

# ANTIOXIDANT EFFECTS OF DAMS' FEEDING SYSTEM DURING LACTATION AND THE INCLUSION OF QUEBRACHO IN THE FATTENING CONCENTRATE IN LAMBS' TISSUES

P. J. Rufino-Moya, M. Joy and M. Blanco \*

Centro de Investigación y Tecnología Agroalimentaria de Aragón. Instituto Agroalimentario de Aragón-IA2-(Universidad de Zaragoza-CITA). Avda. Montañana 930, 50059 Zaragoza, Spain

\*Corresponding author email: mblanco@aragon.es

**Abstract** – The aim of the present study was to assess the influence of the feeding system of the dams during lactation and the inclusion of quebracho in the fattening concentrate on polyphenols content and antioxidant (AO) status of the muscle and the liver of light lambs. Three feeding systems during lactation were studied: dam-lamb pairs grazed alfalfa, dam-lamb pairs grazed sainfoin and dam-lamb pairs stalled and dams were fed a total mixed ration (TMR). After weaning ( $42\pm 2$  days), lambs were fed a fattening concentrate with or without quebracho (QUE) until 22-24 kg. The total polyphenols were measured by Folin-Ciocalteu method, and AO activity was analyzed by DPPH, ABTS and FRAP assays. The feeding system during lactation only affected the ABTS value in muscle ( $P<0.05$ ). The inclusion of quebracho in concentrate increased polyphenols content in the muscle ( $P<0.05$ ) but had no effect on AO status. In the liver, QUE concentrate did not affect polyphenols content but decreased DPPH and FRAP ( $P<0.05$ ). The polyphenols contents in fresh forage and QUE concentrate did not improve the AO status of animal tissues. Studies to elucidate the mechanisms of action of polyphenols in animal tissues should be carried out.

Key Words – condensed tannins, polyphenols, sainfoin.

## I. INTRODUCTION

Oxidative process in animal tissues is the most important factor responsible for quality deterioration. Strategies to delay this process through dietary antioxidants (AO) are studied. Fresh legume forages contain AO compounds, such as polyphenols, which are deposited in animal tissues [1]. Alfalfa (*Medicago sativa* L.) and sainfoin (*Onobrychis viciifolia* Scop.) have high nutritive value, however, they differ in the presence of condensed tannins (CT), a type of polyphenols, present in sainfoin but not in alfalfa [2]. Some studies have shown that the inclusion of quebracho (*Schinopsis balansae*), rich in CT, in lamb's diet improved the AO capacity of the muscle [3] and the liver [4] of lambs.

The aim of this study was evaluate the effects of the dams feeding system during lactation and the inclusion of quebracho in the fattening concentrate on polyphenols content and AO status of the tissues of light lambs.

## II. MATERIALS AND METHODS

### II.I. Animal management and sampling

Sixty-three dam-lamb pairs of Rasa Aragonesa breed were used in the trial. During lactation, 21 pairs were stalled and dams were fed a total mixed ration (TMR), 21 pairs grazed alfalfa (Alfalfa) and 21 pairs grazed sainfoin (Sainfoin). After weaning ( $42\pm 2$  days), lambs were fed fattening concentrates ad libitum; half of the lambs of each lactation feeding system a commercial concentrate (Control) and the other half a concentrate with 5% added quebracho (SYLVAFEED ByPro Q, Adial Nutrition. Girona, Spain) with 75% of condensed tannin (QUE). Lambs were slaughtered when they reached 22-24 kg LW (70 days). After a 24 h at 4°C,

carcasses were split; samples of the Longissimus dorsi muscle and the liver were removed, freeze-dried, ground and stored at -80°C in total darkness.

## II.II. Sample extraction and chemical analyses

To determine total polyphenols of the feedstuffs, the samples were subjected to two successive extractions with 5 mL of acetone:water (1:1; pH:2) [5] and the extracts were analyzed using the Folin-Ciocalteu method as reported Julkunen-Tiitto [6].

The extraction method for determination of total polyphenol content in animal tissues and of AO activity of all the samples was performed as described by Roncero-Ramos et al [7] with the following modifications. Dried samples were sonicated with methanol:water for 10 minutes and homogenized in orbital shaker for 60 minutes. Samples were centrifuged. Then the supernatant was recovered and the residue was re-extracted with acetone–water (70:30, v/v). The combination of these two extracts was used for the further determinations. The AO activity was assessed through the free radical scavenging activity (DPPH and ABTS assays) and the ferric reducing antioxidant power (FRAP) [7]. Trolox was used as standard for DPPH, ABTS and FRAP assays.

## II.III. Statistical analyses

The statistical analyses were performed using SAS. A general linear model was used with the feeding system during lactation, the inclusion of quebracho in the fattening concentrate and its interaction as fixed effects. LSmeans were compared using the Tukey test.

## III. RESULTS AND DISCUSSION

Sainfoin had 3- and 5-fold greater polyphenols content than alfalfa and TMR (Table 1). Besides, Sainfoin also had 7 to 8-fold greater AO capacity due to the polyphenol and CT contents [8], whereas the AO activity of Alfalfa and TMR was similar. The Control concentrate had 11- and 25-fold lower polyphenol content and AO activity, respectively, than Que concentrate, which is in agreement with Hagerman et al. [9].

In the muscle, polyphenols content tended to be affected by the feeding system during lactation ( $P=0.05$ ), being highest in TMR, intermediate in Alfalfa and lowest in Sainfoin lambs. The bioavailability of phenolic compounds in lactating lambs has been scarcely studied. Goats fed sulla (*Sulla coronarium* L), that has polyphenolic compounds and high CT content, presented greater polyphenols content and AO activity on milk than goats fed hay and barley meal [10]. Then, there is a transfer of polyphenols through the milk of the ewes to the muscle of the lactating lamb, according to Moñino et al. [4]. Similarly, there was a transfer of polyphenols through the goat milk to the plasma of sucking goat [11], however, the deposition in the muscle was not studied.

Table 1 Polyphenols content and antioxidant activity of the feedstuffs.

	Polyphenols <sup>1</sup>	DPPH <sup>2</sup>	ABTS <sup>2</sup>	FRAP <sup>2</sup>
Ewes' feedstuffs				
TMR	6.1±0.04	15.0±0.9	34.0±0.6	30.8±0.7
Alfalfa	10.6±0.7	16.7±1.0	35.6±2.2	36.3±2.6
Sainfoin	32.1±1.8	147±11	228±11	20±11
Lambs' fattening concentrate				
Control	3.6±0.2	5.4±0.5	15.6±0.6	11.7±0.4
QUE	38.9±1.0	194±2.8	283±6	237±8

<sup>1</sup> mg tannic acid equivalent/g DM; <sup>2</sup> µmol trolox equivalent/g DM

The polyphenols content in the liver was not affected by the feeding system during lactation ( $P>0.05$ ). No other similar studies are found in the literature, to our knowledge. In fattening lambs fed ryegrass, polyphenol content in the liver was greater than that in lambs fed concentrates [12]. The different result compared to the present study could be related to the type and the quantity of polyphenols ingested, and the type of digestion, lactating lambs vs. weaned lambs. Another fact to take into account is the method of analysis detects phenolic compounds but also other reducing agents, thus it may overestimate the content of polyphenols [13].

The feeding system during lactation had no effect on the AO status of the muscle and the liver ( $P>0.05$ ), except for ABTS in the muscle ( $P<0.05$ ). The TMR and Alfalfa lambs presented greater ABTS values than Sainfoin lambs ( $P<0.05$ ). These unexpected results could be related to the type of polyphenols in the dams' diet, which might affect differently the AO status of the tissues. The deposition of polyphenols and AO status was greater in the liver than in the muscle, as reported by Bodas et al. [14]. Besides, the extraction to determine AO in animal tissues could be incomplete, being the AO values underestimated [15].

Table 2 Effect of the system feeding during lactation and the inclusion of quebracho in the fattening concentrate on the polyphenols content and antioxidant status of the muscle and the liver

	Polyphenols <sup>1</sup>	DPPH <sup>2,3</sup>	ABTS <sup>2</sup>	FRAP <sup>2</sup>
<b>Muscle</b>				
Lactation feeding system				
TMR	152	0.47	1.33 <sup>a</sup>	0.28
Alfalfa	149	0.42	1.33 <sup>a</sup>	0.25
Sainfoin	144	0.45	1.25 <sup>b</sup>	0.25
Fattening Concentrate				
Control	145 <sup>b</sup>	0.43	1.29	0.25
QUE	151 <sup>a</sup>	0.47	1.32	0.27
Root SEM	10.4	0.11	0.11	0.06
P-value				
Forage	0.05	0.32	0.02	0.18
Concentrate	0.03	0.11	0.21	0.19
<b>Liver</b>				
Lactation feeding system				
TMR	446	0.71	2	1.14
Alfalfa	446	0.66	2	1.13
Sainfoin	444	0.72	2	1.2
Fattening Concentrate				
Control	446	0.80 <sup>a</sup>	2	1.29 <sup>a</sup>
QUE	444	0.61 <sup>b</sup>	2	1.02 <sup>b</sup>
Root SEM	38	0.24	0.1	0.28
P-value				
Forage	0.98	0.68	0.99	0.68
Concentrate	0.83	0.002	0.92	0.001

<sup>1</sup>  $\mu\text{g}$  gallic acid equivalent/ g FM; <sup>2</sup>  $\mu\text{mol}$  trolox equivalent/ g FM; <sup>3</sup> The interaction between the forage fed to the ewes and the fattening concentrate was significant ( $P=0.03$ ) in the muscle. Within a row, different superscript letters indicate significant differences ( $P < 0.05$ )

The QUE concentrate in the fattening period increased polyphenols content in the muscle ( $P<0.05$ ) as reported with an inclusion of 9% of quebracho in the concentrate of lambs [3]. On the other hand, the inclusion of quebracho in the fattening concentrate did not affect the polyphenols content in the liver ( $P>0.05$ ) as reported with a 9.6% of quebracho included in the concentrate [4]. The abovementioned study suggested that the profisetinidin tannins from quebracho might not be degraded nor absorbed in the gastrointestinal tract. However, Luciano et al. [3] suggested that quebracho might provide other smaller phenolic compounds that could have been directly absorbed and transferred to the muscle.

The AO status of the muscle was not affected by the inclusion of quebracho in concentrate ( $P>0.05$ ). Conversely, the inclusion of a 9% of quebracho in the fattening concentrate during 60 days increased FRAP and ABTS values in the muscle [3]. This different response could be related to the short fattening period (around 30 days) and to the low inclusion of (5%) quebracho in the current experiment .

Unexpectedly, the DPPH and FRAP values of the liver were higher in Control than in QUE concentrate lambs ( $P<0.05$ ), whereas ABTS values were similar. Conversely, the inclusion of 9% quebracho during 70 days increased FRAP in the liver of fattening lambs [4]. As it has been previously explained, in the present experiment, the inclusion of quebracho could have been too low or fed a too short period to have effect on the AO status of animal tissues compared to other experiments. Besides, the FRAP assay could react to a wide spectrum of reducing compounds [13] and ABTS assays could react with any hydroxylated aromatic compound independently of its real AO potential [16].

#### IV. CONCLUSION

Polyphenols content and AO status of the muscle and liver of light lambs did not increase when dams grazed fresh forage during lactation. The effects of grazing could disappear because after weaning, lambs were concentrate-fed indoors during approximately 30 days. The inclusion of quebracho in the fattening concentrate of lambs increased the concentration of polyphenols in the muscle but not in the liver. The AO status of both tissues was not increased with quebracho inclusion in the fattening diet. More research is needed to elucidate how dietary factors affect the oxidative status, assessing polyphenol contents and AO ability by HPLC and analyzing endogenous AO enzyme activities of animal tissues.

#### ACKNOWLEDGEMENTS

The authors gratefully acknowledge the staff of the CITA Research Centre for technical support. Research and M. Blanco contract funded by Spanish Ministry of Economy and Competitiveness, INIA (project RTA2012-0080-C00-00) and the European Regional Development Funds. P.J. Rufino enjoy predoctoral contracts funded by INIA.

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