

## Article

# Effects of Tannic Acid Supplementation on Growth Performance, Nutrients Digestibility, and Blood Metabolite in Lohi Male Lambs

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**Abstract:** The objective of the current study was to assess the effects of tannic acid supplementation on growth performance, nutrients digestibility, and blood urea nitrogen in the Lohi male lambs. Fifteen animals with an average age of 9-10 months and live body weight of  $26.4 \pm 0.52$  kg were selected randomly. Three total mixed rations (TA0, TA4, TA8) having tannic acid 0, 4 g and 8 g/animal/day, respectively were given to the lambs ( $n = 5/\text{treatment}$ ) for a period of 60 days under a Completely Randomized Design. Data regarding dry matter intake, body weight gain, nutrients digestibility, feed efficiency and blood urea nitrogen were collected. No significant difference ( $p > 0.05$ ) was observed among the treatments in dry matter intake, body weight gain, apparent digestibility of nutrients, and feed efficiency. The blood urea nitrogen levels were significantly lower (17.0 mg/dL) in the TA8 treatment group compared to the TA0 group (22.1 mg/dL;  $SE = 1.3$ ), indicating the protein bypass characteristic of tannic acid. It was concluded that supplementation of tannin may not have appreciable effects on growth performance and digestibility of the nutrients in lambs, but bypass value of protein was increased.

**Keywords:** tannic acid; average daily gain; digestibility

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## 1. Introduction

Sheep production is a significant component of animal agriculture globally, particularly in regions where land is unsuitable for crop cultivation. It plays a vital role in supporting the livelihoods of rural communities [1]. While sheep farming is found in nearly all parts of the world, it is particularly prevalent in arid and semi-arid regions. In these areas, nutritional stress poses a significant challenge to ruminant livestock production. As rainfall diminishes, grazing land deteriorates quickly, often leaving cereal crop residues as the primary feed source [2]. In such circumstances, strategies to enhance feed efficiency become critically important.

Several strategies have been employed in the past to improve the growth performance of ruminants using various feed additives. However, tannic acid supplementation has shown the most promising results [3]. This is attributed to its ability to preserve rumen fermentation of dietary protein and ensure the availability of essential amino acids in the small intestine [4]. Low to moderate tannic acid supplementation may change the site of protein degradation and increase the flow of metabolizable amino acids to the small

intestine [5]. Tannic acid is generally considered as an anti-nutritional factor, but a low concentration of tannic acid changes the rumen fermentation [6] and it becomes beneficial as it alters the microbial protein synthesis [7]. Tannic acid form complex with starch, protein, some minerals and vitamins in the rumen at a moderate pH that dissociates in the duodenum and abomasum at lower pH [8]. Protein binds with tannic acid by hydrogen bonding at acidic pH. Tannic acid and protein complex are stable at pH closer to neutral which is found in ruminal condition and dissociates at lower pH found in abomasum and duodenum [9].

Tannic acid supplementation has been reported to enhance the quality of meat [10]. The increase in bypass proteins due to tannic acid products is associated with improved animal production. Tannic acid functions in the rumen by reducing the solubility of feed proteins or selectively inhibiting ruminal proteolytic bacteria. Previous studies have shown that diets rich in polyphenols and condensed tannins can reduce the environmental impact of sheep production by decreasing methane (CH<sub>4</sub>) emissions and urinary nitrogen (N) excretion [4]. Tannins have also been found to enhance dietary protein utilization, body weight gain, milk and wool production, and overall animal health when fed to animals [11].

Therefore, the current study was designed to evaluate the impact of various concentrations of tannic acid on protein digestibility and, consequently, the growth performance of Lohi lambs.

## 2. Materials and Methods

### 2.1. Study Site, Animals, Housing, and Management

The trial took place at the Small Ruminants Training and Research Center, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, Pakistan. Lohi male lambs were selected from the Lohi breed flock of the center. These lambs, totaling 15 in number, had an average weight of  $26.4 \pm 0.52$  kg and were aged between 9 and 10 months. Throughout the experimental period, the lambs had unrestricted access to fresh, clean water. They were dewormed for both ecto- and endo-parasites at the beginning of the trial. Additionally, the lambs were vaccinated in accordance with the research center's protocol to safeguard their health and prevent endemic diseases.

### 2.2. Treatment Groups

The lambs were randomly divided into three treatment groups: TA0, TA4, and TA8, each consisting of five lambs, using a completely randomized design. All groups were fed a total mixed ration (TMR) diet. The control group (TA0) received no tannic acid, while the TA4 and TA8 groups were supplemented with 4g and 8g of tannic acid (commercial preparation) per animal per day, respectively. The lambs underwent a 10-day adaptation period before the study commenced, and the experimental phase lasted for 60 days. The total mixed ration (TMR) diet was isocaloric, with a metabolizable energy (ME) content of 2.54 Mcal/kg, and isonitrogenous, with a crude protein (CP) content of 14.65%. It comprised a mixture of oat silage, Rhode grass hay, and concentrate ration, with varying tannic acid levels for the different groups. Each lamb was housed in an individual pen, received individual feed, and data were collected individually. The nutritional composition of the basal diet is presented in Table 1. The lambs were fed at a rate of 4% of their body weight on a dry matter basis.

### 2.3. Data Collection

The feed offered and refused, if any, was recorded daily for each animal. Dry matter intake was calculated using the following formula:

$$\text{Dry matter intake (DMI)} = \text{DM offered (g)} - \text{DM refused (g)}$$

At the beginning of the research trial, the initial body weight of each animal was recorded using a digital weighing balance, and then weights were measured weekly.

**Table 1.** Ingredients and chemical composition of basal diets.

Ingredients	Inclusion level on dry matter basis, %
Oat silage	45.0
Rhode grass hay	03.0
Maize grain	20.0
Soybean meal	10.0
Canola meal	10.0
Molasses	10.0
Mineral mixture	02.0
Tannic acid (g/day) <sup>1</sup>	
Chemical composition	
Dry matter	59.26
Crude protein	14.65
Metabolizable energy, Mcal/kg	2.54
Neutral detergent fiber	36.98
Acid detergent fiber	21.2
Ether extract	4.16

<sup>1</sup> Tannic acid supplementation was zero, 4 g and 8 g per animal per day in TA0, TA4, and TA8 treatment groups, respectively. All the groups were fed the same basal diet.

Weight gain was calculated using the following formula:

$$\text{Weight gain (kg)} = \text{Final body weight (kg)} - \text{Initial body weight (kg)}$$

Feed efficiency was calculated using the following formula:

$$\text{Feed efficiency} = [\text{total weight gain (kg)} \div \text{total dry matter intake (kg)}] \times 100$$

Blood samples were collected from the jugular vein of the animals at the beginning and end of the experiment using 10 ml sterilized disposable syringes. The samples were transferred to EDTA-containing tubes for biochemical analysis. Plasma was separated from the blood samples, properly labeled, and stored at -20°C until further analysis. The plasma was used to analyze blood urea nitrogen (BUN) using a kit (Randox Urea Kinetic kit from Randox Laboratories Ltd., Crumlin, UK), with the aid of a spectrophotometer (Epoch2, BioTek, Winooski, VT, USA).

#### 2.4. Digestibility Trial

In the final week of the experiment, a digestibility trial was conducted. The animals were placed individually in digestibility cages. Daily dry matter intake was recorded, and all fecal material was collected and weighed. Fecal samples were packed into polythene bags and stored at -20°C until analysis. The samples were then analyzed for proximate analysis to determine digestibility of dry matter, crude protein (CP), ether extract (EE), crude fiber (CF), and organic matter (OM) contents, following the AOAC [12] guidelines. These digestibility analyses were done in the laboratory of Department of Animal Nutrition, University of Veterinary and Animal Sciences. Digestibility of DM and nutrients was calculated using the following formula:

$$\text{Digestibility, \%} = [(\text{Nutrient intake, g/d} - \text{Nutrient in faeces, g/d}) / \text{Nutrient intake, g/d}] \times 100$$

#### 2.5. Statistical Analysis

The data were analysed under one-way analysis of variance in a Completely Randomized Design using GLM procedure of SAS (SAS for Academics: SAS Institute Inc., Cary, NC, USA). Means were compared using the PDIF option with Tukey's adjusted *p*-values. Statistical significance was declared at *p* ≤ 0.05.

### 3. Results and Discussion

In the present study, lambs' performance was evaluated in terms of dry matter intake (DMI), body weight gain, and feed efficiency. The average daily DMI and average daily gain showed no significant differences among the treatment groups (Table 2). The average daily dry matter intake (DMI) and daily weight gain were 1143 g and 168 g per animal, respectively, across all treatment groups. These findings align with previous research. For instance, Hervás et al. [13] observed that adding Quebracho tannic acid up to 1.5 g/kg of body weight did not affect DMI in sheep. Similarly, Frutos et al. [14] found no significant increase in voluntary DMI in sheep when offered soybean meal treated with hydrolysable tannic acid at 20.8 g/kg DM of feed. Additionally, Pathak et al. [15] reported that supplementing tannic acid in the diet of sheep infected with *Haemonchus Contortus* at 1.5% of dry matter did not significantly increase the daily dry and organic matter intake. A low concentration of tannin had no effect on dry matter intake [16], while a higher concentration of tannin in the diet (50 g/kg) had negative effects on feed intake [17]. Luciano et al. [10] found that lambs offered quebracho tannic acid (89.3 g/kg of dietary DM) in fresh vetch (*Vicia sativa*) had reduced DM intake compared to lambs fed a concentrate-based diet.

**Table 2.** Effects of feeding various levels of tannic acid based TMR on growth performance of Lohi lambs.

Items	Treatment groups <sup>1</sup>			SEM	P value
	TA0	TA4	TA8		
Total DMI (kg)	68.95	69.06	71.15	2.3	0.79
Average daily DMI (g)	1130	1132	1166	38	0.78
Initial body weight (kg)	27.02	26.18	26.10	0.95	0.75
Final body weight (kg)	36.32	36.28	37.36	1.23	0.74
Weight gain (kg)	9.30	10.10	11.26	0.98	0.44
Average daily gain (g/day)	153	166	185	16	0.41
Feed efficiency	0.135	0.145	0.158	0.012	0.48

<sup>1</sup>TA0, TA4, and TA8 had zero, 4 g and 8 g of Tannic acid per animal per day, respectively.

In the current study, no significant difference was observed in body weight gain and average daily gain among the treatment groups (Table 2). Similar findings were reported in a feedlot fattening trial on male lambs supplemented with tannin extract for 70 days, where growth performance was not enhanced by tannic acid supplementation over a longer period [18]. Liu et al. [19] evaluated the effects of coconut oil and chestnut tannins on the growth performance of sheep but found no significant effects on daily dry matter intake (DMI) and average daily gain (ADG). These results are consistent with previous studies that examines the effects of adding 1% or 3% of Quebracho tannin into alfalfa hay on the growth performance of Najdi lambs [20] and on the average daily gain of Awassi lambs fed a partial replacement diet of barley grain with tannin-rich carob pods [21]. While the TA8 group showed a numerically higher average daily gain, this difference was not statistically significant. The lack of a significant effect of tannin supplementation may be attributed to the study's limited statistical power, likely due to the small number of lambs per treatment group.

Supplementation with tannic acid did not lead to significant differences in feed efficiency among the three treatment groups. This finding aligns with the results of Liu et al. [19], who found that coconut oil and chestnut tannins did not significantly affect the feed conversion ratio (FCR) in sheep. Similarly, Rojas et al. [18] concluded that long-term tannic acid supplementation in the diet had no significant impact on feed efficiency.

**Table 3.** Effects of feeding various levels of tannic acid supplementation nutrient digestibility and blood urea nitrogen in Lohi lambs.

Items	Treatment groups <sup>1</sup>			SEM	<i>p</i> -Value
	TA0	TA4	TA8		
Dry matter, %	60.65	61.99	63.31	7.38	0.76
Crude protein, %	64.91	65.86	67.70	6.18	0.65
Ether extract, %	73.92	71.04	67.84	11.01	0.52
Crude fiber, %	62.13	65.21	66.74	8.23	0.50
Organic matter, %	60.12	62.38	63.97	7.35	0.56
BUN, mg/dL	22.1 <sup>a</sup>	18.9 <sup>b</sup>	17.0 <sup>b</sup>	1.3	< 0.001

<sup>1</sup> TA0, TA4, and TA8 had zero, 4 g and 8 g of TA per animal per day, respectively.

The percentage digestibility of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), and organic matter also did not show any significant differences among the treatment groups (Table 3). The concentration of BUN showed a highly significant difference ( $p < 0.001$ ) among the three treatment groups. Specifically, the group TA8 exhibited a significantly lower level of BUN ( $17.0 \pm 1.3$  mg/dL) compared to the group TA0 ( $22.1 \pm 1.3$  mg/dL: means  $\pm$  SE; Table 3). This decrease could be attributed to an increase in the bypass protein value of the supplemented groups relative to the control. Similar findings have been reported by other researchers, where plasma BUN levels were lower in lambs fed tannic acid as a supplement [22] and in female sheep fed sericea lespedeza forage containing condensed tannic acid [23] compared to control groups. Consistent with these results, lower BUN values have been observed in sheep [24] and male lambs [25], when fed a diet supplemented with tannic acid. The BUN levels correspond to a numerically higher growth rate in the tannic acid supplemented group, although this difference is not statistically significant.

#### 4. Conclusions

In conclusion, the supplementation of tannic acid in the diet of Lohi male lambs showed a non-significant effect on performance, including dry matter intake, body weight gain, and feed efficiency. However, tannic acid supplementation significantly reduced blood urea nitrogen levels, suggesting a potential impact on protein metabolism. Further research is needed to explore the underlying mechanisms and optimize the use of tannic acid as a feed supplement in sheep farming practices.

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