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Effects of Quebracho Tannin Extract on *in-vitro*-rumen Fermentation and Chemical Composition of Liquid-Associated Microbes

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Introduction

Condensed tannins (CT) can form complexes with feed proteins depending on their size and structure. Thereby they slow down or even prevent the degradation of dietary protein by rumen microorganisms and thus could increase the amount of dietary amino acids available to the animal in the small intestine (Bunglavan and Dutta, 2013). The growth of rumen microbes is determined by available energy from carbohydrates degradation and ammonia-nitrogen (NH_3-N) from degraded dietary N. Rumen microbes provide around 60 to 80% of protein (Dewhurst *et al.*, 2000).Condensed tannins have been introduced as feed additives to enhance utilization of feed protein (Hervás *et al.*, 2004). Quebracho tannins extract (QTE) contains almost 76% CT, and has been proposed as a potentially useful additive for protecting feed protein from degradation in the rumen (Hervás *et al.*, 2004). Although there are advantages QTE, it may also exert disadvantages effects for instance, impairing carbohydrates degradation. Condensed tannins have previously been studied for their effects on *in vitro*ruminal fermentation, methane production and microbial protein synthesis (Bhatta *et al*., 2009). However, little attention was giving to the changes in chemical composition of rumen microbes as affected by CT. Therefore, the objective of this study was to investigate the effect of various levels of QTE on gas production and chemical composition of rumen microbial fraction using ANKOM RF system. Thus the hypothesis was that QTE affect the chemical composition of rumen microbial fraction and substrate fermentation in the rumen.

Material and Methods

Experimental treatments were a control with no tannins (control: basal diet as total mixed ration based on silage and concentrate (65:35)), and QTE added to provide 15 and 30 g/kg DM. Each substrate was incubated in three runs, with three replicates per run (n=9). Two grams of substrate were incubated in 300 ml of incubation medium

(phosphate buffer and rumen fluid, 2:1 ratio) in a water bath at 39°C using an Ankom RF system. Rumen inoculum was collected from two fistulated lactating Jersey cows. After 24 h, total content of the flasks was centrifuged at 500 g (4°C, 10 min) to separate feed particles. The supernatant was decanted and centrifuged at 20000 g (4^{o}C, 8 min) to separate liquid associated microbes (LAM). This fraction was lyophilized, ground, and analyzed for nitrogen and carbon contents. The data were analyzed by a general linear model with QTE level (0, 15, and 30 g/kg DM) as main effect and run as block effect. Polynomial contrasts were performed to find linear and quadratic effects.

Results and Discussion

Effects of quebracho tannin extract on gas production

The results showed that the total gas production (GP) was not affected by the addition of QTE, when compared with the control (0 QTE).Previous studies ofVieira and Borba (2011)and Beauchemin *et al*. (2007) using similar concentration of QTE to ours (20 and 25 g/kg DM) found no decrease in GP, thus confirming our findings. However higher concentration of QTE (50 g/kg DM) decreased GP (Vieira and Borba, 2011). In contrast, Min *et al*. (2006) conducted an *in vitro* study on gas and methane production from ground fresh wheat forage study using ruminal fluid from steers fed QTE (13 and 26 g/kg DM), they found that GP was decreased. Moreover, Tan *et al*. (2011) demonstrated that in an *in vitro* study using rumen fluid from cattle fed guinea grass and concentrate (in 60:40 ratio) total GP was decreased with increasing amount of CT from *Leucaena* at level 20g/kg DM. The main differences between our finding and those studies we may refer to is the different CT sources, the source of rumen fluid, the type of substrates and the ratio of feed component. Along the same line, the reduction of GP is attributed to the ability of CT (QTE) to bind to nutrients, rumen microbes, microbial enzyme, inhibit the activity of rumen microbes and microbial growth (Min *et al*. 2006). With regard to the mechanisms how tannins affect GP from ruminants, several studies have been indicated that the reduction might happen due to decrease in fibre degradation, through decline in the population of fibre-degrading bacteria and fungi which digest fibre (Jayanegara *et al.*, 2015). In the current study a possible decrease in substrate degradation might be implied from the reduction in total SCFA production the main product of the degradation of carbohydrates in the rumen (Jayanegara *et al.*, 2015), which was in line with a numerical decrease in GP and asymptotic GP.

Effects of quebracho tannin extract on nitrogen and carbon content

In the present study the results show that N content was higher with quebracho tannins extract 15 g/kg dry matter (QTE15) than quebracho tannins extract 30 g/kg DM (QTE30) and there were no differences between QTE15 and the control, suggesting that supplementation with 15 g/kg QTE might be more efficient for N flowing out the rumen.

The differences in N content may refer to differences in growth stage and nutrition of specific species of rumen microbes' populations also the varying microbial species within different populations (Abecia *et al*., 2014). Bunglavan & Dutta (2013) demonstrated that 15 g/kg of DMCT is the optimum level for tannins to exert remarkable effects. The experiment was carried out with adequate level of crude protein in feed samples (almost 21 g/kg of DM).

Table 1. Effects of quebracho tannin extract at different levels on gas produced from buffered rumen fluid and nitrogen and carbon after 24 hours of *in vitro* incubation.

Variable	Feed[1]			P-value[*]	
	Control	QTE15	QTE30	Linear	Quadratic
Total GP[2] (ml/g DM)	349.6 ± 17.2	337.2 ± 14.5	333.5 ± 17.7	0.14	0.65
A[2](ml/g DM)	391.2 ± 17.4	369 ± 19.8	369.1 ± 22.6	0.1	0.24
B[2]	1^{a} ± 0.005	0.99^{b} ± 0.008	0.99^{b} ± 0.005	0.04	0.11
N[2] (mg/100 mg)	5.88^{ab} ± 0.52	6.23^{a} ± 0.42	5.84^{b} ± 0.51	0.83	0.02
C[2] (mg/100 mg)	40.4^{ab} ± 1.28	41.3^{a} ± 1.57	39.9^{b} ± 1.66	0.43	0.03

[*]Means within a row with different superscripts differ (P < 0.05).

[1]QTE15, quebracho tannins extract 15 g/kg dry matter (DM); QTE30, quebracho tannins extract 30 g/kg DM

[2]GP, gas production; A, asymptotic gas production; B is a model parameter without biological interpretation; N, nitrogen; C, carbon

The results showed that the concentration of C quadratically affected by QTE (P = 0.03) with QTE15 being higher than both QTE30 and the control. Carbon content serves as an indication of the organic material present in a sample, which could also give an indication of the total microbial mass growth during fermentation. Even though there was a quadratic effect of QTE addition on C content of LAM, these changes do not seem to be of a major biological significance.

Conclusions and Outlook

The QTE addition to ruminant diets may be beneficial in protecting feed protein from rumen degradation, but the rate and extent of carbohydrate degradation might be impaired as well. Chemical composition of microbial matter may be unaffected, but reductions in rumen microbial protein synthesis cannot be excluded.

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