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Effects of quebracho tannin supplementation in early lactation dairy cow rations on milk yield parameters, rumen fermentation, digestibility and blood parameters

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ABSTRACT. This study investigated the effects of condensed tannin (Quebracho Colorado) supplementation on milk yield and composition, intake, blood and rumen parameters, and digestibility in early–lactating dairy cows. Twenty–four cows were assigned to three groups: control (CON, n = 8), T1 (60 g of tannin, n = 8) and T2 (120 g of tannin, n = 8). Milk yield was recorded twice daily (at 08:00 and 16:00). Blood and rumen fluid samples were collected on days 0, 15 and 30 for the analysis of ammonia, pH, volatile fatty acids and blood metabolic profile. Over the last 5 days, faecal samples were collected to determine digestibility and conduct wet sieving particle size evaluation. The results showed no significant differences between the T1 and T2 groups in terms of milk yield, but both groups had higher milk yield than the CON group ($P < 0.05$). The energy–corrected milk yield (ECMY) and ECMY to dry matter intake (DMI) ratio (ECMY/DMI) were the highest in the supplemented groups. Blood analysis revealed higher blood Ca levels in CON than T1, and lower Mg levels in CON compared to T2. Protein digestibility was the highest in the T2 group. Rumen ammonia levels were the highest in the CON group and the lowest in the T2 group ($P < 0.05$). Acetic, propionic and butyric acid levels increased in the T1 and T2 groups. Faecal sample from the T2 group had lower particle retention on the second (middle) sieve ($P < 0.05$). So, the addition of 60 and 120 g of tannin exerted a positive effect on milk yield, feed utilisation, ruminal ammonia, volatile fatty acids, milk parameters, and protein digestibility. Therefore, quebracho tannin can be considered as a potential means to mitigate the effects of negative energy balance in dairy cows.

Introduction

Tannins are a group of naturally occurring heterogeneous phenolic compounds with varying structures capable of binding and precipitating proteins. They are broadly classified into hydrolysable and condensed tannins. Hydrolysable tannins can be degraded by weak acids, bases, hot water, gastrointestinal esterases, and enzymes secreted by bacteria, yeast and fungi. The breakdown of hydrolysable tannins by rumen microorganisms results in the production of gallic acid, phloroglucinol, pyrogallol and finally butyrate and acetate through enzymatic processes. Pyrogallol and gallic acid have been reported to exert toxic effects on ruminants (Aydin and Üstün, 2007). Condensed tannins are more complex and have a higher molecular weight than hydrolysable tannins, with some reaching up to 20 000 (Zhang et al., 2023). They consist of polymers or oligomers of flavonoid units, such as flavan-3-ol, and are resistant to breakdown by hydrolysis (Aydin and Üstün, 2012). Rumen microorganisms lack the

enzymes necessary to break down condensed tannins (Besharati et al., 2022). Therefore, it has been reported that condensed tannins do not show similar toxic effects as hydrolysable tannins.

Protein complexes formed with condensed tannins are resistant to microbial degradation at neutral pH levels (pH 5.5–7.0) in the rumen, consequently, this process reduces protein decomposition by microorganisms. Tannins decrease the amount of ammonia production in rumen and elevate undegraded protein levels. Tannin–protein complexes dissociate in the abomasum due to pH changes, typically ranging between 2.5 and 3.5. This process leads to protein digestion in the small intestine and subsequent absorption of the degradation products (Ningrat et al., 2017). The utilization of secondary plant compounds to improve nitrogen use efficiency by reducing nitrogen degradability in rumen is a well–established strategy in ruminants. Among these molecules, tannins have been extensively studied in recent years (Broderick et al., 2017). However, studies on the effects of tannins in ruminant nutrition have yielded contradictory results. The impact of condensed tannins on ruminants may vary depending on factors such as the tannin's structure, quantity, the ratio of roughage to concentrate in the diet, and the adaptation of the animals to the tannin–supplemented feed. Consequently, there are conflicting findings regarding the administration of tannins in dairy cow rations. Nonetheless, condensed tannins at moderate and low concentrations have been shown to have beneficial effects on performance (Wang et al., 1996). By decreasing the rate of ruminal protein degradation, tannins can reduce ruminal ammonia production and increase the proportion of undegraded protein (RUP) fraction, potentially enhancing nitrogen efficiency (Broderick et al., 2017). According to Silanikove et al. (1994), the presence of 1–4% condensed tannins reduces protein breakdown in the rumen and increases the absorption of non–ammonia nitrogen and essential amino acids in the abomasum (Silanikove et al., 1994). Wang et al. (2015) indicated that in order to avoid reductions in feed intake and dry matter (DM) digestibility, while still effectively reducing ruminal protein degradability, the optimal concentration of total condensed tannin (CT) for ruminants should be 50 g/kg. In another study, the use of *Leucaena Leucocephala* - based CT at a concentration of 46 g–eq of tannic acid/kg DM resulted in reduced rumen degradable protein and increased rumen undegradable protein contents (Soltan et al., 2012; Pineiro–Vázquez et al., 2015). Ahnert et al. (2015) supplemented heifer rations with quebracho tannin extract at levels ranging from 1 to 6% and observed that the apparent total tract digestibility of protein decreased at dosages exceeding 2% but enhanced the digestibility of other nutrients at concentrations above 4%. However, the addition of high concentrations of tannins may lead to reduced rumen activity, nutrient digestibility, feed intake, and amino acid absorption in the intestine (Frutos et al., 2004; Waghorn, 2008). For instance, while 1% tannin addition had no effect on organic matter digestibility of concentrate feed, a significant decrease was observed when tannin was supplemented at 3 and 5% (Gerlach et al., 2018). Suppression of microbial enzyme activities and intestinal digestion due to high tannin supplementation has been also reported (Gemede and Ratta, 2014). The reduction in DM intake caused by tannins is attributed to their complexation with lignocellulose, which suppresses cellulose digestion and prevents microbial digestion either directly by cellulolytic microorganisms or by inhibiting fibrolytic enzyme activities that cause physical fullness of the rumen and alterations in volatile fatty acid production (Patra and Saxena, 2011; Piñeiro-Vázquez et al., 2015; Hatew et al., 2016).

Conflicting results have been reported in studies on the effects of condensed tannins on milk yield and milk composition. The wide variation in condensed tannin concentration and chemical structure requires more standardised products to ensure a predictable mode of action. Dschaak et al. (2011) found that supplementation with a 3% condensed tannin (quebracho) did not affect milk production but reduced DM intake (DMI). In another study, low levels of quebracho–chestnut tannin extract (0.45% of DM) increased the milk protein content, but higher levels (0.9% and 1.8% DM) could have detrimental effects, as they were shown to reduce DMI milk protein content, milk yield and nutrient digestibility (Aguerre et al., 2020). Some researchers have indicated that an adaptation period longer than 3 weeks is required to stabilise N-related effects, such as milk protein content (Barros et al., 2017; Zanton, 2016). It was reported that the addition of 0.45% tannin to the ration decreased feed conversion, and had a positive effect on milk protein content, while a quebracho–chestnut tannin mixture decreased urinary N excretion to the environment without affecting milk yield (Aguerre et al., 2020). Increased faecal N levels were detected when tannin was applied at doses up to 6% in the ration (Liu et al., 2013). On the other hand, Śliwiński et al. (2004) reported that tannin extracts from quebracho or chestnut tree were not effective in improving performance in dairy cows.

Numerous studies have demonstrated both the beneficial and detrimental effects of tannins on rumen fermentation and productivity of dairy cows at different lactation periods. However, supplementation of condensed tannins in postpartum dairy cow rations has not been extensively investigated. Therefore, the aim of this study was to evaluate the impact of quebracho tannin supplementation on feed intake, performance, milk composition, rumen fermentation parameters, faecal particle size and digestibility in early lactation dairy cows.

Material and methods

Cows and experimental design

The experimental protocol (Approval No.: 2021/062) was approved by the Selcuk University Ethics Committee on Animal Experimentation (Konya, Turkey). Twenty–four Holstein lactating cows were used in the experiment and were divided into the control and experimental groups 5 days after calving. Fifteen primiparous cows were equally distributed among the groups, while the remaining 9 animals were in their 2nd or 3rd lactation. The trial lasted 30 days for all animals. The animals were fed individually twice in the morning and evening with a total mixed ration (TMR) consisting of maize silage, alfalfa, straw, barley, cotton seed meal, and a concentrate mixture. The chemical compositions of these ingredients are given in Table 1.

Table 1. Chemical compositions of feedstuffs used in the experimental total mixed ration (% on dry matter (DM) basis)

	Maize silage	Alfalfa hay	Wheat straw	Barley	Cotton seed meal	Concentrate mixture
DM, %	27.32	87.74	87.39	89.5	91.88	88.93
Ash, %	6.35	9.30	8.51	2.39	7.97	8.68
Ether extract, %	2.10	2.32	2.13	1.93	5.78	3.85
Crude fibre, %	32.72	31.03	36.13	9.70	25.05	11.09
Crude protein, %	6.99	15.29	4.75	11.53	24.94	21.61
Metabolizable energy, kcal/kg	1838	1855	1634	2911	2306	2763

Nutritional requirements of dairy cows in early lactation (0 to 35 day postpartum) period were formulated according to the recommendations of the National Research Council (NRC, 2001). When calculating the cows' requirements, a 5–10% incremental feed allowance was applied. All cows in the experiment had free access to water and TMR. Dairy cows were milked twice daily at 09:00 and 16:00. TMR was offered twice a day at 08:00 and 17:00 with 5 to 10% orts. Orts were removed and weighed before morning feeding each day to calculate DM intake (DMI). Tannin (MGM-S®, UNITAN SAICA Inc., Buenos Aires, Argentina) containing herbal extracts (Quebracho Colorado) was mixed into the TMR at a dose of 60 and 120 g/day for all animal in the experimental groups (T1 and T2, respectively). The tannin was evenly distributed over the top of the TMR to ensure complete consumption by the animals. Animals had *ad libitum* access to water via automatic drinkers.

Determination of milk yield and composition

Milk yields of animals in the groups were individually recorded during both morning and evening milkings from birth, and these records were maintained throughout the study. Milk samples were collected from all animals weekly, and subsequently analysed for milk protein, fat and lactose using an automatic analyser (Milkana). Milk yield parameters were calculated based on the milk yields obtained.

Energy–corrected milk yield (ECMY) was calculated using the following formula, as recommended by the National Research Council (NRC, 2001):

$$\text{ECMY} = \text{milk yield (kg/day)} \times [(0.0929 \times \% \text{ fat}) + (0.0547 \times \% \text{ protein}) + (0.0395 \times \% \text{ lactose})] / 0.7$$

Determination of rumen fermentation properties

Rumen fluid samples were collected through a probe from the animals at the beginning, middle and end of the experiment, 6 h after the morning feeding. Upon collection, the rumen fluid samples were immediately tested for pH. Additionally, 1 ml of rumen fluid sample was added to the Eppendorf tube containing 0.2 ml of 25% meta–phosphoric acid. Another 1 ml aliquot of the sample was mixed in the Eppendorf tube with 20 µl of sulphuric acid for NH_3–N analysis (Cobellis et al., 2015; Weatherburn 1967).

Blood sampling and metabolic profile test

Blood urea nitrogen (BUN), calcium (Ca), magnesium (Mg), phosphorus (P), aspartate aminotransferase (AST), gamma–glutamyl transferase (GGT), glucose, cholesterol, total protein, albumin and ketone values were determined by collecting 20 ml blood samples from the coccygeal vein. Sampling was conducted at the beginning of the experiment, on day 15, and at the end of the experiment, precisely 6 h after morning feeding.

Determination of digestibility

Faecal samples of each animal were collected through the rectum during the last five days of the experiment. To determine digestibility, 200 g of faeces were collected through the rectum on day 26 at 8:30, day 27 at 10:30, day 28 at 12:30, day 29 at 14:30, and day 30 at 16:30. Following the faecal collection protocol, the samples were dried at 55 °C for 72 h. Subsequently, they were ground through a 1 mm sieve and thoroughly mixed. Total nutrient digestibility was calculated based on the analyses of the ration, leftover feed, faecal samples and acid–insoluble ash analyses.

Wet sieving evaluation of manure

During the last 5 days of the study, faeces were collected from the middle–clean areas of each fresh faecal pile 0, 2, 4, 6 and 8 h after feeding. Faeces were collected at 08:30 on day 1, 10:30 on day 2, 12:30 on day 3, 14:30 on day 4 and 16:30 on day 5. A Nasco Digestion Analyzer commercial system (Nasco) was used for particle size assessment. This system consisted of three stainless steel screens with sieve sizes of 4.76 mm, 2.38 mm and 1.59 mm. Evaluation criteria used as a reference included <10% of faeces remaining in the upper sieve, <10–20% in the middle sieve, and >50% in the lowest sieve (Kljak et al., 2019).

For this assessment, 500 g of the collected and mixed faeces were transferred into the mung beaker and diluted up to the marked line and allowed to stand for 10 min. The diluted faeces were then poured onto the top sieve of the sieve set into the digestion device bucket, and the water from the bucket was discarded after waiting for the water to pass through all 3 sieves. The faeces in the upper sieve were washed with pressurised water (10 l/min) from above using a hose nozzle in a circular motion for 30 s, and the filtered water was drained from the bucket once again. The same washing process was repeated until the water easily passed through the upper sieve and no small particles remained. After these washes, the top sieve was removed. If there was a high concentration of faeces with small particles in the second, middle sieve, a 30-s shower wash was carried out until these particles passed through to the lower sieve. Following this procedure, the middle sieve was removed, and the same process was repeated for the last sieve until all small particles were removed. The remaining particles in each sieve were then weighed and evaluated.

Determination of pH values

To determine the pH of the manure, a 20 g sample was homogenised with 180 ml of distilled water for 1 min in a laboratory blender. It was then filtered through filter paper and the pH of the manure filtrate was determined using a digital pH meter.

Nutrient analyses

Crude protein, DM, ether extraction and crude ash analyses of TMR and manure samples collected in the study were conducted in accordance with the methods of analysis specified in AOAC (2005). DM levels of all samples were determined by drying at 60 °C for 48 h. Crude protein contents of the samples were determined in fresh samples before drying. After drying the samples, NDF, ADF and ADL levels were determined using an Ankom 200 Fibre Analyzer (Goering and Van Soest, 1970).

Statistical analyses

ANOVA was employed to compare group averages of the data obtained in the experiment; LSD multiple comparison test was used to determine differences between group averages. The data analysis was conducted using SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.), with a significance level set at 5%.

Results

The effects of tannin addition on DMI, milk yield (MY) parameters and milk composition of dairy cows are given in Table 2. As anticipated, there was a progressive increase in DMI across all groups over the weeks. The differences between the groups in terms of DMI at the end of the experiment were not significant. Weekly increments in milk yields were observed across all three groups with recorded values of 19.75, 23.68 and 25.14 l in the CON, T1 and T2 groups, respectively. While no discernible distinction was noted between the T1 and T2 groups, a statistically significant difference emerged when comparing the CON group to both T1 and T2 groups, yielding 3.93 and 5.39 litres more milk, respectively ($P < 0.05$).

The feed conversion efficiency (FCE) was calculated as the ratio of litres of milk produced to feed intake (DM), and the values were 1.62, 1.81 and 2.06 for the CON, T1 and T2 groups, respectively. Notably, the T2 group had a higher FCE ratio compared to the CON group ($P < 0.05$). Additionally,

Table 2. Effect of tannin supplementation in the diet on dry matter intake (DMI), milk yield (MY) and MY parameters of cows

Item	Control	T1	T2	SEM	*P*–value
DMI, kg/day	12.67	13.52	13.41	0.30	0.457
Feed conversion efficiency	1.62^{b}	1.81^{ab}	2.06^{a}	0.07	0.009
ECMY/DMI	1.63^{b}	1.92^{ab}	2.09^{a}	0.08	0.01
Yields, kg/day					
MY	19.75^{b}	23.68^{a}	25.14^{a}	0.23	0.000
ECMY	19.70^{b}	24.80^{a}	25.02^{a}	0.60	0.000
milk fat	0.71^{b}	0.94^{a}	0.93^{a}	0.03	0.001
milk protein	0.64^{b}	0.77^{a}	0.79^{a}	0.02	0.001
milk lactose	0.94^{b}	1.11^{a}	1.15^{a}	0.03	0.002
solids non–fat	8.59	8.57	8.55	0.06	0.96
Milk composition, %					
milk fat	3.55	3.91	3.72	0.08	0.171
milk protein	3.20	3.23	3.20	0.02	0.814
lactose	4.72	4.70	4.61	0.03	0.217

ECMY/DMI – energy–corrected milk yield/dry matter intake ratio; control – control group, T1 – group with 60 g of tannin supplementation, T2 – group with 120 g of tannin supplementation; SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

the energy–corrected MY to DMI ratio (ECMY/DMI) was also higher in the T2 group compared to the CON group. Moreover, significantly higher fat, protein and lactose contents in milk were recorded in tannin–supplemented groups compared to the CON (Table 2).

Blood parameters determined at the beginning, midpoint and end of the experiment are shown in Table 3. A significant difference ($P < 0.05$) was observed between Ca levels in the CON (8.68 mg/dl) and T1 (8.23 mg/dl) groups, as well as in Mg levels between the control (1.76 mg/dl) and T2 (1.92 mg/d) groups. There were no differences in blood Ca and Mg levels between the groups supplemented with the tannin. While the blood Ca levels of animals in the control group were higher than in the group with the addition of 60 g/day of tannin (T1), blood Mg levels were lower than those of T2.

Table 3. Effect of tannin administration with diet on blood parameters of cows

Item	CON	T1	T2	SEM	*P*–value
BUN, mg/dl	10.66	10.43	10.18	0.31	0.16
Ca, mg/dl	8.68^{a}	8.24^{b}	8.41^{ab}	0.08	0.02
Mg, mg/dl	1.76^{b}	1.85^{ab}	1.92^{a}	0.03	0.02
P, mg/dl	5.45	5.00	5.31	0.09	0.10
AST, IU/l	77.5	94.0	72.54	6.78	0.39
GGT, IU/l	22.71	22.50	22.58	0.53	0.99
Glucose, mg/dl	64.33	67.58	62.67	1.69	0.49
Cholesterol, mg/dl	99.29	90.33	96.50	3.41	0.36
Total Protein, g/dl	7.01	6.72	6.88	0.10	0.31
Albumin, g/dl	2.93	2.94	3.06	0.03	0.20
Ketone, mmol/l	0.42	0.52	0.45	0.03	0.43

BUN – blood urea nitrogen, AST – aspartate aminotransferase, GGT – gamma glutamyl transferase; control – control group, T1 – group with 60 g of tannin supplementation, T2 – group with 120 g of tannin supplementation; SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Tannin supplementation enhanced crude protein (CP) digestion, and the CP digestibility coefficient of the T2 group was significantly higher than in the other groups (Table 4).

Faecal and rumen pH were not significantly different between the three groups, and rumen pH remained within normal limits. Meanwhile, NH_3 levels in the rumen fluid collected from the CON animals were the highest (2.1 mmol), and the lowest (0.94 mmol) in the T2 group. Although no differences were recorded between the tannin–supplemented groups regarding acetic, propionic and butyric acid levels, the control group displayed the lowest level of volatile fatty acids (Table 5). Total volatile fatty acid (VFA) levels were determined at 26.20, 32.83 and 35.08 mmol/l in the control, T1 and T2 groups, respectively.

Table 4. Effect of tannin administration with the diet on nutrient digestibility coefficients of cows, %

Item	CON	T1	T2	SEM	*P*–value
Dry matter	67.93	68.16	70.31	0.49	0.09
Organic matter	69.13	69.26	71.37	0.47	0.08
Crude protein	71.50^{b}	71.59^{b}	74.62^{a}	0.57	0.04
Neutral detergent fibre	55.33	54.43	55.38	0.91	0.90
Acid detergent fibre	53.10	49.68	52.56	1.01	0.34

control – control group, T1 – group with 60 g of tannin supplementation, T2 – group with 120 g of tannin supplementation; SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 5. Effect of tannin supplementation in the diet on faecal and rumen pH and NH_3 and volatile fatty acids (VFA) levels in the rumen of cows

	Control	T1	T2	SEM	*P*–value
Faeces pH	7.12	7.18	7.17	0.02	0.31
Rumen pH	6.73	6.64	6.51	0.05	0.08
NH_3, mg/dl	2.10^{a}	1.43^{b}	0.94^{c}	0.12	0.000
VFA, mmol/l					
acetic acid (A)	17.83^{b}	20.11^{a}	21.18^{a}	0.34	0.000
propionic acid (P)	5.29^{b}	8.03^{a}	8.98^{a}	0.27	0.000
butyric acid	2.05^{b}	3.74^{a}	3.60^{a}	0.23	0.000
total VFA	26.20^{b}	32.83^{a}	35.08^{A}	1.16	0.000
A/P	4.45^{a}	2.75^{b}	2.76^{b}	0.12	0.000

control – control group, T1 – group with 60 g of tannin supplementation, T2 – group with 120 g of tannin supplementation; SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Figure 1. Image of residue left in three sieves of the Nasco Digestion Analyzer after washing the faeces

The study results showed that the average percentages for the CON, T1 and T2 groups were 14.31, 18.10, 13.98, 25.66, 26.80, 19.92, and 60.03, 55.10, 66.09% in the 1st, 2nd and 3rd sieve, respectively (Figure 1). The differences between the groups was found to be insignificant in the 1st and 3rd sieve, while in the 2nd sieve, a statistically significant difference between the T1 and T2 groups was identified ($P < 0.05$) (Table 6). The amount of faeces remaining in the upper sieve, with a mesh size of 4.76 mm, of the Nasco Digestion Apparatus was found to be higher than the desired level of <10% (Figure 2).

Table 6. Effects of tannin use in the ration on Digestive Assembly (Nasco) wet faecal ratios, %

Group	Sieve 1 (top)	Sieve 2 (middle)	Sieve 3 (bottom)
CON	14.31	25.66[a]	60.03
T1	18.10	26.80[a]	55.10
T2	13.98	19.92[b]	66.09
SEM	1.30	1.31	2.40
P–value	0.37	0.03	0.18

control – control group, T1 – group with 60 g of tannin supplementation, T2 – group with 120 g of tannin supplementation; SEM – standard error of the mean; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

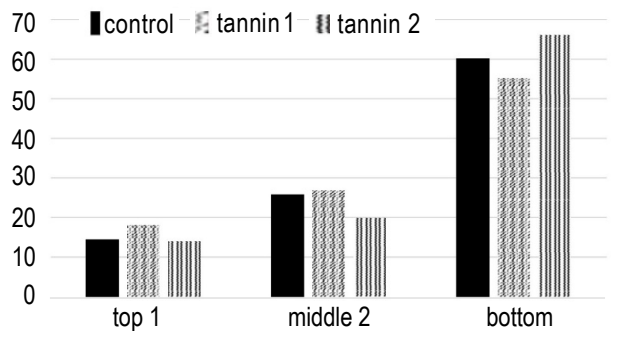

Figure 2. Effect of tannin addition on the proportion of wet faeces remaining on sieves, %

control – control group, T1 – group with 60 g of tannin supplementation, T2 – group with 120 g of tannin supplementation

Discussion

Previous studies demonstrated that the addition of tannins to the ration at levels lower than 3% DM neither decreased nor increased DMI (Carulla et al., 2005; Baah et al., 2007; Benchaar et al., 2008; Liu et al., 2013). Similarly, Zhang et al. (2019) reported no significant impact on DMI when tannins were added to the diet of lactating dairy cows at a level of 3% of DM. However, supplementation of condensed tannins at higher concentrations may negatively affect DMI due to their impact on palatability. The levels of tannin utilised in this study had no effect on DMI. Aguerre et al. (2020) reported a linear decrease in FCE and protein and fat–corrected FCE with increasing tannin levels. Conversely, the observed increase in milk yield and FCE with tannin supplementation is believed to be due to the preservation of feed protein from degradation in the rumen, increased flow from the rumen, and enhanced absorption of essential amino acids in the small intestine (Bermingham et al., 2001). Similar to the positive effect of tannin supplementation on milk yield and milk parameters observed in our study, Wang et al. (1996) found increased milk yield, protein, and lactose levels in ewes fed tannin from *Lotus corniculatus*. Tannin supplementation contributes to a reduction in the amount of protein degraded in the rumen and increases the flow of bypass protein. Tannins may also alleviate the toxic effect of high rumen ammonia concentrations and improve nitrogen efficiency (Pal et al., 2015; Adejoro et al., 2019). The beneficial effects of tannins on ruminant yield are contingent upon the quantity and quality of protein in the ration. This positive effect on yield can be attributed to protein binding by condensed tannins, thereby increasing the source of metabolisable protein (Patra and Saxena, 2011). Many studies using condensed tannins have reported decreased BUN levels, increased MY, milk protein, lactose, and milk fat contents, while also maintaining animal health, which is consistent with our findings (Soltan, 2009; Dey and De, 2014; Anantasook et al., 2015; Huang et al., 2021). However, it is worth noting that while low concentrations of tannins may have a slight positive impact on milk protein, studies have reported that the addition of quebracho or chestnut tannin extracts to the ration exert no effect on the composition or yield of milk components (Śliwiński et al., 2004; Dschaak et al., 2011; Benchaar et al., 2008; Aguerre et al., 2020). The enhanced protein digestibility attributed to tannins has resulted in an increase in milk protein.

The breakdown of ration protein with condensed tannin supplementation occurs at low pH (<3) in the abomasum, increasing passage rate, digestion, and absorption of amino acids in the small intestine. Consequently, the heightened availability of dietary protein in the small intestine likely contributes to the observed increase in milk yield (Waghorn, 2008). Since the ability of tannins to form stable complexes with proteins is pH–dependent, tannins passing through the digestive tract may first interact with dietary components and then with digestive products (Jones et al., 1994). Stable tannin–protein complexes in the rumen are formed at pH 3.5–7.0, but they dissociate in the duodenum and abomasum (Makkar, 2003; de Oliveira et al., 2011). As the digesta progresses through the small intestine, the pH increases due to various secretions, especially those from the pancreas with high levels of bicarbonate ions (Huntington et al., 2006). In slightly alkaline to slightly acidic pH environments of the ileum and large intestine, tannins can re–complex with undigested dietary components and endogenous proteins from digestive secretions (Bae et al., 1993; Frutos et al., 2004). The effects of condensed tannins on N metabolism in ruminants vary depending on the plant source and concentration utilised. For instance, it was determined that the addition of quebracho tannin to diets based on highly concentrated feed did not affect nutrient digestibility and N flux from the small intestine (Baah et al., 2007). On the contrary, Beauchemin et al. (2007) found that the inclusion of 1 and 2% quebracho tannin linearly increased the total tract digestibility of CP. Tannins can render protein and amino acids rumen–protected, thereby enhancing absorption in the small intestine, consequently improving N utilisation efficiency by evading rumen fermentation (Lima et al., 2019).

In line with our findings, some studies determined that the addition of condensed tannin did not affect rumen pH levels (Benchaar et al., 2008; Aguerre et al., 2020). In the present study, the impact of tannins on the activity of certain proteolytic bacteria in the T1 and T2 groups has resulted in lower ammonia (NH_3) concentrations in the rumen. This result can be attributed to the formation of tannin–protein complexes formed under rumen pH conditions. Additionally, tannins can form complexes not only with proteins but also to some extent with carbohydrates and minerals (Addisu, 2016). High tannin content was found to decrease feed intake and nutrient digestibility, while low and moderate tannin addition could increase digestive protein efficiency (Frutos et al., 2004). Complexes formed between tannins and proteins protect the proteins from degradation in the rumen and facilitate their release in the lower digestive segments of the digestive tract, consequently reducing the concentration of ammonia in the rumen (Ningrat et al., 2017). Protein degradation is reduced when tannin–protein complexes are formed in the rumen, thus inhibiting the growth and activity of proteolytic bacteria (McSweeney et al., 2001; Dschaak et al., 2011; Broderick et al., 2017). Similar to the present study, other works also reported that quebracho tannin extracts reduced protein degradation in the rumen by forming tannin–protein complexes, consequently lowering rumen NH_3 levels (Frutos et al., 2004; Getachew et al., 2008). This decrease in ruminal protein degradation is associated with lower ruminal ammonia production and increased flow of non–ammonia N to the duodenum (NASEM, 2016). However, it is important to note that tannins may also adversely affect post–ruminal protein digestibility through the formation of tannin–enzyme complexes, post–ruminal tannin–diet protein complexes, and hinder the absorption of protein in the intestines via tannin–mucosa interactions (Frutos et al., 2004; Patra and Saxena, 2011). Roughages containing condensed tannins at concentrations lower than 50 g/kg DM are generally considered as beneficial for protein utilisation without adversely affecting feed intake and nutrient digestion (Barry and McNabb, 1999; Waghorn, 2008).

In an *in vitro* study evaluating three different doses of two distinct tannin sources (Gambier leaves), it was determined that adding tannin sources up to 15% led to increased NDF and ADF digestibility, while higher doses had a reducing effect on digestibility (Rice–Evans et al., 1996).

The contradictory results regarding VFA and total VFA concentration appear to be influenced by various factors, including the level and source of tannin, the effect between tannin and diet composition, and the adaptation time of rumen microorganisms to tannins. For instance, Dschaak et al. (2011) found that supplementing diets containing high levels of roughage (59%) with 3% condensed tannin led to an increase in the molar ratios of acetic, propionic and butyric acids, along with a decrease in the acetic/propionic acid ratio (Dschaak et al., 2011). In our study, where all groups were fed diets consisting of 50–60% roughage throughout the experiment, tannin addition exerted similar effects on VFA. However, it is worth noting that although quebracho tannin addition had no effect on total DM digestibility, it was reported that tannin addition up

to 2% DM decreased rumen total VFA concentration, as well as the proportion of acetic acid and the acetic acid to propionic acid ratio (Beauchemin et al., 2007). Carulla et al. (2005) found that although *Acacia mearnsii* extract reduced the total digestive tract digestibility of organic matter in sheep, there was no significant change in total VFA concentration, acetic acid percentage decreased, while the proportion of propionic acid increased. It is hypothesised that the increase in DM consumption with tannin supplementation, although not statistically significant, may have contributed to the increase in VFA levels.

Blood urea nitrogen can provide information on how well the proportion of rumen degradable proteins is balanced with available energy to optimise microbial protein synthesis. An imbalance in carbohydrate and protein degradability can lead to poor animal health and reduced production performance (Lager and Jordan, 2012). Blood albumin and globulin concentrations are recognised as inflammation markers in dairy cows. In our study, blood albumin concentrations in all groups were within the normal range reported for dairy cows (Alberghina et al., 2011). Blood glucose concentration is an important marker of energy balance and lactose production in the mammary glands. Tannin addition did not affect the blood glucose levels of the animals in this study.

With respect to blood parameters, the lack of differences between the groups in glucose, BUN and cholesterol concentrations is likely due to the fact that tannin supplementation has no effect on feed intake and digestibility of many nutrients.

This phenomenon can be explained by the high utilisation of roughage with high lignin content and low digestibility, as in the case of straw, in early lactation animals, despite the inclusion of tannin in the ration. The observation that the rate of faeces flowing through the sieves exceeded 50% in the washing process involving three sieves of the Digestion Analyser is a consequence of the high proportion of NDF in the ration and the influence of tannin.

Conclusions

In summary, the addition of tannin to the rations of dairy cows at the beginning of lactation exerted a positive effect on milk yield and milk parameters, while also enhancing protein digestibility and subsequently increasing the availability of dietary protein in the small intestine. However, further studies utilising various tannin sources and proportions in lactating dairy cows are needed to fully understand and optimise the potential benefits of tannin supplementation.

Conflict of interest

The Authors declare that there is no conflict of interest.

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