Meat quality of light lambs is more affected by the dam's feeding system during lactation than by the inclusion of quebracho in the fattening concentrate

Journal:	*Journal of Animal Science*
Manuscript ID	E-2017-1595
Manuscript Type:	Meat Science
Date Submitted by the Author:	29-Mar-2017
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Key Words:	sainfoin, alfalfa, indoor, lipid oxidation, fatty acids

Running head: Ewes' feeding affects meat quality of lambs

Meat quality of light lambs is more affected by the dam's feeding system during lactation than by the inclusion of quebracho in the fattening concentrate[1]

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[1]Appreciation is expressed to the staff of the CITA de Aragon for their help with the data collection and their assistance in the lab. This study was supported by the Ministry of Economy and Competitiveness of Spain and the European Union Regional Development Funds (INIA RTA2012-080-00 and INIA RZP2013-00001-00) and the Research Group Funds of the Aragon Government (A49). M. Blanco is supported by INIA-EFS and S. Lobón by a doctoral grant from the Aragón Government**.** J.R. Bertolín is supported by MINECO of the Spanish Government.

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ABSTRACT: The inclusion of natural antioxidants in the diet, through fresh forages or condensed tannins might prolong meat shelf life and modify the meat quality. The aim of this study was to evaluate the effect of the dam's feeding system during lactation and the inclusion of quebracho in the fattening concentrate of lambs on meat color, chemical composition and lipid oxidation. Ewes and lambs were fed indoor or allowed to graze on alfalfa or sainfoin until the lambs reached 42 d old. Thereafter, the weaned lambs were fed concentrates with 5% or without quebracho until reaching 22-24 kg BW.

Indoor lambs presented greater intramuscular fat content and lower tocopherol content than Alfalfa and Sainfoin lambs ($P < 0.01$). Regarding meat color of Longissimus thoracis et lumborum muscle, Indoor lambs presented greater lightness, yellowness and hue angle values than Alfalfa and Sainfoin ($P < 0.05$).. The redness was affected by the interaction between feeding system during lactation and the time of storage, but on average, Alfalfa and Sainfoin lambs had greater redness than Indoor lambs ($P < 0.05$). The lipid oxidation from 5 to 14 d of display observed in Sainfoin lambs was the lower than, Indoor lambs ($P < 0.05$). The profile of fatty acid of intramuscular fat of Sainfoin and Alfalfa lambs were more appropriate to that of recommended by human healthy institutions, than Indoor lambs. The inclusion of quebracho in the fattening concentrate had mild effects on meat quality. When light lamb production system is based in two phases, lactation and fattening, the feeding system of dams during lactation had greater effect on meat quality (colour, lipidic oxidation and fatty acid profile) of light lamb than the inclusion of quebracho at 5% in the concentrate during the fattening period. Dams grazing Sainfoin provides a more stable meat, and it would be cheaper to feed the dams with fresh forages with high α-tocopherol content than supplementing the concentrate of the lambs with synthetic α-tocopherol.

Key words: sainfoin, alfalfa, indoor, lipid oxidation, fatty acids

INTRODUCTION

The deterioration of meat is mainly caused by discoloration and lipid oxidation. The selling period for the meat of light lambs of several Protected Geographical Indications in Spain is short, at 6-8 d, which results in economic losses that could be reduced if the shelf life of the meat is extended. The inclusion of antioxidants such as vitamin E in lamb diets has extended the meat's shelf life (Wulf et al., 1995; Ripoll et al., 2013; Muíño et al., 2014). Another source of natural antioxidants that can be used in ruminant diets is condensed tannins (**CT**) (Vasta and Luciano, 2011), which are secondary compounds of plants.

In Mediterranean areas, where ewes and lambs are usually stalled during lactation, ewes receive hay, and lambs have access only to their dam's milk. Thereafter, weaned lambs are fed fattening concentrates and straw until slaughter to obtain a homogeneous product and to provide easy flock management as the herd size grows (Bernués et al., 2011). Grazing on alfalfa (*Medicago sativa*), a natural source of vitamin E, increased the meat shelf life compared to an indoor feeding system (Ripoll et al., 2013). Other sources of forages of interest for inclusion in the ration of lactating ewes is sainfoin (*Onobrychis viciifolia*), a high-quality forage with a chemical composition similar to alfalfa except for the CT content (Theodoridou et al., 2011). However, during the fattening period, lambs are confined, and an external source of CT must be added to the concentrate. Quebracho (*Schinopsis lorentzii*) is a good source of CT that can be incorporated easily in the concentrate. The inclusion of quebracho positively affected the meat color and antioxidant status; however, its effect on the lipid oxidation it is still unclear (Luciano et al., 2009b, 2011).

The aim of the study was to evaluate the effects on the light lamb meat quality of the dam's feeding system during lactation (grazing on alfalfa, grazing on sainfoin or fed indoor) and the inclusion of quebracho in the fattening concentrate of the weaned lambs.

MATERIALS AND METHODS

The experiment was conducted in the facilities of CITA Research Centre in Zaragoza (41°3´N, 0°47´W, 216 m above sea level). The Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (**CITA**) approved the experimental procedures, which were in compliance with the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Animal Management and Experimental Design

The experiment had a 3 x 2 factorial design, comprising three dam feeding systems during lactation (indoor, grazing on alfalfa and grazing on sainfoin) and the inclusion of quebracho in the fattening concentrate of the weaned lamb (**Control** and **QUE**).

After lambing, ewe-lamb pairs were randomly assigned according to the ewe's BW (46.6 ± 1.4 kg) and BCS (2.43 ± 0.07) to 1 of the 3 feeding systems during lactation. Each treatment was divided into 2 replicates.

- **Indoor**: 21 ewe-lamb pairs were housed indoor as is usual in this system. Each replicate was stocked in a pen (n = 10 or 11 pairs/pen; 15 m^2/pen). The ewes received a dry total mixed ration ad libitum. The main components of the total mixed ration were barley straw (50%), corn grain (11.6%), barley grain (11.5%), legume grain 17% CP (9.3%), rapeseed meal (7%), soybean meal (3.3%) and sugar-beet molasses (3.5%).

- **Alfalfa**: 21 ewe-lamb pairs were rotationally grazed on alfalfa. The 2 replicates were stocked in separate paddocks (n=10 or 11 pairs/paddock; 1005 m^2/paddock).

- **Sainfoin**: 21 ewe-lamb pairs were rotationally grazed on sainfoin. The 2 replicates were stocked in separate paddocks (n=10 or 11 pairs/paddock; 1005 m^2/paddock).

During lactation, the lambs had access to the dams' milk and to the dams' ration (forage in the paddock when they were stocked or the total mixed ration when they were indoor). Since the first week after birth, the lambs had free access to concentrate so they could adapt to it, thus avoiding potential problems in early weaning. The commercial concentrate was offered

to lambs only through a creep concentrate feeder. The lambs were weaned when they reached 42 ± 2 d of age.

After weaning, during the fattening period, the lambs were permanently stalled in pens and were fed concentrates plus straw ad libitum. Half of the lambs from each paddock/pen received a commercial concentrate (Control), and the other half received the commercial concentrate with 5% added quebracho (as-fed basis; SYLVAFEED ByPro Q, Adial Nutrition. Girona, Spain) (QUE). Water and mineral blocks were always offered ad libitum. Both concentrates were pelleted. The main components of the Control concentrate were corn (35%), soybean meal (23.8%), wheat (20%) and barley (15%). The main ingredients of the QUE concentrate were corn (40%), soybean meal (26.3%), wheat (20%) and quebracho (5%). Concentrates were isoenergetic and isoproteic. The chemical compositions of the feedstuffs used in the present study are reported in Table 1.

The groups within each replicate were balanced by BW at weaning. Each group of animals was allocated in a pen, with a total of twelve pens (6 or 5 lambs per pen, 6 m^2/pen) in the experiment. Lambs were slaughtered weekly upon reaching the target slaughter weight (22-24 kg BW).

Slaughter and Meat Sampling Procedures

After slaughter, the light lamb carcasses were chilled at 4°C for 24 h in total darkness. Then, the carcasses were split. The rectus abdominis muscle of the left carcass and both longissimus thoracis et lumborum (**LTL**) muscles of the carcass were removed. The pH of the LTL muscle was measured at the 4th vertebral region with a pH meter equipped with a Crison 507 penetrating electrode (Crison Instruments S.A., Barcelona, Spain). The LTL muscle from the 4th to the 6th lumbar vertebrae of the left carcass was sliced and packed to determine the chemical composition and α-tocopherol content. The same portion from the right carcass was sliced and packed to analyze the fatty acid composition. The LTL muscles from the 6th to the

13th thoracic vertebrae were sliced into 2.5-cm samples and randomly assigned to seven display times (0, 2, 5, 7, 9, 12 and 14 d), placed in trays and wrapped with oxygen-permeable PVC film and kept in darkness at 4°C until being measured for color. The 0 d samples were allowed to bloom also in darkness at 4°C for 1 h before being measured. Immediately after the color measurement, the samples were vacuum-packed and frozen (-20°C) until lipid oxidation analysis.

Instrumental Meat Color and Heme Pigment Estimations

The color of the rectus abdominis and LTL muscles was measured using a Minolta CM-2006d spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan) in the CIELAB space with a measurement area diameter of 8 mm, including a specular component and a 0% UV, standard illuminant D65 that simulates daylight (color temperature of 6504 K), observer angle 10º and zero and white calibration. The integrating sphere has 52 mm of diameter and the measurement area was cover with a dust cover CM-A149. The lightness (**L***), redness (**a***) and yellowness (**b***) were recorded. The hue angle (**H°**) was calculated as $H° = \tan^{-1}\left(\frac{b^*}{a^*}\right) \times 57.29$, expressed in degrees, and Chroma (**C***), as $C^* = \sqrt{(a^{*2} + b^{*2})}$.

The color of the rectus abdominis muscle was measured after the fascia covering was removed, and the color of the LTL muscle was measured on the surface. In both muscles, the color was measured at 2 locations randomly selected to obtain a mean value with a representative reading of surface color. The measurements were averaged. To standardize the measurements, a white tile was placed behind the muscles.

The relative contents of metmyoglobin (**MMb**) and oxymyoglobin (**MbO_2**) were estimated by the ratios K/S572/525 and K/S610/525, respectively, as described in Ripoll et al. (2013). Both ratios decrease when the pigment content increases. The vertical axis of the figure was inverted to obtain the correct visual impression when viewing the curves.

Chemical Analysis

Meat samples were lyophilized and then minced to determine the DM, CP, intramuscular fat (**IMF**), the α-tocopherol contents and the fatty acid (**FA**) profile. Samples were weighed before and after lyophilization to obtain the DM content. The IMF content was determined following the Ankom Procedure (AOCS, 2005) with an XT10 Ankom extractor (Ankom Technology, Madrid, Spain). The content of CP (Nitrogen x 6.25) was determined following the Dumas Procedure (AOAC, 1999) using a nitrogen analyser (Model NA 2100, CE Instruments, Thermoquest SA, Barcelona, Spain).

The content of α-tocopherol was determined by liquid chromatography ultra-high resolution (Waters UHPLC Acquity H-Class System; Milford, MA) coupled to a fluorescence detector (Waters 2475 Multi λ Fluorescence Detector, working with λ_{exc} = 295 and λ_{emis} = 330). The extraction of α-tocopherol in the feedstuffs was performed according to Val et al.(1994) and in the LTL muscle according to the UNE-EN ISO 12822 Official Method (2014). The quantification of the α-tocopherol was performed using the chromatographic procedure proposed by Chauveau-Duriot et al.,(2010). The lipid oxidation was determined using the Thiobarbituric Acid-Reactive Substances method (TBARS), as described in Ripoll et al. (2013).

Fatty acids from the feedstuffs were derivatized and extracted according to the method described by Sukhija et al., (1988) (extracted with heptane instead of bencene) and by Lee et al., (2012) in IMF. The determination of these fatty acids methyl esters was carried out by the use of Bruker 436 Scion gas chromatograph equipped with a cyanopropyl capillary column (BR-2560, 100 m x 0.25 mm ID x 0.20 μm thickness, Bruker), flame ionization detector and Compass CDS software. Fatty acid content was expressed as a percentage of the total amount of the identified fatty acids. After individual FA determination, the sum of the saturated fatty acids (**SFA**), mono-unsaturated FA (**MUFA**), poly-unsaturated FA (**PUFA**), PUFAn-6 and PUFAn-3 were calculated. The PUFA: SFA and PUFAn-6:n-3 ratios were calculated.

161 ***Statistical Analyses***

162 Data were analyzed with the SAS statistical software (SAS v.9.3). Data on the pH, DM, CP,
163 IMF, α-tocopherol contents and FA composition of the LTL muscle and the color of the
164 rectus abdominis muscle were analyzed through ANOVA with a GLM procedure for the
165 dam's feeding system during lactation, the inclusion of quebracho in the fattening
166 concentrate, and the interaction as the fixed effects. The color, pigments and lipid oxidation
167 of the LTL muscle were analyzed with a mixed model (MIXED procedure) with repeated
168 measurements of the dam's feeding system during lactation, the inclusion of quebracho in the
169 fattening concentrate, the time of display and the interactions as fixed effects and the lamb as
170 the random effect. The degrees of freedom were adjusted with the correction of Kenward-
171 Roger to account for unequal observations or missing values. To model the error, different
172 variance-covariance matrices were tested, and the one with the lowest Aikake and Bayesian
173 information criteria was chosen. Multiple comparisons among treatments were performed by
174 the Tukey method. The interaction between the dam's feeding system during lactation and the
175 inclusion of quebracho in the fattening concentrate was removed from the model when it was
176 not significant ($P > 0.05$), and the analyses were repeated. The least squares means and
177 standard errors were obtained, and differences were considered when $P < 0.05$. Trends were
178 discussed when $0.10 < P \leq 0.05$.

179 **RESULTS**

180 ***Meat pH, Chemical Composition and α-tocopherol Contents of the LTL Muscle***

181 The dam's feeding system during lactation did not affect the pH, DM or CP content ($P >$
182 0.05) but affected the IMF and α-tocopherol contents of the LTL muscle (Table 2). Indoor
183 lambs presented the greatest, Alfalfa intermediate and Sainfoin the lowest IMF ($P < 0.01$). In
184 contrast, Indoor lambs had lower α-tocopherol content than the Alfalfa and Sainfoin lambs (P
185 < 0.001), whose content did not differ between them ($P < 0.05$).

The inclusion of quebracho in the fattening concentrate of the lambs did not affect the pH, DM, CP and IMF contents ($P > 0.05$) but decreased the α-tocopherol content by 21% when compared to the Control lambs ($P < 0.05$).

Instrumental Color and Haeminic Pigments of the Muscles

The dam's feeding system during lactation only affected the redness of the rectus abdominis muscle ($P < 0.05$), which was greater in Sainfoin, intermediate in Alfalfa and lower in Indoor lambs (Table 3). The inclusion of quebracho in the fattening concentrate had no effect on the color of the rectus abdominis muscle.

The dam's feeding system during lactation affected the lightness ($P < 0.05$), yellowness ($P < 0.05$) and Hue angle ($P < 0.01$) of the color of the LTL muscle (Table 4). On average, the LTL muscle of the Indoor lambs had greater lightness, yellowness and hue angle values than the Alfalfa and Sainfoin lambs (Fig. 1). Redness was affected by the interaction between the dam's feeding system during lactation and the display time ($P < 0.01$). Redness remained more or less stable throughout the display time in the Alfalfa and Sainfoin lambs, whereas in Indoor lambs, it increased between 0 and 5 d of display and thereafter decreased to the initial values. On average, Alfalfa and Sainfoin lambs had greater redness than Indoor lambs ($P < 0.05$).

The interaction between the dam's feeding system during lactation and the display time affected the MMb ($P < 0.05$) and MbO_2 ($P < 0.01$) (Fig. 2). The LTL muscles of the Indoor lambs had a different evolution than those of the Alfalfa and Sainfoin lambs, but without a clear effect of the dam's feeding system during lactation. On average, Indoor lambs had lower MMb than did the Alfalfa lambs ($P < 0.01$) and a lower MbO_2 than did the Sainfoin lambs ($P < 00.5$). In addition, the MbO_2 and redness were highly correlated ($r = -0.87$; $P < 0.001$).

The inclusion of quebracho in the fattening concentrate had no effects on the color parameters and the MMb content of the LTL muscle (Table 4). However, the interaction of the QUE with the display time affected the MbO_2 content ($P < 0.05$). The MbO_2 content increased between 0 and 2 d, peaked at 7 d and decreased until 14 d of display, although not significantly, in Control lambs. The MbO_2 content increased between 0 and 2 d, remained steady until 5 d and decreased linearly until 14 d in QUE lambs (Fig. 2). On average, the inclusion of quebracho in the fattening concentrate reduced the MbO_2 ($P < 0.05$).

Lipid Oxidation of the LTL Muscle

Lipid oxidation of the LTL muscle was affected by the interaction between the dam's feeding system during lactation and the display time ($P < 0.001$) but not by the inclusion of quebracho in the fattening concentrate (Table 4). Lipid oxidation of the LTL muscle did not differ among feeding systems during lactation until displayed for 7 d, when it was greater for Indoor, intermediate for Alfalfa and lower for Sainfoin lambs (Fig. 3; $P < 0.05$). Lipid oxidation increased similarly in the 3 feeding systems between 7 and 12 d of display, being greater in the Indoor, and Alfalfa than in Sainfoin lambs ($P < 0.05$). Between 12 and 14 d of display, the lipid oxidation increased considerably in the Sainfoin lambs, was intermediate in the Indoor and was low in the Alfalfa (43, 24 and 10%, respectively); consequently, the Alfalfa treatment had an intermediate lipid oxidation, similar to the rest of the treatments ($P > 0.05$), but the Indoor were still different ($P < 0.05$) from the Sainfoin.

Fatty Acid Composition of the LTL Muscle

The dam's feeding system during lactation greatly affected the FA profile of the LTL muscle (Table 5). The fatty acid profile of the Alfalfa and Sainfoin lambs differed only in the percentages of the individual PUFAn-3 ($P < 0.05$), which were lower in Alfalfa lambs than in the Sainfoin lambs. The LTL muscle of the Alfalfa and Sainfoin lambs presented lower C18:1n-9 and greater C18:2n-6, C18:3n-3, CLA, C20:5n-3 and C22:5n-3 percentages than of

the Indoor lambs ($P < 0.01$). The inclusion of quebracho in the fattening concentrate increased the percentages of C16:0, C18:1 and C18:2n-6 and decreased the percentages of C15:0, C17:0, and C17:1 ($P < 0.05$).

The percentages of some relevant FA, such as C18:1n-9, C20:4n-6, C20:5n-3, C22:5n-3 and C22:6n-3, were affected by the interaction between the dam's feeding system during lactation and the inclusion of quebracho in the fattening concentrate ($P < 0.05$). The inclusion of quebracho decreased the C20:4n-6, C20:5n-3, C22:6n-3 and PUFAn-3 in the Sainfoin but not in the Alfalfa and Indoor lambs ($P < 0.05$).

Indoor lambs presented greater MUFA and lower PUFA than the Alfalfa and Sainfoin lambs ($P < 0.001$). However, the effect of the inclusion of quebracho in the fattening concentrate on total PUFA, PUFAn-6 and PUFAn-3 depended on the dam's feeding system during lactation ($P < 0.01$). The inclusion of quebracho increased the total PUFA and PUFAn-6 only in the Alfalfa lambs ($P < 0.05$) and decreased the PUFAn-3 only in the Sainfoin lambs ($P < 0.05$). Regardless of the inclusion of quebracho, the Sainfoin lambs had the greatest, the Alfalfa intermediate and the Indoor the lowest PUFAn-3 ($P < 0.001$). The ratio n-6:n-3 was greater in the Indoor lambs, intermediate in the Alfalfa and lower in the Sainfoin ($P < 0.001$).

DISCUSSION

Meat pH, Chemical Composition and α-tocopherol Contents

The range of pH values of the LTL muscle was narrow (from 5.54 to 5.57), similar to that reported in light lambs of the same breed (Ripoll et al., 2012) and of other local breeds (Díaz et al., 2002; Carrasco et al., 2009). These pH values correspond to a normal range, ruling out dark-cutting or stress problems (Carrasco et al., 2009).

The effect of the dam's feeding system during lactation was noticeable in the IMF and α-tocopherol contents of the LTL muscle, despite the subsequent fattening period of 28 (±2) d.

The greatest IMF content of the Indoor lambs could be related to the greater intake of the concentrate (Data not shown) and to less exercise (Velasco et al., 2001).

As in the current experiment, the greater α-tocopherol content in the muscle is deposited when ruminants are fed fresh forages or concentrates rich in α-tocopherol (Ripoll et al., 2013; Jose et al., 2016). The present results highlight the importance of the diet of the dam during the suckling period on the content of α-tocopherol in the muscle of the light lamb, regardless of the type of concentrate fed during the period of fattening. The α-tocopherol from the feedstuff ingested by the dam and deposited into the muscle of the suckling lamb through the milk remains after a fattening period which lasted 30 d. Most likely, it would be cheaper to feed the dams with fresh forages with high α-tocopherol content than supplementing the concentrate of the lambs with synthetic α-tocopherol.

Instrumental Color and Heminic Pigments

In the current experiment, the dam's feeding system during lactation had different effects on the rectus abdominis and LTL muscle colors, probably because of differences in thickness (Lanza et al., 2006). The greater lightness in the LTL muscle of Indoor lambs compared to Alfalfa lambs has been reported previously in light lambs fed Indoor concentrates or grazing alfalfa with their dams until slaughter (Ripoll et al., 2012; Ripoll et al., 2013) and in grazing weaned lambs (Díaz et al., 2002). Other factors such as pH, IMF, carcass weight and age at slaughter are responsible for differences in lightness between forage- and concentrate-fed ruminants (Priolo et al., 2001). In the current experiment, pH was similar between the feeding systems, but differences in the IMF were observed, as well as in the carcass weight (data not shown).

Grazing animals are characterized as experiencing greater physical activity, resulting in a greater concentration of heme pigments, which involve darker (Renerre, 1986) and redder meat (Carrasco et al., 2009), features that are often rejected by Spanish consumers (Bernués

et al., 2012). The redness seems to be more appropriate than lightness when assessing color acceptability. Hopkins (1996) reported a low correlation (r = 0.18) between the lightness and the acceptability scores. Khliji et al. (2010) reported that redness values explained the variation of consumer scores better than the lightness values. These authors suggested that when the redness values are equal to or exceed 9.5, consumers will consider the meat color acceptable. The meat of the present study in all treatments was above this limit. The greater redness observed in the rectus abdominis and in the LTL muscles in lambs from grazing ewes (alfalfa and sainfoin) was also observed in light lambs, whether under the grazing system or stalled indoor (Carrasco et al., 2009). The differences in redness are partially related to the greater intake, mainly through milk, of carotenoids and α-tocopherol (Ripoll et al., 2008) and partially to the physical activity, as discussed above.

Discoloration of lamb meat occurs when the hue angle increases quickly at the end of the display time, and this characteristic is a good descriptor of meat discoloration (Carrasco et al., 2009; Luciano al., 2009a). In the current experiment, the greater hue angle of Indoor lambs in contrast to Alfalfa and Sainfoin lambs confirms the protective effect of the herbage-based diets against meat discoloration compared to the concentrate-based diets (Luciano et al., 2012). In the present study, the greater MMb of Alfalfa and Sainfoin lambs than Indoor lambs agrees with the greater content of pigments in the grazing animals, caused by the physical activity; thus, these lambs had more pigment to be oxidized. The MMb followed the typical, almost logarithmic, evolution previously reported by Ripoll et al. (2013), whereas the deoxymyoglobin remained steady (Lagerstedt et al., 2011; Alberti et al., 2014). Hence, MbO_2 also increased in the first days and, then, decreased from approximately the 5th d of display (Ripoll et al., 2013).

In the current experiment, the lack of effect of the inclusion of quebracho in the fattening concentrate on the hue angle of the LTL muscle agrees with the results obtained when 8.9%

309 quebracho was included in the concentrate of the fattening period, which lasted 60 d (Luciano
310 et al., 2011). The source of condensed tannins can influence their effect on meat color. An
311 increase in the lightness of muscle occurs when CT are from other sources, such as those in
312 *Acacia cyanophylla* (Priolo et al., 2002) or *Hedysarum coronarium* (Priolo et al., 2005),
313 because the CT reduced the production of hemoglobin (Priolo and Vasta, 2007). However,
314 when *Cistus ladanifer* was fed to lambs, no effects were observed on the meat color
315 (Francisco et al., 2015). In the present study, the short fattening period and the low dose and
316 type of condensed tannins may have impaired the possible effect of the CT from quebracho
317 on the meat color.

318 ***Lipid Oxidation***

319 The dam's feeding system during lactation had a greater effect on lipid oxidation than the
320 inclusion of quebracho in the fattening concentrate. The threshold of lipid oxidation for
321 acceptable meat was set at 1 mg MDA/kg of muscle in light lambs in Spain (Ripoll et al.,
322 2011). This threshold was reached after the LTL muscles of the Indoor, Alfalfa and Sainfoin
323 lambs were stored for 5, 7 and 11 d, respectively. These results highlight the importance of
324 the dams' diets during lactation on the shelf life of the light lamb meat, even though the
325 lambs were finished on concentrates for a short feeding period. The lipid oxidation of the
326 LTL muscle could be partially related to the contents of α-tocopherol (González-Calvo et al.,
327 2015) and polyphenols (Vasta and Luciano, 2011) in the muscle, as the contents of each in
328 the diet reduced lipid oxidation in the lamb meat (Francisco et al., 2015; Liu et al., 2016). The
329 mechanisms of action of CT on lipid oxidation are not completely known (Vasta and
330 Luciano, 2011). CT could be involved to the defense antioxidants mechanism of meat.
331 Moreover, the CT may protect other antioxidants from oxidation, such as α- tocopherol
332 (Yamamoto et al., 2006; Iglesias et al., 2012), which would explain the lower lipid oxidation
333 observed in the Sainfoin lambs in the current experiment.

No effects of CT from quebracho in the fattening concentrate were observed on the lipid oxidation, as reported in lambs fed 80 g/kg of quebracho (Brogna et al., 2014) or in the minced meat from lambs fed 8.9% of quebracho in the concentrate (Luciano et al., 2009b). The different effect of CT from quebracho and from Sainfoin can be partially related to the α-tocopherol content, which was low in the QUE lambs, and to the type of CT. Furthermore, the CT from quebracho was not absorbed in the gastrointestinal tract, although it induced antioxidant effects in animal tissues (López-Andrés et al., 2013).

Fatty Acid Composition of the LTL Muscle

The dam's feeding system during lactation had more effect on the FA profile of the IMF than the inclusion of quebracho in the fattening concentrate. The meat of Alfalfa and Sainfoin lambs was healthier, from a human point of view, than that of Indoor lambs, increasing the CLA and PUFAn-3 contents. The population has focused its attention on healthy FA, highlighting the CLA because of their possible beneficial effects on human health because it reduces the incidence of cancer, diabetes and atherosclerosis (Pariza et al., 1999; Jeung et al., 2005; Park and Pariza, 2007). Sainfoin lambs presented greater PUFAn-3 than Indoor and Alfalfa lambs, probably due to the presence of the CT in the Sainfoin (Girard et al., 2016). Condensed tannins improve the FA profile in ruminants, thereby reducing the ruminal biohydrogenation and increasing Δ^9-desaturase protein expression in the muscle (Vasta et al., 2009).

The effects of the inclusion of quebracho on the FA profile, expressed as g/100 g muscle, of lamb meat has been only studied with an inclusion of 8.9% quebracho for 60 d (Vasta et al., 2009) and 80 g of quebracho/kg during 12 d (Brogna et al., 2014), which was too short to show differences. Thus, their results are not completely comparable with the present results, expressed as g/100 g FA. In the current experiment, the inclusion of quebracho in the fattening concentrate changed individual FA, some of them depending on the dam's feeding

system during lactation. Results are inconsistent, whereas Vasta et al. (2009), showed no effect of CT on total SFA and MUFA, we found a significant effect of the dams feeding system during the lactation on PUFA and MUFA, but did not on SFA. In the above-mentioned study, the inclusion of quebracho especially affected the FA involved in the biohydrogenation pathway, particularly the C18:2n-6 and C18:1n-7, which partially agrees with the results of the current experiment. The reasons for the discrepancy of the effect of inclusion of quebracho depending on the dams feeding system remain unclear. The inclusion of quebracho increase the total PUFA and PUFA n-6 in the Alfalfa lambs and decrease the total PUFA n-3 in the Sainfoin, but not in the remaining lambs. Vasta et al. (2009) observed different effect of tannins on ruminal biohydrogenation, being stronger when they are included into concentrates, rather than were mixed with herbage.

CONCLUSIONS

The dams' feeding system affected the meat quality of light lambs more than the inclusion of quebracho in the fattening concentrate, highlighting the importance of the lactation period. Rearing lambs with their dams in alfalfa and sainfoin is recommended more than the indoor system, because grazing during lactation increased the meat color stability and improved the meat FA profile, which are important to human consumers. Grazing on sainfoin is especially recommended because this system extended the meat shelf life more than 4 d and resulted in a more appropriate FA profile. The inclusion of 5% quebracho in the fattening concentrate had mild effects on the meat quality. Further studies should be carried out to clarify the effect of condensed tannins (type, dose, and intake) and the residual effect of the dams' diet during the lactation period on the meat quality.

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520

521 **Table 1**. Chemical and fatty acid composition of the feedstuffs used in the experiment

	Ewe feedstuffs			Lamb concentrates	
Item	TMR[1]	Alfalfa	Sainfoin	Control[2]	QUE[2]
Chemical composition, g/kg DM					
Moisture	924.0	919.5	916.0	886.1	889.1
CP	109.0	189.7	161.3	195.4	210.1
NDF	427.0	454.9	451.9	177.7	176.5
ADF	200.0	310.2	352.0	43.6	41.1
ADL	25.3	66.8	82.7	6.0	6.3
Ash	47.9	90.4	78.4	54.3	53.8
Condensed tannins, cyanidin eq.	0.8	1.5	21.9	0.6	3.7
α-tocopherol, µg/g DM	5.5	132.2	396.9	4.9	3.1
Fatty acids, %FAME[3]					
C14:0	2.8	0.9	0.5	0.5	0.6
C16:0	31.2	24.7	23.4	25.2	28.5
C16:1n-7	0.5	0.4	0.3	0.1	0.2
C17:0	0.3	0.5	0.4	0.1	0.1
C18:0	7.5	6.6	6.6	2.9	3.4
C18:1n-9	23.1	3.5	3.5	29.8	30.8
C18:2n-6	26.6	18.5	16.3	37.6	33.0
C18:3n-3	2.8	35.6	40.8	2.4	2.0

522 [1] TMR = Total mixed ration fed to the Indoor ewes.

523 [2] Control = commercial concentrate; QUE = commercial concentrate with 5% quebracho.

524 [3] FAME = Identified total fatty acid methyl esters.

525

526

527 **Table 2**. Effect of the feeding system during lactation (L) and the inclusion of quebracho in
528 the concentrate during fattening (C) on the pH and chemical composition of the meat.

	L			C			*P* value	
	Indoor[1]	Alfalfa[1]	Sainfoin[1]	Control[2]	QUE[2]	RMSE	L	C
pH	5.54	5.56	5.57	5.56	5.56	0.03	0.42	0.64
DM	24.82	24.66	24.38	24.72	24.52	0.69	0.12	0.27
Intramuscular fat, %FM[3]	2.86[a]	2.39[ab]	2.28[b]	2.45	2.57	0.23	0.01	0.44
CP, %FM[3]	18.27	18.49	18.27	18.42	18.27	0.59	0.38	0.30
α-tocopherol, mg/kg FM[3]	0.45[b]	1.03[a]	1.26[a]	1.02[x]	0.81[y]	0.33	0.001	0.02

529 [a,b] Means within a row with different superscripts differ significantly among feeding system
530 during lactation ($P < 0.05$).

531 [x,y] Means within a row with different superscripts differ significantly among fattening
532 concentrate ($P < 0.05$).

533 [1] Indoor = Ewes fed a total mixed ration; Alfalfa = grazing on alfalfa; Sainfoin = grazing on
534 sainfoin.

535 [2] Control = commercial concentrate; QUE = commercial concentrate with 5% quebracho.

536 [3] FM = Fresh matter.

537

538 **Table 3**. Effect of the feeding system during lactation (L) and the inclusion of quebracho in
539 the fattening concentrate (C) on the color of the rectus abdominis muscle.

	L			C			*P* value	
	Indoor[1]	Alfalfa[1]	Sainfoin1	Control[2]	QUE[2]	RMSE	L	C
Lightness(L*)	46.92	45.87	45.31	45.8	46.3	3.16	0.24	0.57
Redness (a*)	9.25[b]	10.19[ab]	10.44[a]	10.19	9.74	1.5	0.03	0.24
Yellowness (b*)	8.89	8.69	8.55	8.78	8.64	1.93	0.84	0.77
Hue angle (H°)	43.28	40.31	39.34	40.38	41.6	8.34	0.28	0.57
Chroma (C*)	13.03	13.46	13.62	13.62	13.1	1.51	0.42	0.20

540 [a,b] Means within a row with different superscripts differ significantly among feeding system
541 during lactation ($P < 0.05$).

542 [1] Indoor = Ewes fed a total mixed ration; Alfalfa = grazing on alfalfa; Sainfoin = grazing on
543 sainfoin.

544 [2] Control = commercial concentrate; QUE = commercial concentrate with 5% quebracho.

545

546 **Table 4**. *P* values of the effects of the feeding system during lactation (L), the inclusion of
547 quebracho in the fattening concentrate (C) and the display time (Time) on the instrumental
548 color, heme pigments and lipid oxidation in the longissimus thoracis et lumborum muscle.

	L	C	Time	L×Time	C×Time
Lightness (L*)	0.02	0.69	0.001	0.21	0.86
Redness (a*)	0.005	0.09	0.001	0.01	0.17
Yellowness (b*)	0.02	0.60	0.001	0.49	0.20
Hue angle (H°)	0.002	0.23	0.001	0.31	0.20
Chroma (C*)	0.18	0.15	0.001	0.01	0.24
Metmyoglobin	0.001	0.45	0.001	0.04	0.20
Oxymyoglobin	0.02	0.04	0.001	0.007	0.048
Lipid oxidation	0.001	0.10	0.001	0.001	0.33

549

Table 5. Effect of the feeding system during lactation (L) and the inclusion of quebracho in the fattening concentrate (C) on fatty acids composition of LTL muscle of light lambs.

	L			C		L x C							*P* –Value		
						Indoor[1]		Alfalfa[1]		Sainfoin[1]					
%FAME[3]	Indoor[1]	Alfalfa[1]	Sainfoin[1]	Control[2]	QUE[2]	Control[2]	QUE[2]	Control[2]	QUE[2]	Control[2]	QUE[2]	RMSE	L	C	LxC
C10:0	0.23	0.24	0.27	0.26	0.24	0.24	0.22	0.24	0.24	0.29	0.26	0.06	0.06	0.22	0.78
C12:0	0.45	0.47	0.55	0.49	0.49	0.44	0.46	0.46	0.47	0.57	0.53	0.16	0.10	0.91	0.74
C14:0	4.42	4.27	4.81	4.44	4.56	4.20	4.63	4.33	4.19	4.79	4.83	1.04	0.21	0.67	0.66
C15:0	0.47^{b}	0.55^{a}	0.57^{a}	0.56^{x}	0.51^{y}	0.51^{rs}	0.43^{s}	0.57^{r}	0.54^{rs}	0.59^{r}	0.55^{r}	0.09	0.001	0.03	0.63
C16:0	24.92	24.54	24.05	24.09^{y}	24.91^{x}	24.40^{rs}	25.43^{r}	24.29^{rs}	24.79^{rs}	23.60^{s}	24.50^{rs}	1.18	0.07	0.008	0.75
C16:1	1.99^{a}	1.85^{ab}	1.78^{b}	1.87	1.88	1.93	2.05	1.88	1.82	1.81	1.76	0.26	0.04	0.97	0.46
C17:0	1.22	1.3	1.36	1.46^{x}	1.13^{b}	1.42^{rs}	1.03^{u}	1.49^{r}	1.11^{tu}	1.46^{rs}	1.25^{st}	0.17	0.06	0.0001	0.16
C17:1	0.63	0.62	0.57	0.72^{x}	0.49^{y}	0.81^{r}	0.46^{t}	0.72^{rs}	0.51^{st}	0.63^{rst}	0.51^{st}	0.18	0.52	0.0001	0.11
C18:0	14.09	14.27	14.42	14.43	14.08	14.34	13.84	14.54	13.98	14.41	14.41	1.05	0.61	0.19	0.65
C18:1	4.56	4.69	4.53	4.17^{y}	5.01^{x}	4.22^{rs}	4.91^{rs}	3.93^{s}	5.48^{r}	4.41^{rs}	4.67^{rs}	0.99	0.85	0.002	0.11
C18:1n-9	36.03^{a}	33.76^{b}	33.05^{b}	34.86^{x}	33.71^{y}	36.71^{r}	35.36^{rs}	35.08^{rst}	32.43^{t}	32.78^{st}	33.33^{st}	2.06	0.0001	0.03	0.049
C18:1n-7	0.32	0.41	0.41	0.4	0.36	0.36	0.28	0.47	0.35	0.38	0.45	0.19	0.20	0.36	0.21
C18:2n-6	6.97^{b}	7.87^{a}	7.54^{ab}	7.08^{y}	7.83^{x}	6.65^{s}	7.28^{rs}	7.12^{s}	8.66^{r}	7.54^{rs}	7.58^{rs}	1.10	0.03	0.01	0.09
C18:3n-3	0.51^{c}	1.11^{b}	1.54^{a}	1.07	1.04	0.49^{u}	0.53^{u}	1.09^{t}	1.14^{st}	1.64^{r}	1.44^{rs}	0.27	0.0001	0.65	0.23
CLA	0.45^{b}	0.65^{a}	0.65^{a}	0.56	0.61	0.45^{s}	0.45^{s}	0.59^{rs}	0.72^{r}	0.65^{rs}	0.65^{rs}	0.20	0.002	0.39	0.52
C20:4n-6	1.77	1.82	1.92	1.91	1.76	1.82^{rs}	1.72^{s}	1.71^{s}	1.92^{rs}	2.20^{r}	1.64^{s}	0.35	0.366	0.09	0.003
C20:5n-3	0.19^{c}	0.42^{b}	0.67^{a}	0.48^{x}	0.36^{y}	0.19^{t}	0.19^{t}	0.41^{s}	0.42^{s}	0.85^{r}	0.49^{s}	0.16	0.0001	0.006	0.001
C22:5n-3	0.40^{c}	0.66^{b}	0.81^{a}	0.64	0.62	0.41^{t}	0.40^{t}	0.59^{st}	0.74^{rs}	0.91^{r}	0.72^{rs}	0.17	0.0001	0.65	0.01
C22:6n-3	0.18^{b}	0.22^{b}	0.29^{a}	0.26^{x}	0.20^{y}	0.18^{s}	0.18^{s}	0.24^{s}	0.20^{s}	0.36^{r}	0.22^{s}	0.09	0.001	0.01	0.03
SFA[4]	45.80	45.64	46.03	45.74	45.91	45.54	46.04	45.93	45.32	45.71	46.33	1.77	0.77	0.70	0.47
MUFA[5]	43.53^{a}	41.36^{b}	40.4^{b}	42.05	41.47	44.02^{r}	43.06^{rs}	42.09^{rst}	40.58^{st}	40.00^{t}	40.73^{st}	2.04	0.0001	0.27	0.19

PUFA[6]	10.47[b]	12.78[a]	13.44[a]	12.03	12.43	10.19[t]	10.76[t]	11.76[st]	13.80[r]	14.14[r]	12.75[rs]	1.47	0.0001	0.28	0.002
PUFA[6]n-6	8.73	9.7	9.48	9.014	9.6	8.47[s]	9.00[rs]	8.83[s]	10.58[r]	9.74[rs]	9.21[rs]	1.35	0.06	0.09	0.03
PUFA[6]n-3	1.28[c]	2.42[b]	3.31[a]	2.45	2.23	1.27[t]	1.30[t]	2.33[s]	2.50[s]	3.76[r]	2.88[s]	0.59	0.0001	0.13	0.01
PUFA[6]:SFA[4]	0.23[b]	0.28[a]	0.29[a]	0.26	0.27	0.22[t]	0.23[t]	0.26[st]	0.30[r]	0.31[r]	0.28[rs]	0.04	0.0001	0.35	0.003
n-6:n-3	7.00[a]	4.38[b]	3.05[c]	4.64	4.99	7.03[r]	7.00[r]	4.20[st]	4.55[s]	2.68[t]	3.41[st]	1.42	0.0001	0.33	0.69

552 [a,b] Means within a row with different superscripts differ significantly among feeding system during lactation ($P < 0.05$).

553 [x,y] Means within a row with different superscripts differ significantly among fattening concentrate ($P < 0.05$).

554 [r,s] Means within a row with different superscripts differ significantly among feeding system during lactation x fattening concentrate ($P < 0.05$).

555 [1] Indoor = Ewes fed a total mixed ration; Alfalfa = grazing on alfalfa; Sainfoin = grazing on sainfoin.

556 [2] Control = commercial concentrate; QUE = commercial concentrate with 5% quebracho.

557 [3] Identified total fatty acid methyl esters.

558 [4] Saturated fatty acids.

559 [5] Monounsaturated fatty acids.

560 [6] Polyunsaturated fatty acids.

561

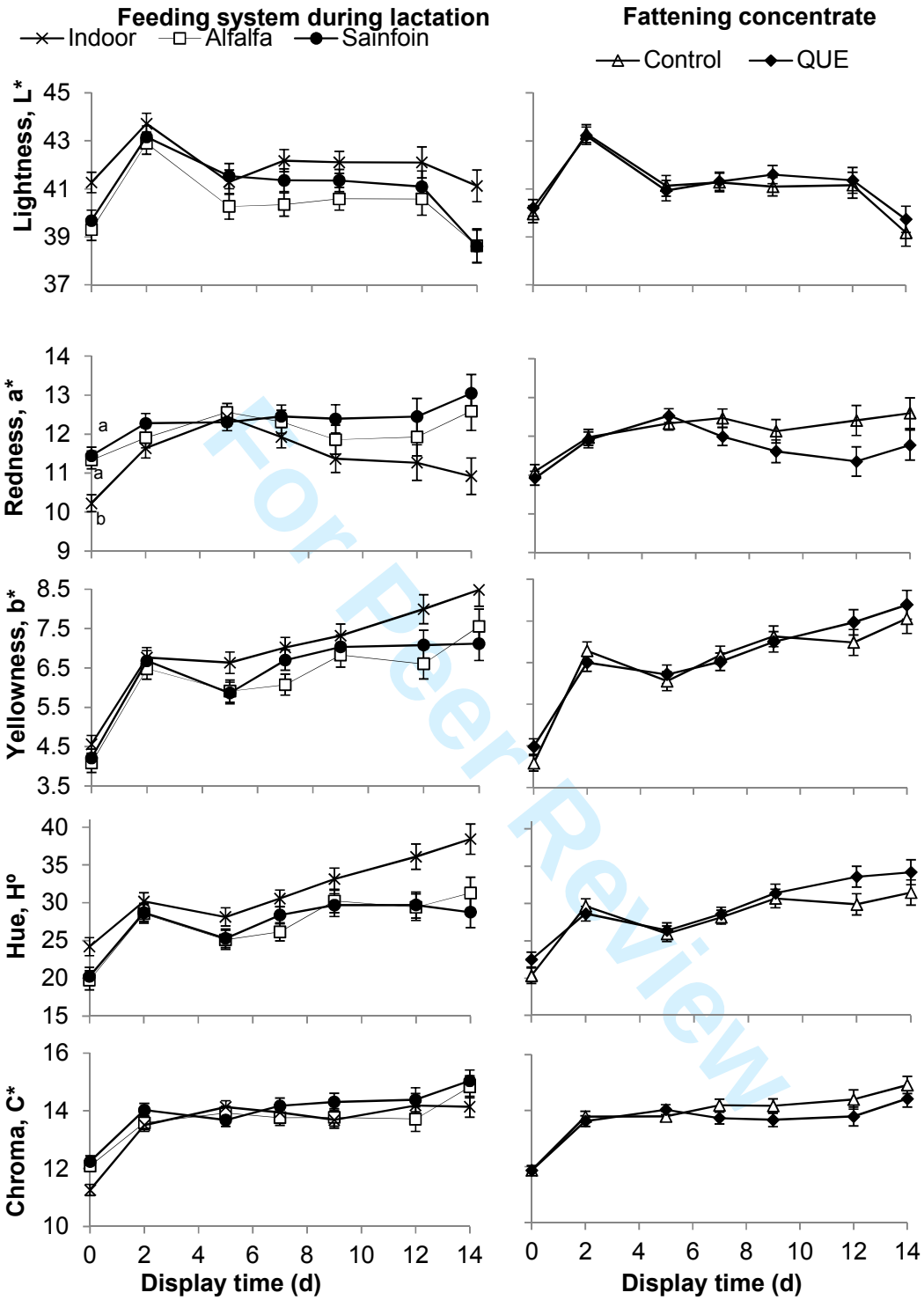
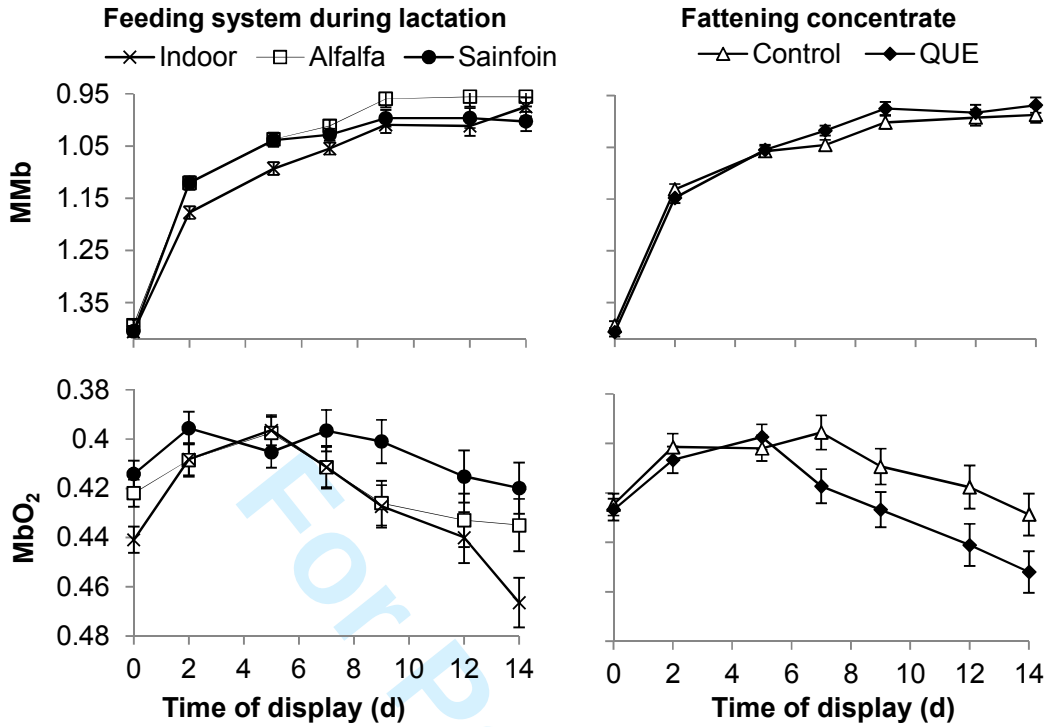


Figure 1. Evolution of the instrumental color of longissimus thoracis et lumborum muscle of light lambs according to the feeding system during lactation and the inclusion of quebracho in the fattening concentrate.

Within a main effect and display time, different letters indicate differences at $P < 0.05$ between treatments.

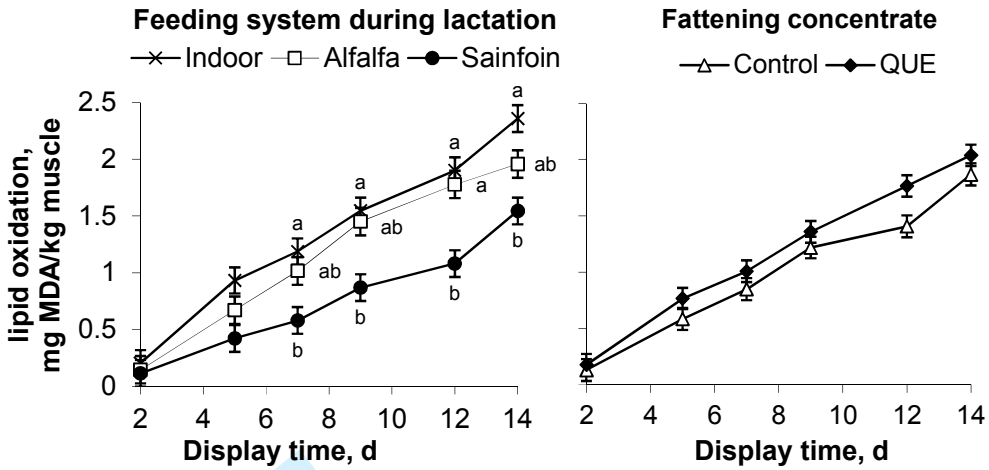


572

573

574 **Figure 2**. Evolution of metmyoglobin (MMb) and oxymyoglobin (MbO_2) of the longissimus
575 thoracis et lumborum muscle of light lambs according to the feeding system during lactation
576 and the inclusion of quebracho in the fattening concentrate.

577



578

579 **Figure 3**. Evolution of the lipid oxidation of the longissimus thoracis et lumborum muscle of
580 light lambs according to the feeding system during lactation and the inclusion of quebracho in
581 the fattening concentrate.
582 Within a main effect and time of display, different letters indicate differences at $P < 0.05$
583 between treatments.
584

Dear Editor,

We send you the manuscript: ***Meat quality of light lambs is more affected by the dam's feeding system during lactation than by the inclusion of quebracho in the fattening concentrate,*** by S. Lobón, M. Blanco, A. Sanz, G. Ripoll, J.R. Bertolín, M. Joy

To be evaluated for Journal of Animal Science review. Hopefully it would be of interest for the review.

Yours Sincerely,

Dr. Margalida Joy

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