treated samples and highest value was found in 1TSBM. Jayanegara *et al.,* (2015) who reported that the higher levels of chestnut and sumach (purified hydrolysable tannin) linearly improved PF ($P \leq 0.05$).

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 in other tannic acid treated SBMs. Whereas, EMMP was higher non-significantly in all tannic acid treated SBM than control and was highest in 1% tannic acid treated SBM. Getachew *et al.,* (2008) reported that the efficiency of microbial protein synthesis increased when 50 and 100 g TA/kg DM was added compared with the control.

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. Tannic acid treatment significantly decreased the rate of degradation at that level. 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. Salawu *et al.,* (1999) found the silage containing tannins had lower ($p < 0.05$) DM disappearance in the rumen than the control silage. Getachew *et al.,* (2008) reported that the TA reduced *in vitro* true degradability of dry matter (P<0.05) at the 60 and 90 g levels but not at 30 g/kg DM compared with the control.

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.,* (2008) concluded that the TA decreased total SCFA (P<0.001) at the rate of 50, 100, and 150 g/kg. Jayanegara *et al.,* (2015) reported chestnut tannins decreased total SCFA (P ≤ 0.05).

ME was lower significantly (P<0.05) in all tannic acid treated SBMs. El-Waziry *et al.,* (2005) noted that the predicted ME which calculated from gas production after 24 h incubation were reduced 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

It was concluded that tannic acid was more effective in protein protection from rumen degradability. SBM treated with 1% tannic significantly (p<0.05) lowered NGP, ammonical nitrogen compared to control and no further effect of higher levels of quebracho and tannic acid. 1TSBM improved PF significantly as compared to control and was comparable with 2TSBM and 3TSBM. So, it is recommended from the study that SBM should be treated with 1% tannic acid to protect protein from ruminal degradation.

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How to cite this article:

Bilal Chamadia, R. S. Grewal, J. S. Lamba, Jasmine Kaur and Neeraj Kashyap. 2020. Effect of Varying Levels of Tannins Treatment on *in vitro* Degradability of Soybean Meal. *Int.J.Curr.Microbiol.App.Sci.* 9(07): 3991-4000. doi: https://doi.org/10.20546/ijcmas.2020.907.469