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Influence of quebracho tannins administration on some blood metabolites in sheep and goats

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Abstract. Eight Merino sheep (49.4±4.23 kg LW) and eight Alpine goats (53.2±2.51 kg LW) were used in two experiments to study the effect of quebracho tannins (Q) on some plasma metabolites. Four sheep and four goats were fed daily 20 g DM of alfalfa hay (3-5 cm) per kg LW (A), whereas the other animals were given the same amount of hay treated with 50 g of Q. Blood samples were taken after 5, 34, and 64 days of starting the administration of the Q diet, to assay some plasma metabolites [i.e. alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), glutamic oxaloacetic transaminase (GOT), and creatinin] as indicators of liver and kidney function. The concentration of the above mentioned plasma metabolites was unaffected by the inclusion of quebracho in the diet for the three sampling times being the mean values for sheep: ALP (IU/l) 56.97 *vs* 58.26 (sed 6.710), GGT (IU/l) 44.45 *vs* 44.48 (sed 4.857), GOT (IU/l) 40.93 *vs* 46.47 (sed 10.682) and creatinin (mg/dl) 0.94 *vs* 1.05 (sed 0.023) for the A and Q diet, respectively; and for goats: ALP (IU/l) 161.64 *vs* 190.12 (sed 47.461), GGT (IU/l) 30.51 *vs* 29.82 (sed 3.426), GOT (IU/l) 41.25 *vs* 44.16(sed 4.218) and creatinin (mg/dl) 0.76 *vs* 0.77 (sed 0.029) for the A and Q diet, respectively. Thus, it appears that the level of tannins used in these experiments does not cause any ill effect either in sheep or in goats even after 64 days of consumption of the Q diet.

Keywords. Quebracho – Sheep – Goats – Toxicity.

Effet de l'administration du quebracho sur les métabolites sanguins chez les moutons et les chèvres

Résumé. *Huit moutons de race Mérino (PV 49,4±4,23 kg) et huit chèvres de race Alpine (PV 53,2±2,51 kg) ont été utilisés dans deux expériences pour étudier l'effet des tanins du quebracho sur quelques métabolites sanguins. Quatre moutons et quatre chèvres ont reçu 20 g/kg PV de MS de foin de luzerne par jour (3-5 centimètres) (groupe A), alors que le reste a reçu la même quantité de foin traité avec 50 g de quebracho (groupe Q). Des échantillons de sang de chaque animal ont été prélevés après 5, 34 et 64 jours d'administration du foin traité au quebracho pour analyser quelques métabolites plasmatiques [la phosphatase alcaline (ALP), la gamma glutamyl-transferase (GGT), la glutamic oxaloacetic transaminase (GOT) et la creatinine] comme des indicateurs de la fonction hépatique et rénale. La concentration des métabolites plasmatiques n'a pas été affectée (P>0,05) par l'incorporation du quebracho dans le régime pour les trois jours d' échantillonnage, avec des valeurs moyennes pour les moutons : ALP (IU/l) 56,97* vs *58,26 (sed 6,710), GGT (IU/l) 44,45* vs *44,48 (sed 4,857), GOT (IU/l) 40,93* vs *46,47 (sed 10,682) et creatinine (mg/dl) 0,94* vs *1,05 (sed 0,023) pour les groupes A et Q, respectivement; et pour les chèvres : ALP (IU/l) 161,64* vs *190,12 (sed 47,461), GGT (IU/l) 30,51* vs *29,82 (sed 3,426), GOT (IU/l) 41,25* vs *44,16 (sed 4,218) et creatinine (mg/dL) 0,76* vs *0,77 (sed 0,029) pour les groupes A et Q, respectivement. En conclusion, le niveau de tanins utilisé dans ces expériences n'a pas provoqué des d'effets négatifs chez les moutons ou les chèvres, même après 64 jours de consommation du régime Q.*

Mots-clés. *Quebracho – Moutons – Chèvres – Toxicité.*

I – Introduction

Ingestion of tannin-containing food by ruminants may reduce digestibility, particularly that of protein, and voluntary food intake (Robbins *et al.*, 1987; Salem *et al.*, 2006). The effect is accentuated as the level of tannins increases (Silanikove *et al.*, 1996). The intoxications with tannins in the literature are failing in most cases to state the type of tannin reported. Such cases are, however, normally due to hydrolysable tannins (Garg *et al.*, 1992; Zhu *et al.*, 1992; Plumlee *et al.*, 1998) and are associated with weather conditions that prevent access to conventional forage (Spier *et al.*, 1987). Tannic acid (Zhu *et al.*, 1992; Plumlee *et al.*, 1998), gallic acid and ellagic acid (Murdiati *et al.*, 1992), all of which are hydrolysable tannins, have been used as models in tannin toxicity studies. In the rumen, microbial tannases hydrolyze galloyl esters to gallic acid that is further metabolized by microbes to pyrogallol and other low-molecular-weight phenols that are absorbed from the rumen (Murdiati *et al.*, 1992). These phenols may be further metabolized by conjugation to glucuronic acid after absorption. Resorcinol glucuronide and the glucuronide of 2-carboxy- 2'4' 4, 6,-tetrahydroxy diphenyl 2, 2'-lactone are the main urinary metabolites from sheep fed tannic acid (Murdiati *et al.*, 1992). The major lesions associated with tannin poisoning are hemorrhagic gastroenteritis, necrosis of the liver, and kidney damage with proximal tubular necrosis (Holliman, 1985; Filippich *et al.*, 1991). Little is known on the toxicity of condensed tannins, although they occur in a wide range of plant species commonly consumed by ruminants and have received more attention than any other group of plant secondary metabolites in relation to effects on digestibility (Foley *et al.*, 1999). Condensed tannins (i.e. quebracho which contains 76% of condensed tannins) have been suggested as feed additives for improving the digestive utilization of dietary protein (Schwab, 1995; Frutos *et al.*, 2000). It was observed that sheep dosed intra-ruminally (0.75 g/kg live-weight/ d) for 60 days remained healthy throughout the experiment and no signs of intoxication were found either at necropsy or on histological examination (Frutos *et al.*, 2000). Despite this apparent lack of toxicity, recent studies in ruminant nutrition (Barry and McNabb, 1999; Hervas *et al.*, 2003), gave rise to concern as to the possible importance of dose-dependent effects, particularly those of secondary compounds such as condensed tannins. The purpose of the present study was to examine the influence of feeding quebracho tannins treated alfalfa hay during 64 days on some blood metabolites as indicators of the hepatic and kidney functions in sheep and goats.

II – Materials and methods

Two experiments [Expt. 1 (Sheep) and Expt. 2 (Goats)] were completely conducted at the experimental station and laboratory of Animal nutrition of the University of León-Spain. Eight adult Merino sheep (49.4±4.23 kg LW) and eight Alpine goats (53.2±2.51 kg LW) were fed 20 g DM/kg LW of alfalfa hay (A; chopped at 3-4 cm) for 15 days. During this period of adaptation, all animals were housed in individual pens (0.70 x 1.10 x 1 m). After this period of adaptation to the basal diet (A), level of ingestion and experimental conditions, four sheep and four goats were given hay treated with quebracho (Q; 50 g/kg DM of alfalfa hay) for 64 days, whereas the others animals were given untreated alfalfa hay (A) and used as controls. At 6:00 am of each day of the experiments, 50 g of a commercial extract of Q tannins (Roy Wilson Dickson Ltd., United Kingdom) were dissolved in 200 ml of water and sprayed on to the weighed amount (kg DM) of hay and thoroughly hand mixed with alfalfa hay just before to feeding animals at the morning (at 8:00 am). Feeds were distributed once daily and animals had continuous clean water and a block of vitamin-mineral corrector to free disposition. Blood samples were taken after 5, 34, and 64 days of starting the administration of Q treated alfalfa hay to animals, to assay some plasma metabolites [i.e. alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), glutamic oxaloacetic transaminase (GOT), and creatinin] as indicators of liver and kidney function. Blood samples were collected before feeding the animals in heparinized tubes of 10 ml (Venoject®-Terumo® Europe V. N., Bélgium] and immediately centrifuged (P-Selecta S-478, España) at 3000 rpm for 15 mins and the plasma was kept in plastic tubes of 2.5 ml (Tapval-Aquisel, Spain) at -20ºC. A 400µl aliquot of plasma was taken for assaying the afore mentioned plasma metabolites (i.e. ALP, GGT,

GOT and creatinin) using an autoanalyser (Hitachi 704, Japan). Data were analysed by ANOVA using a split-plot in time design in which diets (A and Q) were the main plot and time after feeding quebracho (P1, P2, P3) the subplot (Steel and Torrie, 1980). The dietary (D), period (P) effect and the interaction of D x P were added to the model. Statistical analyses were performed by procedures of the Statistical Analysis Systems Institute (SAS, 1993).

III – Results and discussion

The activity of the previously mentioned plasma enzymes and the concentration of creatinin in plasma were not affected by the inclusion of quebracho in the diet neither in sheep nor in goats (Fig. 1).

The values of ALP were reduced (P<0.05) in P3 in comparison with P1 in sheep and the plasma concentration of creatinin in goats was lower (P<0.05) in P3 than in P1. These declines were in both cases a consequence of changes produced in the animals receiving the Q diet as it is shown in Table 1. On the other hand there was a significant (P<0.05) decrease in the plasma level of GGT in sheep consuming the Q diet from P1 to P2 and P3.

These results are in agreement with other studies (Hervas *et al.*, 2003) that indicate that dose of quebracho higher than the ones used in these experiments are required for producing intestinal mucosa damage and subsequent systemic toxicity. Ruminants, like sheep and goat, seem to have the ability of developing some mechanisms to tolerate the negative effects of condensed tannins. Some of these mechanisms are possibly: (i) pre-ruminal, by increasing the saliva production, (Salem *et al.*, 2001); (ii) ruminal, by increasing the number of tannin-resistant organisms (Bae *et al.*, 1993; Nelson *et al.*, 1998; Krause *et al.*, 2005); or (iii) post-ruminal, by increasing the intestinal mucus production as a defense against intestinal damage (Waghorn, 1996) which could avoid the Q tannins absorption and its systemic toxicity effects.

Table 1. Activity (IU/l) of some plasma enzymes and concentration (mg/dl) of creatinin in sheep and goats fed on alfalfa hay alone (A) or with a daily dose of 50g quebracho /kg DM (Q) at three experimental periods

	Diet (D)		Sampling periods (P)			Sed d(p)	Sed p(d)
			P1	P2	P3		
Sheep	ALP	A	62.25	51.48	57.17	8.280	5.839
		Q	64.95[a]	59.26[ab]	50.59[b]		
	GGT	A	44.00	45.88	43.47	6.805	5.835
		Q	53.30[a]	40.25[b]	39.90[b]		
	GOT	A	47.97	35.80	39.03	12.270	7.383
		Q	47.68	52.63	39.13		
	Creatinin	A	0.94	0.94	0.94	0.108	0.050
		Q	1.04	1.06	1.04		
Goats	ALP	A	145.76	169.40	169.75	52.811	28.472
		Q	183.77[ab]	228.66[a]	157.93[b]		
	GGT	A	30.00	30.70	30.83	4.042	2.617
		Q	27.98	30.35	31.13		
	GOT	A	44.85	40.73	38.18	5.405	4.131
		Q	45.58	45.10	41.80		
	Creatinin	A	0.79	0.77	0.73	0.040	0.033
		Q	0.80	0.75	0.75		

[a b] Means with different superscript within rows differ significantly (P<0.05).

Sed d(p) Sed p(d): Standard error of the differences between diets within periods and between periods within diets, respectively.

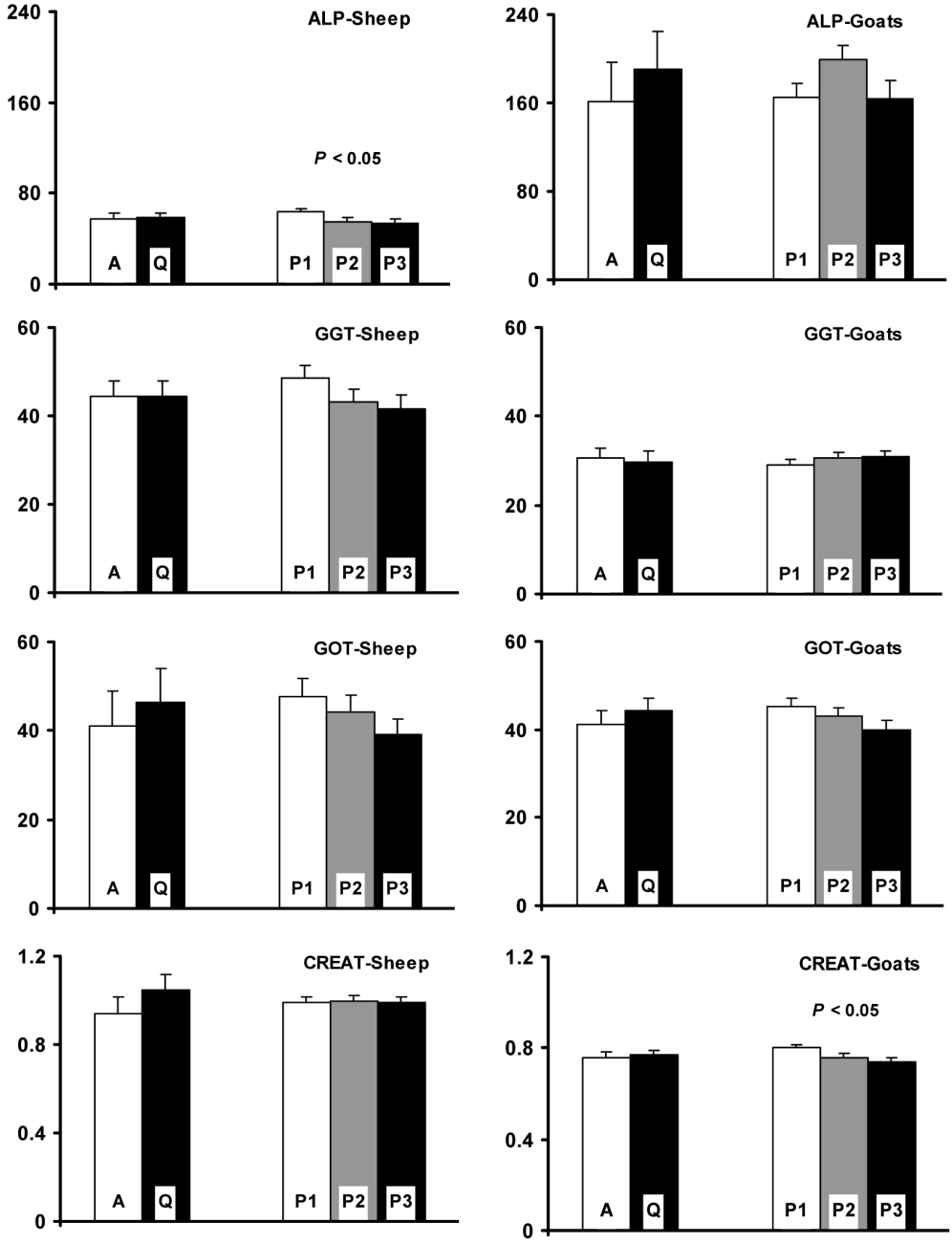

Fig. 1. Activity (IU/l) of some plasma enzymes and concentration (mg/dl) of creatinin in sheep and goats fed on alfalfa hay alone (A) or with a daily dose of 50 g quebracho/kg DM (Q) at three experimental periods (P1, P2 and P3).

IV – Conclusions

Our results suggested that the level of tannins used in these experiments did not cause any ill or systemic toxicity either in sheep or in goats even after 64 days of consumption of the Q diet.

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