Effect of Condensed Tannins in *Lotus uliginosus* cv. E-Tanin and/or Extracts of Quebracho and Chestnut in Carcass and Lamb Meat

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Summary
The effect of condensed tannins (CT) in the pasture (*Lotus uliginosus* cv. E-Tanin) and/or quebracho and chestnut extracts on lamb carcass and meat quality was evaluated. Two experiments were conducted in consecutive years with 60 crossbreed lambs each year. The animals were stratified by live weight (LW) and randomly assigned to the treatments: white clover grazing (WC); WC plus concentrate supplementation (WC + C); WC plus concentrate supplementation and quebracho and chestnut extract (WC + C + CT); *Lotus uliginosus* grazing (E-Tanin); E-Tanin plus concentrate supplementation (E-Tanin + C); E-Tanin plus concentrate supplementation and quebracho and chestnut extract (E-Tanin + C + CT); with two repetitions each year. Pasture type affected loin weight, which was greater (P < 0.05) in lambs grazing WC than E-Tanin. Meat color redness (a*) was greater (P < 0.05) in supplemented treatments with C (with or without CT) than those exclusively grazing. Intramuscular fat content was not affected (P > 0.05) by the pasture, the supplementation, or the year. The proportion of dihomo-gamma-linolenic acid (C20:3-n6) was greater (P < 0.05) in lambs grazing E-Tanin compared to WC. Supplementation affected (P < 0.05) the saturated (SFA) and monounsaturated fatty acids (MUFA) proportions, and the omega 6:omega 3 ratio. Estimation of dry matter intake under grazing conditions would allow a better understanding of the effect of CT on carcass and meat quality.

Keywords: lamb, pasture, condensed tannins, carcass and meat quality

Efecto de taninos condensados de *Lotus uliginosus* cv. E-Tanin y/o extractos de quebracho-castaño en canal y carne de cordero

Resumen
Se evaluó el efecto de los taninos condensados (TC) en la pastura (*Lotus uliginosus* cv. E-Tanin) y/o extractos de quebracho y castaño en canal y carne de corderos. Se realizaron dos experimentos en años consecutivos con 60 corderos cruzada cada año. Los animales fueron estratificados por peso vivo (PV) y asignados al azar a los tratamientos: pastoreo de trébol blanco (TB); pastoreo de TB más ración (TB + R); pastoreo de trébol blanco más ración y extracto de quebracho y castaño (TB + R + TC); pastoreo de *Lotus uliginosus* (E-Tanin); pastoreo de E-Tanin más ración (E-Tanin+R); pastoreo de E-Tanin más ración y extracto de quebracho y castaño (E-Tanin + R + TC), cada uno con dos repeticiones cada año. La pastura afectó el peso del bife, que fue mayor (P < 0.05) en los animales que pastorearon TB respecto a aquellos sobre E-Tanin. La intensidad de rojo (a*) del color de la carne fue mayor (P < 0.05) en los tratamientos suplementados con R (con y sin TC) respecto a aquellos exclusivamente pastoreles. El contenido de grasa intramuscular no fue afectado (P > 0.05) por el tipo de pastura, suplementación, o año. Los corderos que pastorearon E-Tanin presentaron una mayor (P < 0.05) proporción del ácido dihomo-gamma-linolénico (C20:3-n6) que aquellos sobre TB. La suplementación afectó (P < 0.05) las proporciones de ácidos grasos saturados (AGS) y monoinsaturados (AGM), y la relación omega 6:omega 3. Estimaciones del consumo en condiciones de pastoreo permitirían conocer mejor el efecto de los TC en la calidad de la canal y la carne.

Palabras clave: cordero, pastura, taninos condensados, calidad de canal y carne
Introduction

It is quite challenging to achieve consistency and homogeneity in meat produced from lambs reared under grazing systems. Therefore, the inclusion of supplementation is an alternative that allows achieving heavier carcasses and greater levels of fat coverage and may also affect the quality of the meat\(^{(9)}\). Pasture species used in the animal fattening phase have been reported to affect meat characteristics\(^{(2)(3)}\). Diet of the animal affects the fatty acid profile of intramuscular fat, which is associated with its potential effect on human health and the development of flavor when meat is cooked\(^{(4)(5)(6)}\). Production system also affects meat flavor and differences were reported even between different species of pastures\(^{(7)}\). On the other hand, animals in grazing systems generally produce darker meat than those animals fattened with high-concentrate diets\(^{(8)}\).

Tannins are water-soluble phenolic compounds, with molecular weights between 500 and 3000 Da which, apart from the usual phenolic reactions, have special properties such as the ability to precipitate alkaloids, gelatin and other proteins\(^{(9)}\). Tannins are frequently found in fruit trees, in temperate pastures (mainly legumes), and other species such as sorghum and corn, commonly used in cattle feeding. Condensed tannins (CT) interact with proteins forming complexes that prevent their degradation in the rumen but allow digestion in the abomasum and small intestine\(^{(10)}\). However, the diversity of effects of tannins on digestion is due, partly, to differences in the chemical reactivity of different types of tannins\(^{(11)}\). In terms of meat quality, CT have been reported to increase meat lightness (L*) compared to control animals\(^{(12)}\) and affect the fatty acid profile of intramuscular fat due to its effect on the ruminal biohydrogenation\(^{(13)}\).

Currently, the CT are becoming of interest as part of ruminant diets, due to their potentially beneficial effect on animal health and performance, as well as on product quality.

The objective of the present study was to evaluate the effect of condensed tannins present in Lotus uliginosus cv. E-Tanin and in extracts of Shinopsis balanceae (quebracho) and Castanea sativa (chestnut), on carcass and meat quality characteristics of heavy lambs, fattened under grazing conditions.

Material and Methods

The experiments were carried out at Glencoe Research Station, INIA Tacuarembó, located in Molles de Paysandú (32° 00'24" S, 57° 08' 01" W) during two years: 9/10 to 15/12/2015 (Year 1) and 26/05 to 13/09/2016 (Year 2). The experimental area was two plots of 4 ha sown with Lotus uliginosus cv. E-Tanin (E-Tanin) and Trifolium repens cv. Zapicán (white clover, WC).

E-Tanin was chosen due to its moderate to high content of condensed tannins (53 g/kg DM, average from different physiological states and seasons (Reyno, 2018, com pers.) when it represents 70 % of a pasture). White clover was used as control whose CT content is negligible (1.3 g/kg DM\(^{(14)}\)).

Animals, management and experimental treatments

Sixty Texel and Australian Merino crossbreed lambs were used (30 females and 30 castrated males) in Year 1 and Year 2 respectively and the stocking rate was 8 lambs/ha. The animals were stratified by live weight (LW) and randomly assigned to six treatments with two repetitions each: improved pasture with white clover (Trifolium repens cv. Zapicán; WC); WC plus concentrate supplementation (WC+C); WC plus concentrate supplementation plus quebracho and chestnut extract (WC+C+CT); improved pasture with Lotus uliginosus cv. E-Tanin (E-Tanin); E-Tanin plus concentrate supplementation (E-Tanin+C); E-Tanin plus concentrate supplementation plus quebracho and chestnut extract (E-Tanin+C+CT). The commercial quebracho (Schinopsis balanceae) and chestnut (Castanea sativa) extract (Silvafeed® Bypro de Silvateam S.A.) with high CT content was provided at 1 % of the concentrate, and lambs were supplemented at 1 % of LW every morning at the same time. The grazing system consisted of 14 days plots occupation and 14 days resting periods and lambs had quality water available in each plot.

The experimental protocol was reviewed and approved by the Ethics Committee on the Use of Animal for Research of INIA (CEUA by its Spanish acronym), registered at the National Commission of Animal Experimentation (CNEA by its Spanish acronym) with the record number 0009/11, INIA 2015.46.

The initial average LW of lambs in Year 1 was 39.7 kg for the group on WC and of 37.3 kg for the group assigned to E-Tanin, whereas during Year 2, the average LW was

\[ \text{LW of lambs in Year 1} = 39.7 \, \text{kg} \]
\[ \text{LW of lambs in Year 2} = 37.3 \, \text{kg} \]
28.7 kg and 28.2 kg for those grazing on WC and E-Tanin, respectively. Forage dry matter availability in Year 1 at the beginning of the experiment was of 3149 and 2218 kg DM/ha for WC and E-Tanin, respectively, and of 2151 and 2904 kg DM/ha in Year 2 for the same pastures. Crude protein content in Year 1 was 18.3 % in WC pasture and 15.0 % in E-Tanin pasture, whereas in Year 2 it was of 21.5 and 14.1 % for these same pastures.

The content of CT in Year 1 was of 15.9 g/kg DM in October and 58.9 g/kg DM in November when the legume was a pool of native pasture and E-Tanin, and of 2.2 and 2.1 g/kg DM in a pool of native pastures and WC during the same period. During Year 2, the concentrations of CT were of 8.4 g/kg DM in June and 19.6 g/kg DM in September for the improved pasture with E-Tanin, and of 0.4 and 0.9 g/kg DM for the pasture with WC in the aforementioned months.

Determinations

All the animals from each year experiment were slaughtered at the same day in a commercial abattoir. Hot carcass weight (HCW) was registered in the slaughter. Before fabrication, thickness of the subcutaneous tissue at the GR point (on the 12th rib and at 11 cm from the carcass midline) was determined(15), and carcass length (CL), leg perimeter (LP), and leg length (LL) were measured. The weight of the following valuable cuts was determined in the fabrication room: loin (on the lumbar section), boneless leg with rump (BLR), and frenched rack (eight ribs; FR)(16). Subsequently, the carcass left loins were collected and vacuum-packaged for their transfer to the Meat Technology Laboratory at INIA Tacuarembó, where they were aged at 0-2 ºC for five days.

After aging, the vacuum sealed bags containing loin samples were opened. Meat color was determined in triplicate in each sample after 45 minutes blooming, using a Konica Minolta (model CR 400) colorimeter in the CIELab color space (L*: lightness, a*: redness, and b*: yellowness). Subsequently, a 5 cm loin sample was cut to determine the Warner-Bratzler shear force (WBSF, kg) using a Warner-Bratzler (G-R Electric Manufacturing Co, Manhattan, KS; model D2000) equipment with a triangular section blade. For the WBSF determination, the samples were placed in polyethylene bags and cooked in a water bath until reaching an internal temperature of 70 °C. Six square section pieces of 1.2 cm of side parallel to the longitudinal direction of muscle fibers were taken, and WBSF was performed perpendicular to fibers orientation; an average value for each sample was calculated. The loin portion not used for WBSF measurements remained frozen at -20 °C for subsequent analysis of fatty acid profile of the intramuscular fat. Lipid extraction was carried out using chloroform-methanol according to the procedure of Bligh and Dyer(17). Fatty acids were methylated in cold with methanolic potassium(18). A Konik HRGC 4000B equipment with a capillary column of 100 m long (SP 2560, Supelco, Bellefonte, USA; 0.25 mm internal diameter, 0.20 µm of thickness) was used for the chromatographic analysis. Nitrogen was the carrier gas with a flow of 1 ml/min. The injection volume was of 1 µl, and a flame ionization detector was used (FID). Peak recognitions in the chromatogram were performed using a Supelco™ 37 Component FAME Mix pattern, and identifying fatty acids by comparing their retention times with the standards (Supelco, Bellefonte, USA). Fatty acids were expressed as percentage of total fatty acids identified.

Statistical analysis

A split-plot randomized complete block design was used in this research, where the forage species represented the whole plot (E-Tanin or WC) and the sub-plot by the supplementation (without C, with C, C plus CT) which was randomly assigned. Data analysis was performed using the Statistical Analysis System (SAS) PROC MIXED, 9.4 version (SAS Institute, Cary, NC, USA). A mixed model was used for the carcass and meat quality variables that included the fixed factors: forage species, supplementation (C and C+CT) and the interaction between these factors, while the year was considered as a random effect as well as its interaction with the forage species and the supplementation. The final LW was used as covariate on the analysis of HCW, while the HCW was a covariate on the analysis of the GR, CL, LP, LL and loin weights, FR and BLR. Shapiro-Wilk(20) test was conducted to determine normality in the distribution of the response variables and the extreme or atypical values were verified using the studentized residual plots. Degrees of freedom of the denominator were calculated using the Kenward-Roger(21) method. Tukey’s test(22) was used for mean treatment comparisons with a significance level of $\alpha = 0.05$. 


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Results and Discussion

Carcass quality

The type of pasture only affected the loin weight, which was heavier (P < 0.05) in the case of animals fattened on WC than in those grazing E-Tanin (Table 1). Although in this study the subcutaneous tissue thickness at the GR level was not affected (P > 0.05) by the type of pasture, Purchas and Keogh(23) reported that for equal carcass weight, lambs grazing *Lotus uliginosus* presented lower subcutaneous fat thickness than those grazing WC. Supplementation did not affect carcass quality (P > 0.05). The year and its interactions with the type of pasture and supplementation did not have a significant effect (P > 0.05) on any of the carcass quality parameters. Nevertheless, HCW was affected (P < 0.05) by the pasture x supplementation interaction where lambs grazing exclusively on E-Tanin presented a lower HCW (P < 0.05) than WC and WC + R.

Meat quality

Regarding the effects of pasture type and supplementation on meat quality parameters, lean color redness (a*) was the only characteristic affected wherein supplemented treatments (with and without CT) registered greater values (P < 0.05) than those exclusively on pasture (Table 1). Color is a pivotal meat characteristic since consumers purchase decision is based mainly on this attribute(24)(25). In this regard, Ren erre(26) indicated that a* represents a relevant parameter of meat color associated with its discoloration (conversion of oxymyoglobin to metmyoglobin), characterized by a decrease in its value. Luciano and others(27) found that grazing lambs produced meat packaged under modified atmosphere that presented greater values of a* (greater redness) than those under a confinement system. In addition, O’Sullivan and others(28) reported that steers on grazing systems produced meat with greater values of a* than supplemented animals or in confinement when using modified atmosphere packaging. These findings would not fully support the results of the current study, although it is important to highlight that a confinement fattening system was not assessed in this research. On the other hand, Luciano and others(29) reported that the semimembranosus muscle of lambs (105 days old) presented greater values of a* when supplemented with tannins. In the current study, L* was unaffected (P > 0.05) by CT, whereas Priolo and others(30) reported a greater meat lightness (greater values of L*) of lambs that grazed on sulla (*Hedysarum coronarium* L.) which contains CT.

The year and its interactions with pasture type and supplementation did not have a significant effect (P > 0.05) on meat quality parameters. In regard to pasture x supplementation interaction, animals on E-Tanin + C and E-Tanin + C + CT presented greater values of a* (P < 0.05) than those grazing on E-Tanin exclusively, while no differences were found (P > 0.05) with animals that grazed on WC with or without supplement (Table 1).

Fat content and fatty acid profile

Intramuscular fat content was not affected (P > 0.05) by pasture type, supplementation or year, nor by the interactions year x pasture and year x supplementation (Table 2). Nevertheless, the interaction pasture x supplementation had a significant effect (P < 0.05) on the intramuscular fat content. In this regard, meat from lambs that grazed exclusively on WC presented a greater (P < 0.05) intramuscular fat content than those that grazed on E-Tanin with or without supplementation and on WC + C treatment. Intramuscular fat deposition is associated with the energy concentration of the diet. Therefore, it is expected that treatments with concentrate supplementation present greater intramuscular fat content although it was not confirmed in the present study. This result may be explained by the fact that animal intake was not evaluated, and a substitution effect of pasture with concentrate could have taken place.

Pasture type only affected the proportions of linoleic (C18:2 n6), linolenic (C18:3 n6), arachidic (C20:0) and dihomo-gamma-linolenic (C20:3 n6) fatty acids, that were greater (P < 0.05) in meat from animals on E-Tanin compared to those on WC (Table 2).

Supplementation affected (P < 0.05) linoleic (C18:3 n6), saturated (SFA) and monounsaturated fatty acids (MUFA) proportions, and the omega 6:omega 3 ratio. Animals exclusively under grazing systems presented a lower proportion (P < 0.05) of omega 6:omega 3 fatty acid ratio than C + CT treatments. Meat from animals supplemented with concentrate results in a greater proportion of omega 6 fatty acids, showing an omega 6:omega 3 ratio less favorable for human health, in relation to exclusively grass-fed animals(31)(32). Vasta and others(33) and Rana and others(34) found that CT supplementation increased polyunsaturated fatty acids (PUFA) and reduced the SFA levels.
### Efecto pastura y taninos en calidad de canal y carne

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#### Table 1. Pasture effect, supplementation, year and their interactions with carcass and meat quality characteristics.

<table>
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<tr>
<th>Pasture</th>
<th>Supplement</th>
<th>Year</th>
<th>Interactions</th>
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#### Table 2. Pasture effect, supplementation, year and their interactions on the fatty acid profile of intramuscular fat.

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<th>Interactions</th>
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#### Table 3. Pasture effect, supplementation, year and their interactions with carcass and meat quality characteristics.

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### Table 4. Pasture effect, supplementation, year and their interactions on the fatty acid profile of intramuscular fat.

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# References

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proportions in intramuscular fat due to an inhibitory effect of CT on ruminal biohydrogenation carried out by microorganisms; this was not observed in the present study probably because the intake levels of CT were lower than the ones reported by the aforementioned authors. On the other hand, conflicting results were found on CT supplementation regarding the proportion of conjugated linoleic acid (CLA) in intramuscular fat; it has been reported that the percentage of CLA increased\(^{[33][34]}\), decreased\(^{[35]}\) or was not affected\(^{[13]}\) compared to control treatments.

The year affected (P < 0.05) the docosapentaenoic acid (C22:5 n3) proportion, while the year x pasture interaction had a significant effect (P < 0.05) on myristoleic (C14:1), palmitic (C16:0), stearic (C18:0), linoleic (C18:2), linolenic (C18:3) and arachidonic (C20:4 n6) fatty acids and the omega 6:omega 3 ratio. In addition, the eicosapentaenoic acid (C20:5 n3) and the omega 6:omega 3 ratio were significantly affected (P < 0.05) by the year x supplementation interaction.

**Conclusions**

Carcass and meat quality characteristics were not greatly affected by CT intake from either legumes or extracts. Several factors could have influenced this such as differences in the intake of the supplements by animals under grazing conditions, which was not assessed in the current study. Regarding the fatty acid profile of intramuscular fat, no clear evidence was found on the inhibitory effect of CT on the ruminal biohydrogenation, which would have direct consequences for the nutritional value of the meat. It is important to emphasize that this study was made under grazing conditions and that it is one of the first studies in Uruguay to assess the effect that CT in pastures has on lamb carcass and meat quality. Intra and interannual variability of CT concentration in pastures with E-Tanin could have affected CT intake in lambs under grazing conditions and thus the results of the study.

Therefore, under the conditions of the current study, it is possible to conclude that pasture type or the use of supplements such as concentrate and CT, would not allow to obtain an added-value product in terms of meat quality except meat redness), including the fatty acid profile of the intramuscular fat.

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**Author’s contribution**

All the authors contributed equally to the content.

**References**