Effect of Quebracho Tannins Supplementation on Nutrients Utilization and Rumen Fermentation Characteristics in Sheep

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ABSTRACT

This experiment was conducted to evaluate the potential impacts of commercial quebracho tannins (QT) supplementation to male Barki sheep on dry matter intake, digestion coefficients of nutrients, nitrogen balance and rumen fermentation characteristics. Digestibility trial was carried out on Barki sheep (n=12), which were allocated into three groups (4 animals each). The supplementation levels of QT; 0, 26 and 52 g/h/d, were mixed with the concentrate mixture. The results showed that inclusion of 52 g/h/d of QT decreased ($P<0.05$) the dry matter intake, while low dose had no negative impact on dry matter intake as compared to the control group. The low level (26 g/h/d) of QT supplementation decreased ($P<0.05$) digestion coefficients of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) relative to control, while the high level (52 g/h/d) of QT supplementation had no significant impacts on nutrients digestion coefficients, except improving ($P<0.05$) that of ether extract (EE) compared to the control and to the low level of QT supplementation. Moreover, total digestible nutrients (TDN) and digestible crude protein (DCP) decreased ($P<0.05$) by inclusion of 26g QT /h/d compared to the other levels of QT. Faecal and urinary N increased ($P<0.05$) when QT was supplemented at 26g/h/d as compared to the control group, while the high level of QT had no effect on faecal or urinary N execration. Also, the low level of QT caused decrease ($P<0.05$) in the N balance as compared to the other levels of QT. Inclusion of QT at 52g /h/d decreased ($P<0.05$) the ruminal NH_3-N concentration but the reduction in volatile fatty acids (VFA) was not significant when compared to the control group. It can be concluded that low level of QT had negative impacts on nutrients utilization and it is recommend that more studies with lower levels of QT should be conducted.

Key words: quebracho tannins, fermentation, nutrients digestibility, nitrogen balance.

INTRODUCTION

In 2006 the European Union banned the use of antibiotics in livestock feeds due to their risk on human health, antibiotic resistant bacteria may pass to human pathogens (OJEU, 2003). The European Union Directive EC 1831/2003 provided an opportunity to exploit plants, plant extracts and plant secondary metabolites like tannins, essential oils, saponins and flavonoids as natural alternatives to enhance livestock productivity and reduce environmental pollutants such as methane from fermentation process and P and N in manure (Makkar et al., 2009).

Tannins are natural plant secondary compounds that are present in many species and are commonly consumed by ruminants. They are generally defined as water soluble polymeric phenolics that precipitate proteins and are classified into hydrolysable and condensed tannins (CT) (Haslam and Lilley, 1988). Hydrolysable tannins are gallic acid and ellagic acid esters of a core molecule that consists of polyols including sugars and phenolics (*e.g.* catechin). They possess small molecules and may be hydrolyzed in the digestive tract (McLeod, 1974). Condensed tannins consist of oligomers of flavan-3-ols and related flavanol residues, which produce anthocyanidins upon acid degradation (Reed, 1995). Condensed tannins affect nutrient supply to the animal by complexing with digestive enzymes, dietary and endogenous proteins (Barry and Manley, 1986). A unique chemical property of tannins is their affinity to bind to feed proteins and thereby reduce excessive breakdown of protein in rumen (Getachew et al., 2000) and increase availability of high quality protein for absorption in the lower gut of ruminants (Waghorn et al., 1987). Eating tannins makes dietary protein unavailable for ruminal digestion until it reaches the more acidic abomasum and small intestines, modest amounts of tannins improve the protein nutrition of ruminants (Min et al., 2005). In addition to protecting feed proteins from rumen degradation, tannins also play significant roles in the prevention of bloat in ruminants by binding to proteins in the rumen (Tanner et al., 1995), suppressing intestinal parasites (Min and Hart, 2003), cause less emission of greenhouse gases such as methane from animals (Ramirez-Restrepo and Barry, 2005) and increase amino acid absorption (Waghorn et al., 1987).

This high quality bypass-protein enhances immune responses (Min et al., 2004) and improves reproductive efficiency (Min et al., 2001). In addition, a reduced protein digestion in the rumen decreases the rate of ammonia production, a potentially toxic chemical detoxified in the liver

(Chalupa et al., 1970) which represents a metabolic cost to the host (Parker et al., 1995). As a result of the aforementioned benefits on protein metabolism, commercial quebracho tannins have been proposed as an additive for protecting rumen-degradable protein in feeds (Frutos et al., 2000; Getachew et al., 2008). Tannins supplement could also represent a useful alternative for improving nitrogen utilization in ruminants. Therefore, the aim of this experiment was to study the effect of different levels of quebracho tannins on nutrients digestibility, nitrogen balance and ruminal fermentation characteristics in sheep.

MATERIALS AND METHODS

This experiment was conducted at the Milk Production Project, which belongs to the Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Egypt. All analyses were carried out at the Animal Nutrition Laboratory, Department of Animal Production, Faculty of Agriculture, Alexandria University.

Animals and management:

Twelve adult male Barki sheep (live body weight, 41.9 Kg±3.20) were randomly divided into three groups. The first group (Control, n=4) was fed a basal diet, which composed from concentrate mixture and Egyptian clover (*Alexandrium trifolum*) without supplement, the second group (QT_{26}, n=4) was fed the basal diet supplemented with daily 26 g of QT per head and the third group (QT_{52}, n=4) was fed the basal diet supplemented with daily 52 g of QT per head. The commercial QT extract was originated in Argentine. The condensed tannins content of QT was analyzed according to Yazaki and Hillis (1980).

The proximate analyses of concentrate mixture and green clover are presented in Table 1. The animals were fed their ration in groups at a rate of 3% of their average live body weight (50: 50% roughage: concentrate ratio). Animals were housed in metabolic crates under a protective roof and had free access to fresh water throughout the study. The experimental period was 28 days including 21 days adaptation period and 7 days for samples collection. During the 7-days collection period, the sheep were kept in metabolic cages individually and feed refusals, feces and urine were collected to measure nutrient digestibility and N balance.

To measure the digestibility, two representative samples (10% of total quantity) of feces from each animal were collected daily; one of these samples was sprayed by citric acid 10% and stored in the freezer, while the second feces sample was used for determination of the feces dry matter. Just after collection period, stored feces samples days for each animal were pooled, mixed well and sampled for further analyses. Representative portions were dried in a forced air-oven at 50°C for 48 h and ground to pass a 1 mm-screen and stored until analysis. The urine was collected daily during the collection period in plastic buckets containing 100 ml of H_2SO_4 (10%). Also, representative samples (10% of total volume) from each animal were collected daily and kept in the freezer. Just after collection period, urine samples for each animal were pooled, mixed well, sampled and kept frozen at −20◦C until analysis. Thawed urine samples were centrifuged at 2000×*g* for 20 min and sub-samples were analyzed for Kjeldahl N (AOAC 2006). The ruminal fluids were collected via the stomach tube before morning feeding, separated from the feed particles through four layers of cloth sheets, centrifuged at 2000×*g* for 20 min and then kept frozen at -20°C for VFA and ammonia(N) analyses. The samples were chemically analyzed according to AOAC (2006) for DM, OM, CP (CP as 6.25×N) and EE. Contents of NDF and ADF were measured according to Van Soest et al. (1991) and adapted to Mertens (2002). Concentrations of NH_3-N and total VFA were determined according to Preston (1995) and Warner (1964), respectively.

Table 1: Proximate analyses (%) on dry matter basis of the concentrate mixture and Egyptian clover.

Items, %	Concentrate	Egyptian clover
OM	92.1	85.8
CP	15.9	16.9
EE	1.3	1.9
NDF	48.5	52.9
ADF	21.3	42.5
Hemicellulose	27.2	10.4

OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber.

Statistical analysis

Data were analyzed by the generalized linear model procedure (SAS, 2002). The following model was assumed: $Y_{ij}= \mu+ T_i + e_{ij}$ where: μ is the overall mean, T_i is the treatment level, e_{ij} is the random error term. Differences among means were tested using Duncan multiple range test (Steel and Torrie, 1980).

RESULTS

The mean values of the proximate analysis on dry matter basis of the concentrate mixture and Egyptian green clover are presented in Table 1. The results of the proximate analysis showed that OM, CP and EE of concentrate mixture and Egyptian clover were 92.1 vs. 85.8, 15.9 vs. 16.9 and 1.3 vs. 1.9 %, respectively. While, NDF, ADF, and hemicellulose content of the concentrate mixture and Egyptian clover were 48.5 vs. 52.9, 21.3 vs. 42.5 and 27.2 vs. 10.4%, respectively. In addition, the CT content of the investigated commercial QT was 84.6%.

Data of feed intake, nutrients digestion coefficients and feeding values of rations without

(control) or with QT are summarized in Table 2. Quebracho tannins (QT_{52}) supplementation had negative impact on dry matter intake, while QT_{26} did not affect dry matter intake compared to the control group. Digestion coefficients of DM, OM, CP, NDF and ADF decreased ($P<0.05$) when QT_{26} was supplemented with the basal ration, while QT_{52} had no effects on nutrients utilization, but increased the digestion coefficients of EE compared to the control group. In addition, TDN and DCP declined ($P<0.05$) by QT_{26} inclusion compared to the control group, while no response appeared on TDN and DCP with QT_{52} inclusion.

The impacts of quebracho tannins supplementation on N intake, fecal N, urinary N and N balance in Barki sheep are shown in Table 3. Faecal and urinary N increased ($P<0.05$) when QT_{26} was supplemented compared to the control group, while QT_{52} had no effect on faecal or urinary N execration. Also, QT_{26} decreased ($P<0.05$) the N balance compared to QT_{52} and control group.

Ammonia-N and VFA concentrations for control, QT_{26} and QT_{52} supplementations are presented in Table 4. The results revealed that QT_{52} inclusion decreased ($P<0.05$) the ruminal NH_3-N concentration but not significantly reduced VFA compared to the control group. On the other hand, QT_{26} had no effect on NH_3-N concentration but reduced ($P>0.05$) VFA concentration compared to the untreated animals.

DISCUSSION

Addition of the tannins to ruminant diets usually reduces feed intake because of reduced palatability, decreased rate of digestion and development of conditioned aversion (Mueller-Harvey, 2006). The reduction in dry matter intake in the current study may be due to negative feedback of tannins astringency on palatability. The inclusion of QT decreased the CP digestion coefficient. Binding tannins with dietary protein generated stable protein-tannins complex at rumen pH and reduced the proteolytic activity and protein degradation. When tannin-containing plants are eaten, most binding appears to take place during chewing, but additional binding can occur in the rumen, including binding of proteins from other dietary components (Waghorn and Jones, 1989). The QT treated-animals at the current study tended to decrease N balance as stated by (Degen et al., 1997), probably due to the presence of CT, the high proportion of acid detergent insoluble nitrogen which is not easily digested by intestinal enzymes and high urinary N which in turn was attributed possibly to an imbalance of high N relative to a low energy in the rumen. The higher faecal N of the QT_{26} group over that of QT_{52} is yet to be explained through further investigations.

The main requirements for the successful use of tannins are that (1) the tannin-protein complexes should be stable in the rumen, but do not interfere with the digestion of the protein in the lower gut; (2) metabolism of rumen microorganisms should not be adversely affected; and (3) the tannins should be harmless to animals (Reid et al., 1973). There is concern that if tannin concentration in the diet becomes too high, microbial enzyme activities including cellulose (Makkar et al., 1988) and intestinal digestion (Horigome et al., 1988) may be depressed.

Furthermore, tannins can reduce ruminal protein degradability and plant cell wall digestion because they bind with dietary protein and with structural polysaccharides such as cellulose, hemicelluloses and pectin, thereby, slowing their digestion rate. Tannins might also interfere with digestion by binding microbial enzymes (McSweeney et al., 2001) and this might explain why QT supplementation decreased cell walls digestion.

Effects of plant compounds on protozoal, fungi and bacterial populations give rise to changes in formation of fermentation end products such as ammonia and VFA in the rumen.

Table 2: Effects of quebracho tannins (QT) supplementation on dry matter intake (DMI), nutrients digestion coefficients and nutritive value in Braki sheep (Means ± SE).

	Control	QT_{26}	QT_{52}
DMI, g/head/d	785.07±13.7[a]	780.94±13.4[a]	712.96±13.4[b]
Digestion coefficients, %			
Dry matter	80.62±1.47[a]	74.01±1.47[b]	79.23±1.47[a]
Organic matter	82.26±1.72[a]	75.39±1.72[b]	80.07±1.72[a]
Crude protein	78.70±1.45[a]	72.17±1.45[b]	77.14±1.45[a]
Ether extract	81.01±2.13[b]	80.49±2.13[b]	86.28±2.13a
Neutral detergent fiber	79.78± 2.48[a]	71.03± 2.48[b]	76.94± 2.48[a]
Acid detergent fiber	72.34±1.41[a]	58.68±1.41[b]	68.12±1.41[a]
TDN	75.85±0.29[a]	68.15±0.29[b]	73.21±0.29[a]
DCP	12.97±16.82[a]	11.53±16.82[b]	12.60±16.82[a]

Different letters (a, b) in the same row indicate significant differences ($P<0.05$).
TDN: Total digestible nutrients; DCP: Digestible crude protein; SE: Standard error.

Table 3: Effect of quebracho tannins (QT) supplementation on nitrogen fractions in Barki sheep (Means ± SE)

	Control	QT_{26}	QT_{52}
N intake, g/d	20.18±0.65	19.97±0.65	18.91±0.75
Faecal N	4.05±0.24[b]	5.38±0.24[a]	4.34±0.28[b]
Urinary N	3.38±0.59[b]	5.66±0.59[a]	4.02±0.68[ab]
Nitrogen balance, g/d	12.75±1.11[a]	8.93±1.11[b]	10.55±1.28[ab]

Different letters (a, b) in the same row indicate significant differences (P<0.05). SE: standard error

Table 4: Effect of quebracho tannins (QT) supplementation on NH_3-N and volatile fatty acids (VFA) concentration in Barki sheep (Means ± SE)

	Control	QT_{26}	QT_{52}
NH_3-N, mg/dL	23.27±0.84[a]	23.12±0.78[a]	16.86±0.84[b]
VFA, meq/dL	10.11±0.80	8.81±0.86	7.901±0.93

SE: standard error

Thus, saponins, essential oils and tannins usually reduce the amount of ammonia N produced in the rumen, which improves the assimilation of feed amino acidic N by ruminants (Patra and Saxena, 2009). This decrease in ammonia concentration, which is usually accompanied with a reduction in the production of isoacids, is a consequence of a decrease in degradation of feed proteins (Alexander et al., 2008; Hervás et al., 2000). Effects on rumen ammonia concentrations are probably related to a reduction in protozoal numbers (Klita et al., 1996; Newbold et al., 1997), which plays a major role in ruminal feed protein degradation (Jouany, 1996). The impairment of protein metabolism in the rumen may be due to two additive mechanisms (McIntosh et al., 2003; Newbold et al., 2004), in which the first is reduction in protein degradation to peptides (thus plant extracts such as clove bud reduce concentrations of large peptides without affecting ammonia concentrations suggesting reduced peptidolityc activity (Busquet et al., 2005), and the second mechanism is specific inhibition of microbes such as the "hyper ammonia-producing bacteria" and their deaminase activity (Newbold et al., 2004).

The CT content of the investigated commercial QT was 84.6 and this is in agreement with Frutos et al., (2000) who reported that QT contains 76% of CT and it has been suggested as a potentially useful additive for protecting soya bean meal protein against rumen degradation. However, along with this advantageous effect, the QT extract may also exert disadvantageous effects, such as impairing fiber digestion. Therefore, before promoting their use as a feed additive, further research on its effects on rumen fermentation would be desirable. Recent studies on CT (Barry and McNabb, 1999) point out the importance of considering their dosage-dependent effect; while moderate concentrations of CT (2–4.5% DM) can exert beneficial effects on protein metabolism in ruminants, high dietary CT concentrations (>5.5% DM) can depress voluntary feed intake, digestive efficiency and animal productivity. However, effects are not the same for all CT as they depend upon their chemical structure (Min et al., 2003).

Volatile fatty acids production was not affected by QT, possibly due to lack of effect of supplemented tannins on bacteria or to adaptation of rumen microorganisms to tannins (Patra and Saxena, 2011). Moss et al. (2000) showed that propionate formation can be considered as a competitive pathway for CH_4 production. Moreover, Szumacher-Strabel and Cieslak (2012) noted that a limitation of methanogenesis may be due to transformation of readily digestible carbohydrates, such as starch, to propionic acid, which may impact hydrogen transfer and, as a consequence, limit process rate. Indeed propionic acid can be formed by pyruvate conversion to propionate via the succinate pathway or by converting pyruvate to lactate and then propionate via the lactate-acrylate pathway. Formation of VFA's, including propionic acid, in the rumen depends on the substrates available in the rumen and, therefore, the microbes involved (Szumacher-Strabel and Cieslak, 2012).

CONCLUSION

In conclusion, the results of this work demonstrated that QT added to the diets of sheep depressed the apparent digestibilities of nutrients and reduced rumen microbial activities. Further data from experiments on farm animals fed lower doses of QT or tannin-containing forages are required before the potential benefit of browses can be fully assessed.

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الملخص العربى

تأثير إضافة تانينات الكابراتشو علي الإستفادة من المكونات الغذائية وعلى خصائص تخمرات الكرش في الأغنام

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أجريت هذه التجربة لتقييم تأثير إضافة تانينات الكابراتشو التجارى على الكمية المأكولة من المادة الجافة، معاملات هضم العناصر الغذائية، ميزان الآزوت وخصائص التخمرات الميكروبية بالكرش لأغنام البرقى. أجريت التجربة بإستخدام عدد ١٢ من ذكور البرقي الناضجة تم تقسيمها إلى ثلاث مجاميع تتكون كل مجموعة من ٤ حيوانات. وكانت مستويات إضافة الكابراتشو صفر، ٢٦، ٥٢ جم/ حيوان/ يوم على الترتيب تخلط مع مخلوط العلف المركز.

أوضحت النتائج أن إضافة ٥٢ جم/ حيوان/ يوم من الكابراتشو قللت معنوياً الكمية المأكولة من المادة الجافة، بينما لم يكن لإضافة ٢٦ جم/ حيوان/ يوم من الكابراتشو تأثير سلبى على كمية المادة الجافة المأكولة بالمقارنة بالمجموعة الكنترول. أدى المستوى المنخفض (٢٦ جم/ حيوان/ يوم) من الكابراتشو إلى إنخفاض معنوى فى معاملات الهضم للمادة الجافة، المادة العضوية، البروتين الخام، الألياف المقاومة للذوبان فى الجوهر المتعادل والألياف المقاومة للذوبان فى الجوهر الحامضي مقارنة بمجموعة الكنترول، بينما لم يكن للمستوى المرتفع (٥٢ جم/ كبش/ يوم) من الكابراتشو تأثير معنوى على معاملات هضم العناصر الغذائية ولكن حدث تحسن معنوى فى معامل هضم المستخلص الإيثيرى مقارنة بالكنترول و مقارنة بالمستوى المنخفض من الكابراتشو. علاوة على ذلك انخفض مجموع المواد الغذائية المهضومة والبروتين الخام المهضوم معنوياً بإضافة الكابراتشو بمستوى ٢٦ جم/ حيوان/ يوم مقارنة بمجموعة الكنترول ومقارنة بالمجموعة المرتفعة من الكابراتشو. حدثت زيادة معنوية في النيتروجين المفرز فى البول والروث بإضافة ٢٦ جم/ حيوان/ يوم كابراتشو مقارنة بمجموعة الكنترول، بينما لم يكن المستوى المرتفع من الكابراتشو له تأثير على النيتروجين المفرز فى البول والروث. أيضاً أدى المستوى المنخفض من الكابراتشو إلى إنخفاض غير معنوي في ميزان الآزوت مقارنة بالمجموعة المرتفعة من الكابراتشو والمجموعة الكنترول. كذلك أوضحت النتائج أن إضافة ٥٢ جم/ حيوان/ يوم من الكابراتشو أدت إلى إنخفاض معنوى في تركيز الأمونيا بالكرش أما الإنخفاض فى الاحماض الدهنية الطيارة فكان غير معنوى مقارنة بمجموعة الكنترول. من هنا نستنتج أن المستوى المنخفض من الكابراتشو كان له تأثير سلبى على الإستفادة من العناصر الغذائية ونوصى بإجراء مزيد من التجارب بإستخدام مستويات منخفضة من الكابراتشو.