Running title: **Grazing sheep milk and tannins**

Chestnut or quebracho tannins in the diet of grazing ewes supplemented with soybean oil: effects on animal performances, blood parameters and fatty acid composition of plasma and milk lipids.

A. Buccioni[1*], M. Pauselli[2], S. Minieri[3], V. Roscini[2], F. Mannelli[1], S. Rapaccini[1], P. Lupi[1], G. Conte[4], A. Serra[4], A. Cappucci[4], L. Brufani[2], F. Ciucci[4], M. Mele[4].

[1] Dipartimento di Scienze delle Produzioni Agro-alimentari e dell'Ambiente, University of Florence, Piazzale delle Cascine 18, 50144 Firenze, Italy.

[2] Dipartimento di Scienze Agrarie Alimentari ed Ambientali, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy.

[3] Dipartimento di Scienze Veterinarie, University of Pisa, Viale delle Piagge 2, 56124 Pisa, Italy.

[4] Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy.

[*] Corresponding author: arianna.buccioni@unifi.it

Abstract

The aim of the present study was to evaluate the effect of the inclusion of chestnut or quebracho tannin extracts in the diet of grazing ewes supplemented with soybean oil, on the blood plasma and milk fatty acid profile, milk quality traits and animal metabolic profile. Eighteen Comisana ewes at 172±6 days in milking were allotted into 3 experimental groups. Diets were characterized by pasture *ad libitum* administered and by 800 g/head and day of 3 experimental concentrates containing 84.5 g of soybean oil /kg of DM and 52.8 g/kg DM of bentonite (Control diet) or 52.8 g/kg DM of chestnut tannin extract (hydrolysable tannins, CHE diet) or 52.8 g/kg DM of quebracho tannin extract (condensed tannins, QUE diet). The trial lasted 4 weeks after 15 days of adaptation to the feeding regimen. Milk yield was daily recorded while milk composition and blood parameters were weekly analysed. CHE and QUE did not affect the milk yield and composition. Casein Index was affected by diet and it was significant higher in milk from animals fed QUE ($P<0.0259$). The clotting parameters with the exception of a_{30} were affected by tannins: r was higher for QUE milk while k_{20} increased regardless the kind of tannin. Blood parameters were not affected by tannins and the oxidative status of ewes, determined using MDA as indicator, did not present significant differences among groups, regardless the concentrates fed to animals. Fatty acid profile of blood plasma demonstrated that tannin extract, regardless the source, favored the accumulation of vaccenic acid (*trans-11* 18:1) reducing the hematic concentration of stearic acid (18:0). Only few significant differences in milk fatty acid profile were found. In particular, rumenic acid (*cis*-9, *trans*-11 18:2) increased when the concentrates contained polyphenols and the stronger effect is reached with QUE ($P<0.0002$).

Key words: chestnut or quebracho tannins, dairy ewes, fresh forage, soybean oil.

Acronyms: ADF, acid detergent fibre; **ADL**, acid detergent lignin; **ALB**, albumin; **BH**, biohydrogenation; **C**, control concentrate; **CHO**, cholesterol; **CHE**, chestnut tannin; **CI**, casein index; **CLA**, conjugated linoleic acid; **DM**, dry matter; **DMI**, dry matter intake**; FA,** fatty acid; **FCM**, fat corrected milk; **GBL**, globuline, **γ GT,** γ-glutamil-transferase; **GLU**, glucose; **α-LNA**, alpha-linolenic acid; **LA**, linoleic acid; **MDA**, malonaldehyde; **MTP**, milk total polyphenols, **NDF**, neutral detergent fibre assayed with heat stable amylase and expressed inclusive of residual ash; **PUFA**, polyunsaturated fatty acids; **QUE**, quebracho tannin; **RA**, rumenic acid **SGPT**, serum glutamic-pyruvic, transaminase; **SGOT**, serum glutamic-oxaloacetic-transaminase; **T AE**, tannic acid equivalents; **VA**, vaccenic acid.

1 Introduction

Chestnut (*Castanea sativa* Miller*)* trees, widespread in the Mediterranean region, and Quebracho (*Schinopsis lorentzii*) trees, widespread in South America, have their wood or fruits rich in hydrolysable and condensed tannins, respectively. Several authors demonstrated the efficacy of these two extracts in modulating biohydrogenation (BH) of polyunsaturated fatty acids (PUFA) in dairy ewes fed diets based on hay and concentrates supplemented with oils or full fat seeds (Buccioni et al., 2015 a; Hervàs et al., 2003; Toral et al., 2011; Toral et al., 2013). Recently, Buccioni et al. (2015a) and Correddu et al., (2015) reported that the interaction between dietary polyphenols such as chestnut (CHE), quebracho (QUE) or grape seeds (GS) and lipid supplements (soybean oil or extruded linseed) resulted in an increase of the concentration of linoleic (LA; *cis*-9, *cis*-12 18:2), vaccenic (VA, *trans*-11 18:1), rumenic (RA, *cis*-9, *trans*-11 18:2) acids and in a decrease of total saturated fatty acids (SFA) in sheep milk. These effects were probably due to the ability of tannins to interfere with rumen microbial metabolism, as indirectly confirmed by changes in the concentration of volatile fatty acids (VFA) and by changes in rumen microbial communities (Buccioni et al., 2015a; Carreño et al., 2015; Minieri et al., 2014; Pallara et al., 2014; Vasta et al., 2010).

In a previous experiment, Buccioni et al. (2015a) demonstrated that QUE tannins were more efficient than CHE tannins in perturbing rumen BH when diets were based on preserved forages and supplemented with soybean oil as main dietary source of PUFA. However, little information is available on the interaction between different kind of tannin extracts and rumen BH when PUFA contemporary originate from oil and pasture. Since in oil PUFA are mainly present as triglycerides whereas in forage are constituents of structural lipids such as phospholipids (Buccioni et al., 2012), some differences might occur in the BH process due to the timing of lipolysis or to the kind of bacteria strains involved. Consequently, also the interaction between different kinds of tannins and the individual step of the BH process of PUFA may be altered according to the origin of PUFA (triglycerides or phospholipids).

Hence, the aim of the present study was to evaluate the effect of the inclusion of a moderate amount (<2%) of CHE or QUE tannin extracts in the diet of grazing ewes supplemented with soybean oil, on the blood plasma and milk fatty acid (FA) profile and on the milk yield and quality. Moreover, since some toxic effects of dietary tannins have been previously reported in ruminants (Reed, 1995; Hervas et al., 2003), blood parameters as indicators of hepatic function and oxidative status were also considered in the present experiment.

2 Material and methods

2.1 Experimental design.

Animals. Eighteen multiparous Comisana ewes at 172 ± 6 days in milking (DIM) kept at the Experimental Section of the Department of Agriculture, Food and Environmental Science - University of Perugia Italy, were randomly allotted into 3 experimental groups, blocked for body weight (68.1 ± 7.83 kg; BW), age, and milk yield. Each group was formed by 6 ewes and kept in a pen. All animals grazed together eight hour per day on a spontaneous pasture, following the rotational grazing technique. Grazing periods lasted 3 days each by the displacement of the electrified fence, yielding approximately 40 m^2/ewe/day while water was always available. The trial lasted 4 weeks after 15 days of adaptation to the feeding regimen. The handling of the animals was according to Institutional Animal Care and Use Committee (IACUC, 2014) of University of Perugia. The ewes were milked twice daily at 07:30 and 17:30 h using a milking machine (43 kPa; 150 pulsation / min) and daily individual milk yield was recorded.

Diets. The experimental diets were formulated according to the nutrient requirements of a ewe weighing 68 kg and producing 1 kg of milk at 6.5 % of fat (Cannas et al., 2004):

$$MEl = \{[251.73 + 89.64 \times PQ + 37.85 \times (PP/0.95)] \times 0.001 \times Yn\}/kl$$

where MEl is metabolizable energy required for lactation, Mcal/d; Yn is measured milk yield at a particular day of lactation, kg/d; PQ is measured milk fat for a particular day of lactation, %; PP is measured true milk protein for a particular day of lactation, %; and kl is efficiency of ME utilization for milk production, which is equal to 0.644.

Diets were composed by spontaneous pasture managed as described above, 250 g per head and day of chopped grass hay (particle size > 4 cm of length) and 800 g per head and day of a concentrate, which contained 84.5 g of soybean oil / kg DM and 52.8 g / kg DM of bentonite (control diet), or 52.8 g / kg DM of chestnut tannins (CHE diet) or 52.8 g / kg DM of quebracho tannins (QUE diet). All the concentrates ingredients were incorporated into pellets using a pelletting machine (CMS-IEM – Colognola ai Colli, Verona, Italy), pellet diameter was 5 mm, and the pelleting temperature ranged between 35 and 40°C. The chemical composition of feeds is presented in Table 1. The dose of tannins was chosen in order to obtain a tannin concentration in the diet of nearly 16 g / kg of expected DM intake, estimated according to Cannas et al. (2004). Based on results from previous studies published in literature, this dose was considered as safe for the animal and practical for the farmers (Buccioni et al., 2015a; 2015b). One hundred g / head of rolled barley with the 800 g/ head of experimental concentrates were individually offered during milking. Chestnut hydrolysable tannins (750 g / kg DM of equivalent tannic acid; by Gruppo Mauro Saviola srl Radicofani, Siena, Italy), and extract of quebracho tannins (456 g / kg DM of equivalent tannic acid; by Guido Lapi spa Castel Franco di Sotto, Pisa, Italy) were titrated according to Burns (1963). The chromatographic profile of CHE tannin extract is reported in literature by Campo et al. (2012) while the profile of QUE tannin extract is reported by Pash et al. (2001).

2.2 Sampling and analysis.

Feed sampling and analysis. Pasture was sampled hand-plucking one square meter area before each grazing period while hay, experimental concentrates and their orts were measured daily, pooled and sampled weekly. All collected samples were stored at -80°C until analysis. Samples were freeze dried and then ground for chemical analysis by mill Cyclotec 1093 (PBI International, Milan, Italy), using

a mesh size of 1 mm. Crude protein (CP), ether extract (EE) and ash were determined according to the AOAC methods 976.06, 920.39 and 942.05, respectively (AOAC, 1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were determined according to Van Soest et al. (1991), using heat stable amylase and sodium sulphite, and expressed inclusive of residual ash. Metabolizable energy (ME) and Net energy for lactation (NEl) were calculated according to Cannas et al. (2004). Feed FA were extracted according to Folch et al., (1957), esterified according to Christie (1982) with 19:0 (Sigma Chemical Co., St Louis, MO) as the internal standard, and identified using the same procedure described below for FA of milk samples.

Milk sampling and analysis. Individual milk samples from morning and evening milking were collected weekly and allotted into three aliquots for analysis: the first aliquot was processed in order to assess fat, lactose, protein and casein content, by using Milkoscan 6000 FT (Foss Electric, Hillerød Denmark), and total somatic cell count (SCC) according to ISO 13366-2|IDF 148-2 (2006), by using a Fossomatic 5000 (Foss Electric, Hillerød Denmark) and expressed as linear score (linear score = $\log_2$ [SCC / 12,500]; Shook, 1993).

The second aliquot was processed to determine the milk rennet characteristics at 35°C by a Maspress apparatus (Foss Italia, Padua, Italy), according to Zannoni and Annibaldi (1981). The following rennet parameters were determined: clotting time (r) that is the time from rennet addition to the beginning of coagulation, firming time (k_{20}) that is the time needed for the amplitude to reach 20 mm on the recording chart, and curd firmness (a_{30}) that is the amplitude of the trace 30 min after rennet addition. The third aliquot of milk samples was stored at -80°C until analysis for FA extraction and composition by gas-chromatography according to Buccioni et al. (2010). Individual fatty acid methyl esters (FAMEs) were quantified using valeric acid (5:0) and nonadecanoic acid (19:0) methyl esters (cod W275204 and cod N5377, respectively; Sigma Chemical Co., St. Louis, MO) as internal standards and identified by comparison of the relative retention times of FAMEs peaks from samples, with those of the standard mixture 37 Component FAMEs Mix (Supelco, Bellefonte, PA, USA 4:0 -

24:0 (cod 18919 - 1AMP, Supelco, Bellefonte, PA, USA), individual *trans*-9 18:1 and *trans*-11 18:1 (cod 46903 and v1381 respectively, Sigma-Aldrich, St. Louis, Missouri, USA), individual *cis*-9, *trans*-11 18:2 (cod 1255, Matreya Inc Pleasant GAP, PA, USA.), CLA mix standard (cod 05632; Sigma-Aldrich, St. Louis, Missouri, USA) and published isomeric profile (Kramer et al., 1997; Kramer et al., 2004; Cruz-Hernandez et al., 2006). The 18:1 isomers elution sequence was performed according to Kramer et al. (2004). Moreover, standard mix of α-linolenic acid (α-LNA) isomers (cod 47792, Supelco, Chemical Co., St. Louis, MO) and of LA isomers (cod 47791, Supelco, Chemical Co., St. Louis, MO) and published isomeric profiles (Destaillats et al., 2005) were used to identify the isomers of interest. Two bacterial acid methyl ester mix (cod 47080-U Supelco, Chemical Co., St. Louis, MO; GLC110, Matreya, Pleasant Gap, PA) and individual standard for methyl ester of *iso* 14:0, *anteiso* 14:0, *iso* 15:0 and *anteiso* 17:0 (cods 21-1211-11, 21-1210-11, 21-1312-11 and 21-1415-11, Larodan Malmo, Sweden) were used to identify branched FA profile. Inter and intra-assay coefficients of variation were calculated by using a reference standard butter (CRM 164, Community Boureau of Reference, Bruxelles, Belgium) and detection threshold of FA was 0.01g / 100g of FA (Contarini et al., 2013). All FA composition results are expressed as g / 100g of total lipids for milk fat.

At 28th day of the trial, milk samples were collected also for total polyphenol content (milk total polyphenol, MTP) determination. MTP, expressed as tannic acid equivalents (TAE), was measured according to the Folin- Ciocalteu method (Makkar et al., 1993).

Blood sampling and analysis. Samples of blood were collected from each animal at the end of every experimental week by punching the jugular vein. Blood was stored into tubes without anticoagulant and serum was immediately separated by centrifugation (5000 x g for 30 min at 25°C).

For FA profile, blood samples were collected using tubes with anticoagulant (heparin) and plasma was immediately separated by centrifugation (5000 x g for 30 min at 25°C). One millilitre of plasma for each sample was directly methylated using a combination of methods according to Kramer et al.

(1997) modified by Park et al. (2001). The first step consisted of an alkaline methylation with sodium methylate/methanol (0.5 mL of 0.5 M-Sodium Methoxide) to esterify glycerides. The second step involved an acid methylation with HCl/methanol (4 mL of 5% methanolic HCl, 1 h at 50°C) as catalyst to esterify free fatty acids. Fatty acid methyl esters (FAME) were extracted using n-hexane (2 mL) with 9:0 and 23:0 methyl ester (cod 76368 and T9900 respectively, Sigma Chemical Co., St. Louis, MO) as internal standards for quantification, and maintained in vials with hermetic closure to avoid the loss of volatile components. FAME were separated and identified by gas-chromatography using the same programming described above for milk. All FA composition results were expressed as g / 100g of FA.

For metabolic profile, total protein (Colorimetric method BIURET), urea (kinetic enzymatic method), albumine (ALB; colorimetric BCG method), γ-glutamil-transferase (γ-GT; kinetic SZASZ-tris method), serum glutamic-pyruvic-transaminase (SGPT; kinetic UV IFCC method), serum glutamic-oxaloacetic-transaminase (SGOT; kinetic UV IFCC method), glucose (GLU; enzymatic colrimetric method), cholesterol (CHO; enzymatic colrimetric method CHO-POD), creatinine (kinetic enzymatic method – Jaffe), triglycerides (enzymatic colrimetric method) and phosphorus (Ammonium Molybdate method – UV) were detected using diagnostic kits (cods ASR01120; ASR01143; ASR0128012; ASR01194; ASR01219; ASR01229; ASR01202; ASR01101; ASR01150; ASR01134; ASR01181 Assel s.r.l., Rome Italy) with an auto blood-analyzer for hematology (Vegavet AMS, Analyser Medical System, Rome, Italy). Globuline (GBL) content was estimated by the difference between total protein and albumin contents.

Moreover, blood samples, collected at the end of the trial (4th week), were used also to determine the lipid peroxidation in plasma using as indicator the malonaldehyde (MDA). The assays were carried out using commercial kits (QuantiChrom™ TBARS Assay Kit, Bioassay System, cod. DTBA-100) by means colorimetric method, monitoring the change of absorbance at 532 nm with a

spectrophotometer (Placer et al., 1996). The MDA concentrations were expressed as micromoles per millilitre for plasma.

2.3 Statistical analysis.

All data (e.g., animal performance, milk composition, blood metabolic parameters and milk FA profile) recorded over the course of the experiment were processed as completely randomized design with repeated measures using the MIXED procedure of SAS (SAS, 1999):

$$Y_{ijkl} = \mu + D_i + T_j + I_k(D) + (D \times T)_{ij} + e_{ijkl}$$

where y_{ijkl} is the observation; μ is the overall mean; D_i the fixed effect of diet (i = 1 to 3); T_j the fixed effect of sampling time (j = 1 to 4); I_k is the random effect of the ewe nested within the diet (k = 1 to 6); $(D_i \times T)_{ij}$ the interaction between diet and sampling time and e_{ijkl} the residual error. The covariance structure was compound symmetry, which was selected on the basis of Akaike's information criterion of the mixed model of SAS. Statistical significance of the diet effect was tested against variance of sheep nested within diet according to repeated measures design theory (Littell et al., 1998). Multiple comparisons among means were performed using the Tukey's test.

Data related to MTP in milk and MDA in plasma were processed using one way analysis of variance (SAS, 1999) with a model that included diet and experimental error.

$$y_{ij} = \mu + D_i + e_{ij}$$

where y_{ij} is the observation; μ is the overall mean; D_i the diet (i = 1 to 3) and e_{ij} the residual error. Multiple comparisons among means were performed using the Tukey's test.

3 Results

3.1 Animal performance, milk composition and milk total polyphenol content

The dietary supplementation with tannic extracts did not affect the palatability of concentrates, completely consumed by animals.

During the experiment, no refusal was obtained from concentrate consumed, irrespective of the treatment (800g/ head and day). Since the concentrate was individually administrated, the daily intake of both tannins was 40g per head and per day and of soybean oil was 60g per head and per day.

The integration with CHE and QUE did not affect the milk yield and composition (Table 2). Urea concentration showed a significant variation due to only a time effect. An effect due to the interaction between diet and time (DxT) is observed for total protein and casein content. In contrast, Casein Index (CI), expression of the percentage of Casein respect the total protein, was affected by diet and it was significant higher in milk from animals fed QUE ($P<0.0259$; Table 2). The clotting parameters with the exception of a_{30} were affected by tannins. In particular, r was higher for QUE milk while k_{20} increased regardless the kind of tannin. The data are reported in Table 2.

The presence of tannins, regardless the kind of the extract (CHE *vs* QUE), did not affect the total polyphenol content of milk from ewes fed the supplementations. In fact, no significant differences were found among groups (Control, 1.86 g/ TAE; CHE, 1.94g/ TAE; QUE, 2.15g/ TAE; S.E.M, 0.18; P= 0.4675).

3.2 Metabolic profile, oxidative status and fatty acid profile of blood.

Blood parameters were not affected by diets with the exception of phosphorous that was lower in the blood samples of animals fed CHE diet. Significant differences due to time effect (T) are shown by urea content and by transaminase, total Protein, globuline, glucose and triglycerides. The data are reported in Table 3.

The oxidative status of ewes, determined using MDA as indicator, did not present significant differences among groups, regardless the concentrates fed by animals (Control, 1.48 µmol/ml plasma *vs* CHE, 1.49 µmol/ml plasma *vs* QUE, 1.43 µmol/ml plasma; S.E.M. 0.07; P=0.8484).

Fatty acid profile of blood showed that tannins, regardless the typology, favored the increase of VA and, at the same time, the reduction of stearic acid (18:0; SA) concentration. Data are reported in

Table 4. Interestingly, neither LA nor α-LNA concentration was enhanced by CHE and QUE. No significant effects of tannin supplementation were observed also for BH intermediates such as RA, *trans*-11, *cis*-15 18:2, *trans*-10, *cis*-15 18:2 and *cis* and *trans* isomers of 18:1 different to VA (Table 4). Saturated fatty acids (SFA) are lower in blood from animals fed concentrates integrated with tannins and this effect is stronger with CHE. Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) reached higher concentrations in blood from animals fed QUE and minor percentages in C group. Finally, the content of fatty acids with a carbon chain longer than 16 units was comparable in C and QUE groups and lower in CHE (Table 4).

3.3 Milk fatty acid profile.

Only few significant differences among fatty acids as consequence of the dietary tannin supplementation were found (Table 5). In particular, regardless the tannin kind, RA increased when the concentrates contained polyphenols and the stronger effect is reached with QUE ($P<0.0002$). In milk from CHE and QUE groups *cis*-9, *cis*-15 18:2 decreased with tannin supplementation ($P<0.0001$; Table 5).

4 Discussion

In the present trial, quebracho tannins increased the CI of milk ($P=0.0259$), probably as consequence of the slight decrease of whey proteins. Literature reported that tannins, especially if condensed, are able to interfere with amino acid absorption at the gut level because they selectively complex amino acids, affecting protein synthesis in tissues (Min et al., 2003; Patra and Saxena, 2011). The higher clotting time and firming time observed in milk from ewes fed QUE respect milk from no tannins and CHE fed ewes ($P<0.05$), could be probably due to different interaction between casein and bioactive monomers derived by the rumen microbial biodegradation of quebracho proantocyanidins (Gladine et al., 2007) or chestnut gallic acid compounds (Bhat et al., 1998), reaching in small quantities the mammary gland after duodenal absorption. Moreover, commercial tannin extracts from quebracho are not often pure proantocyanidins and might contain other compounds with a simpler

structure (monomers) which could be metabolized in the rumen. Indeed, several authors demonstrated that tannins can be partially metabolized by rumen microorganisms and that their metabolites can be absorbed at the gut level with a transferring in meat and milk of small ruminants (Lopez- Andres et al., 2013; Luciano et al., 2011; Jordan et al., 2010; Singh et al., 2001). The absorption of polyphenolic compounds generally depends on i) their molecular structure that, in turn, affects their solubility; ii) the ability of rumen microbiome in degrading them to compounds with a lower molecular weight; iii) their percentage of inclusion in the animal diet. Chestnut and quebracho tannins are characterized by a very complex structure with a high molecular weight (Campo et al., 2012; Pash et al., 2001).

In the present trial, the lack of significant differences in MTP concentration across groups suggested that the polyphenol substances contained in the tannin extracts were little absorbed in the intestine. This result could be also due to the dietary concentration of the tannin extracts in treated groups (about 1.6 % on DMI). In literature, significant differences have been observed when the concentration of dietary polyphenols from condensed tannins contained in pasture *Hedysarum coronarium* L. has been up to 6.0 % of the DMI (Di Trana et al., 2015), hence with a percentage of inclusion more than three folds higher respect to that used in the present experiment.

Nevertheless, a key role seems to be played by the kind of metabolite deriving from biodegradation of tannins at the rumen level. In fact, in literature, previous findings report a higher clotting time of milk when grape extracts (Felix da Silva et al., 2015) or tea catechins (Haratifar and Corredig, 2014) are added, as consequence of the interaction between polyphenols and proline residues close to the cleavage point of k-casein, reducing the enzyme accessibility. Han et al. (2011) observed that milk clotting parameters is affected by the structure and source of polyphenols while O'Connel et al. (1998) observed an increase of the interference on milk clotting behavior with the increase of the polymeric size of phenolic compounds.

No significant differences were found in oxidative status of blood among groups, probably because animals were at late lactation. A previous study on dairy cows, in fact, suggested that chestnut tannin

might exert antioxidant properties when animals were in the transition period (Liu et al., 2013). In contrast, other studies suggested that condensed tannin, as QUE, may exhibit toxic effect in liver (Min et al., 2003; Patra and Saxena, 2011; Reed, 1995; Hervas et al., 2003). In the present study, data related to blood parameters showed that CHE and QUE did not exert a toxic effect in liver at short term according to optimal values of transaminase.

Fatty acid profile of blood suggested that tannins were able to interfere with BH of dietary PUFA. In fact, plasma samples from CHE and QUE groups contained higher concentration of VA and lower concentration of SA if compared to plasma samples from C group. This pattern suggested that tannins interfere with the last step of BH of α-LNA and of LA affecting the activity of microorganisms responsible of VA hydrogenation, as reported also by previous studies (Buccioni et al., 2015a, Vasta et al., 2010, Hervas et al., 2013). Moreover, our data confirmed that the effect of quebracho is stronger than that of Chestnut tannin in modulating rumen fermentation of PUFA (Table 4).

In the present study, LA mainly is originated from soybean oil triglycerides whereas α-LNA from structural lipids of pasture grasses. Since rumen BH of LA and α-LNA produces different pattern of intermediates (Shingfield et al., 2013), the comparison of plasma lipid fatty acid profile between CHE and QUE sheep might provide information about difference in the BH pattern due to the effect of the type of tannin included in the diet. According to Table 4, among the BH intermediates only VA content significantly varied, suggesting that the effect of CHE and QUE did not differ in the BH of PUFA contained in triglycerides or structural lipids because VA is originated either from LA either from α-LNA, main FA in oil and roughage respectively.

The addition of tannin extract to the diet CHE and QUE did not result in an enhancement of the rumen outflow of α-LNA and LA, as confirmed by the lack of differences in the fatty acid profile of both blood plasma and milk samples. This result did not agree with findings obtained in a previous trial by Buccioni et al. (2015b). These authors found that the intake of α-LNA from pasture and linseed increased concentration of α-LNA in milk from ewes fed a diet supplemented with chestnut tannins, suggesting an effect of CHE extract on the BH process of α-LNA starting from the first step of the

pathway. In a previous trial, Buccioni et al. (2015a) found that the addition of similar amounts of quebracho and chestnut tannin extracts to the diet of dairy ewes resulted in an increase of LA in milk fat, as a consequence of the reduction of LA BH process. Also in this case, therefore, the results of the present experiment did not confirm a reduction of the LA BH from the first step of the process. Since the previous study adopted diets containing hay as forage basis instead of pasture, the role of the forage typology should be better understood in order to interpret the effect of tannin extracts on rumen BH of LA.

In the present experiment, the amount of VA escaped from rumen BH was probably enhanced when dairy sheep are fed diet supplemented with tannin extract, especially in the case of QUE diet (Table 4). This aspect was confirmed by the higher concentration of VA in plasma samples from QUE animals. Since *cis*-9, *trans*-11 CLA is mainly produced in mammary gland by Δ^9desaturation of VA (Bauman and Griinari, 2003), higher concentration of circulating VA probably led to higher amounts of *cis*-9, *trans*-11 CLA in milk fat. Hence, comparing plasma and milk fatty acid composition, it seemed that the activity of Δ^9desaturase (SCD) increased RA concentration in milk reducing the differences in VA concentration found in blood samples (Tables 4 and 5). Interestingly, also the concentration of SA in milk fat did not differ across treatments, despite the percentage of SA in plasma samples did. Similarly, to VA, the content of SA in milk fat is strictly regulated by the uptake of mammary tissue and by Δ^9desaturase enzyme activity, which converts SA to *cis*-9 18:1. In particular, almost 50% of *cis*-9 18:1 secreted in sheep milk originates from SCD activity (Frutos et al., 2014). However, several authors found that rumen fluid fatty acid profile may not fully reflect those of digesta in duodenum when dairy lactating cows fed a diet supplemented with a blend of soybean and fish oils together with tannins from *Vaccinium vitis idaea* (Szczechowiak et al., 2016). Moreover, Szczechowiak et al. (2016) found also that the treatment did not change the expression of five genes regulating the FA metabolism in mammary gland (codifying for acetyl-CoA carbossilase 1, ACACA; fatty acid synthase, FASN; stearoyl-CoA desaturase, SCD; fatty acid desaturase 1, FADS1; fatty acid elongase 5, ELOVL5) and of the gene codifying for lipoprotein lipase (LPL)

regulating the lipoprotein absorption catalyzing the hydrolysis of triglycerides transported in blood stream by very low density lipoproteins and by chilomicrons at the gut level (Malgorzata Brzozowska and Oprzadek, 2016).

5 Conclusion

The supplementation of diet of dairy ewes with chestnut or quebracho tannin extracts was effective in perturbing the last step of rumen BH of LA from soybean oil and α-LNA from pasture, as suggested by the higher amounts of VA in the plasma of animals fed QUE and CHE diets. The lack of effect on BH intermediates other than VA (the last step of the process) may suggest that rumen BH of PUFA was not influenced by the origin of PUFA. The higher amount of circulating VA probably enhanced the RA synthesis in mammary gland by SCD activity, resulting in higher percentages of RA in milk fat from animals fed QUE and CHE diets. Although more studies are needed in order to better understand why in some cases tannin extracts act at the first step of the BH whereas in other cases, as in the present study, the interference happened at the last step of BH, the use of tannin extracts might be an efficient way to improve PUFA content of sheep milk.

Acknowledgments

The authors thanks Mr Giuseppe Ponti, Mr Attilio Mazzocanti, Mr Giovanni Migni and Mr Giuseppe Covarelli of “Department of Agriculture, Food and Environmental Science” University of Perugia, Italy for their technical assistance in animal management during the trial and Nuovo Molino di Assisi s.r.l. (Bastia Umbra, PG, Italy) for providing feed ingredients. This research has been financed by Universities of Florence, Pisa, Perugia and by Gruppo Mauro Saviola srl.

References

AOAC International. 1995. Official Methods of Analysis, International. Gaitersburg, MD.

Bauman, D. E., Griinari, J.M., 2003. Nutritional regulation of milk fat synthesis. Ann. Rev. Nutr. 2, 203-227.

Bhat, T. K., Singh, B., Sharma, O.P., 1998. Microbial degradation of tannins – A current perspective. Biodegradation 9, 343–357.

Buccioni, A., Rapaccini, S., Antongiovanni, M., Minieri, S., Conte, G., Mele, M., 2010. Conjugated linoleic acid (CLA) and C18:1 isomers content in milk fat of sheep and their transfer to Pecorino Toscano D.O.P. cheese. Int. Dairy J. 20, 190–194.

Buccioni, A., Decandia, M., Minieri, S., Molle, G., Cabiddu, A., 2012. Lipid Metabolism in the rumen: new insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. Anim. Feed Sci. and Technol. 174, 1-25.

Buccioni, A., Serra, A., Minieri, S., Mannelli, F., Cappucci, A., Benvenuti, D., Rapaccini, S., Conte, G., Mele, M., 2015b. Milk production, composition, and milk fatty acid profile from grazingsheep fed diets supplemented with chestnut tannin extract and extruded linseed. Small Rum. Res. 130, 200-207.

Buccioni, A., Pauselli, M., Viti, C., Minieri, S., Pallara, G., Roscini, V., Rapaccini, S., Trabalza Marinucci, M., Lupi, P., Conte, G., Mele, M., 2015a. Milk fatty acid composition, rumen microbial population and animal performances in response to diets rich in linoleic acid supplemented with chestnut or quebracho tannins in dairy ewes. J. Dairy Sci. 98, 1145-1156.

Burns, R. E., 1963. Methods of tannin analysis for forage crop evaluation. Technical Bulletin number 32. Georgia Agricultural Experiment Station, Athens.

Campo, M., Pinelli, P., Romani, A., 2012. HPLC/DAD/MS characterization and antioxidant activity of sweet chestnut (Castanea sativa M.) fractions - XXVI International Conference on Polyphenols proceedings, 135-136.

Cannas, A., Tedeschi, L.O., Fox, D.G., Pell, A.N., Van Soest, P.J., 2004. A mechanistic model for predicting the nutrients requirements and feed biological values for sheep. J. Anim. Sci. 82,149–169.

Carreño, D., Hervàs, G., Toral, P.G., Belenguer, A., Frutos, P., 2015. Ability of different types and doses of tannin extracts to modulate in vitro ruminal biohydrogenation in sheep. Anim. Feed Sci. and Technol. 202, 42-51.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, L., Doreau M., 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci. Technol. 109, 828–855.

Christie, W.W., 1982. A simple procedure for rapid *trans*-methylation of glycerolipids and cholesterol esters. J. Lipid Res. 23, 1072-1075.

Contarini, G., Povolo, M., Pelizzola, V., Monti, L., Lercker, G., 2013. Interlaboratory evaluation of milk fatty acid composition by using different GC operating conditions. J. Food Compos Anal. 32, 131-140.

Correddu, F., Gaspa, G., Pulina, G., Nudda, A., 2015. Grape seed and linseed, alone and in combination, enhance unsaturated fatty acids in the milk of Sarda dairy sheep. J. Dairy Sci. 99, 1-11.

Cruz-Hernandez, C., Kramer, J. K. G., Kraft, J., Santercole, V., Or-Rashid, M., Deng, Z., Dugan, M. E. R., Delmonte, P., Yurawecz M. P., 2006. Systematic analysis of trans and conjugated linoleic acids in the milk and meat of ruminants. Pages 45–93 in Advances in Conjugated Linoleic Acid Research. Vol. 3. M. P. Yurawecz, J. K. G. Kramer, O. Gudmundsen, M. W. Pariza, and S. Banni, ed. AOCS Press, Champaign, IL.

Destaillats, F., Trottier, J.P., Galvez, J.M.G., Angers, P., 2005. Analysis of alpha-linolenic acid biohydrogenation intermediates in milk fat with emphasis on conjugated linolenic acids. J. Dairy Sci. 88, 3231–3239.

Di Trana, A., Bonanno, A., Cecchini, S., Giorgio, D., Di Grigoli, A., Claps, S., 2015. Effect of Sulla forage (*Sulla coronarium* L.) on the oxidative status and milk polyphenol content in goats. J. Dairy Sci. 98, 37-46.

Felix da Silva, D., Motumoto-Pintro, P., T., Bazinet, L., Couillard, C., Britten, M., 2015. Effect of commercial grape extracts on the cheese-making properties of milk. J. Dairy Sci. 98, 1552-1562.

Fievez, V., Colman, E., Castro-Montoya, Stefanov, J. M., I., Vlaeminck, B., 2012. Milk odd- and branched-chain fatty acids as biomarkers of rumen function – an update. Anim. Feed Sci. Technol. 172, 51–65.

Folch, J., Lees, M., Sloane Stanley, G. H., 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226, 497–509.

Frutos P., Toral, P.G., Ramos-Morales, E., Shingfield, K. J., Belenguer, A., Hervás, G.. 2014. Oral administration of cobalt acetate alters milk fatty acid composition, consistent with an inhibition of stearoyl-coenzymeA desaturase in lactating ewes. J. Dairy Sci. 97, 1036-1046.

Gladine, C., Rock, E., Morand, C., Bauchart, D., Dourand, D., 2007. Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. Brit. J. Nutrition, 98, 691-701.

Haratifar, S., and Corredig, M., 2014. Interaction between tea catechins and casein micelles and their impact on renneting functionality. Food. Chem.143, 27-32.

Han, J., Britten, M., St-Gelais, D., Champagne, C., P., Fustler, P., Salmieri S., Lacroix, M., 2011. Polyphenolic compounds as functional ingredients in cheese. Food Chem. 124, 1589-1594.

Hervas, G., Perez, V., Giraldez, F.J., Mantecon, A.R., Almar, M.M., Frutos, P., 2003. Intoxication of sheep with quebracho tannin extract. J. Comp. Pathol. 129, 44–54.

IACUC. 2014. Istitutional Animal Care and Use Committee. University of Perugia. www.unipg.it/files/pagine/115/regolamentoOPBApageweb.pdf.

ISO13366-2|IDF148-2. 2006. Milk-Enumeration of somatic cells – Part 2: Guidance on the operation of fluoro – opto- electronic counters.

Jordan, M.J., Monino, M.I., Martinez, C., Lafuente, A., Sotomayor, J.A., 2010. Introdution of distillate rosemary leaves into the diet of the murciano-granadina goat: transfer of polyphenolic compounds to goats' milk and the plasma of suckling goat kids. J. Agric. Food Chem. 58, 8265-8270.

Kramer, J. K. G., Cruz-Hernandez, C., Deng, Z.Y., Zhou, J.Q., Jahreis, G., Dugan, M.E.R., 2004. Analysis of conjugated linoleic acid and *trans* 18:1 isomers in synthetic and animal products. Am. J. Clin. Nutr. 9, 1137S–1145S.

Kramer, J. K. G., Fellner, V., Dugan, M.E.R., Sauer, F.D., Mossoba, M.M., Yurawecz, M.P., 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids. 32, 1219–1228.

Littell, R. C., Henry, P. R., Ammerman, C.B., 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76, 1216-1231.

Liu, H. W., Zhou, D. W., Li, K., 2013. Effects of chestnut tannins on performance and antioxidative status of transition dairy cows. J. Dairy Sci. 96, 5901–5907.

Lopez-Andres, P., Luciano, G., Vasta, V., Gibson, T.M., Biondi, L., Priolo, A., Mueller-Harvey, I., 2013. Dietary Quebracho tannins are not absorbed, but increase the antioxidant capacity of liver and plasma shhep. Br. J. Nutr. 110, 632-639.

Luciano, G., Vasta, V., Monahan, F.J., Lopez-Andres, P., Biondi, L., Lanza, M., Priolo, A., 2011. Antioxidant status, colour stability and myoglobin resistance to oxidation of longissimus dorsi muscle from lambs fed a tannin contasining diet. Food Chem. 124, 1036-1042.

Makkar, H.P.S., Bluemmel, M., Borowy N.K., Becker, K., 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric. 61, 161-165.

Malgorzata Brzozowska, A., Oprzadek, J., 2016. Metabolism of fatty acids in tissues and organs of the ruminants- a review. Anim. Sci. Papers and Rep. 34, 211-220.

Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim. Feed Sci and Technol. 106, 3-19.

Minieri, S., Buccioni, A., Rapaccini, S., Pezzati, A., Benvenuti, D., Serra, A., Mele, M., 2014. Effect of Quebracho tannin extract on soybean and linseed oil biohydrogenation by solid associated bacteria. An in vitro study. Ital. J. Anim. Sci. 13(3267), 604-608.

O'Connel, J., E., Fox, P., D., Tan-Kintia, R., Fox, P., F., 1998. Effects of tea, coffe and cocoa extract on the colloidal stability of milk and concentrated milk. Int. Dairy J. 8, 689-693.

Pallara, G., Buccioni, A., Pastorelli, R., Minieri, S., Mele, M., Rapaccini, S., Messini, A., Pauselli, M., Servili, M., Giovannetti, L., Viti, C., 2014. Effect of stoned olive pomace on rumen microbial communities and polyunsaturated fatty acids biohydrogenation: an in vitro study. BMC Vet Res. 10, 271-286.

Park, P.K., Albright, K.J., Cai, Z.Y., Pariza, M.W., 2001. Comparison of methylation procedures for conjugated linoleic acid artefact formation by commercial (trimethylsilyl)diazomethane. J. Agric. Food Chem, 49, 1158-1164.

Pash, H., Pizzi, A., Rode, K., 2001. MALDI_TOF mass spectrometry of polyflavonoid tannins. Polymer. 42, 7531-7539.

Patra, A.K., Saxena J., 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91, 24-37.

Placer, Z.A., Cushman, L.L., Johnson, B.C., 1996. Estimation of lipid peroxidation, malindialdehyde in biochemical system. Anal. Biochem. 16, 359-367.

Reed, J. D., 1995. Nutritional toxicology of tannins and related polyphenols in forages legumes. J. Anim. Sci. 73, 1516–1528.

SAS Institute. 1999. SAS User's Guide: Statistics. Version 8.0 ed. SAS Institute Inc., Cary, NC.

Shingfield, K.J., Bonnet, M., Scollan N.D., 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal.7, 132–162.

Shook, G.E. 1993. Genetic improvement of mastitis through selection on somatic cell count. Vet. Clin. North Am. Food Anim. Pract. 9, 563–58.

Singh, B., Bhat, T.K., Sharma, O.P., 2001. Biodegradation of tannic acid in an in vitro ruminal system. Liv. Prod. Sci. 68, 259–262.

Szczechowiak, J., Szumacher-Starbel, M., El-Sherbiny, M., Pers-Kamczyc, E., Pawlak, P., Cieslak, A., 2016. Rumen fermentation, methane concentration and fatty acid proportion in the rumen and milk of the dairy cows fed condensed tannins and /or fish-soybeans oils blend. Anim. Feed Sci. Technol. 216, 93-107.

Toral, P.G., Hervas, G., Bichi, E., Belenguer, A., Fruitos P., 2013. Effect of the inclusion of quebracho tannins in diet rich in linoleic acid on milk fatty acid composition in dairy ewes. J. Dairy Sci. 96, 431–439.

Toral, P., Hervas, G., Bichi, E., Belenguer, A., Fruitos P., 2011. Tannins as feed additives to modulate ruminal biohydrogenation: Effects on animal performance, milk fatty acid composition and ruminal fermentation in dairy ewes fed a diet containing sunflower oil. Anim. Feed Sci. and Technol. 164, 199–206.

Van Soest, P.J., Robertson, J.B., Lewis B.A., 1991. Methods fordietary fiber, neutral detergent fiber, and no starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Vasta, V., D. R. Yanez-Ruiz, M. Mele, A. Serra, G. Luciano, M. Lanza, L. Biondi, and A. Priolo. 2010. Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. Appl. Environ. Microbiol. 76, 2549–2555.

Zannoni, M., Annibaldi S., 1981. Standardization of the rennet ability of milk by Formagraph-I. Sci.

Table 1. Ingredients, chemical composition and fatty acids profile of the experimental concentrates, hay and of the pasture administered to the ewes.

			Experimental concentrates [1]		
	Hay	Pasture	Control	CHE	QUE
Ingredients (g / kg of dry matter)					
Barley			213.8	213.8	213.8
Corn			211.3	211.3	211.3
Wheat bran			158.5	158.5	158.5
Soybean meal (44 CP)			126.8	126.8	126.8
Beet pulp			89.8	89.8	89.8
Soybean oil[2]			84.5	84.5	84.5
Bentonite			52.8	-	-
Chestnut tannin extract[3]			-	52.8	-
Quebracho tannin extract[4]			-	-	52.8
Molasses			41.3	41.3	41.3
$CaCO_3$			10.6	10.6	10.6
Sodium bicarbonate			5.3	5.3	5.3
Di-calcium phosphate			5.3	5.3	5.3
Chemical composition (g / kg of DM)					
Chemical composition (g / kg of DM)					
Crude Protein	111.2	156.8	135.0	135.0	135.0
Ether extract	12.0	24.5	96.0	96.0	96.0
NDF	636.4	425.6	181.0	181.0	181.0
ADF	501.3	347.4	74.2	74.2	74.2
ADL	105.7	67.3	12.9	12.9	12.9
Ash	69.6	43.2	85.0	85.0	85.0
ME (MJ / kg DM)	7.8	8.3	10.3	10.3	10.3
NEl (MJ / kg DM)	4.7	5.0	6.6	6.6	6.6
Fatty acids (g / 100g of total fatty acids)					
16:0	35.5	16.3	14.0	14.0	14.0
18:0	5.8	4.2	3.6	3.6	3.6
cis-9 18:1	9.3	11.8	23.3	23.3	23.3
18:2 n-6	28.5	22.2	51.4	51.4	51.4
18:3 n-3	2.8	37.5	5.8	5.8	5.8

[1]CHE: concentrate containing chestnut tannin extract; QUE: concentrate containing quebracho tannin extract.

[2]Fatty acid profile of soybean oil (g / 100g of total fatty acids): 16:0, 11.0; 18:0, 3.6; *cis*-9 18:1, 22.1; *cis*-9, *cis*-12 18:2, 53.7; *cis*-9, *cis*-12, *cis*-15 18:3, 7.2.

[3]Hydrolysable tannins extracted from Chestnut wood (*Castanea sativa)* containing 750 g of equivalent tannic acid/kg DM (provided by Gruppo Mauro Saviola srl Radicofani, Siena, Italy).

[4]Condensed tannins extracted from quebracho (*Schinopsis lorentzii*) containing 456 g of equivalent tannic acid/kg DM (provided by Guido Lapi spa, Castel Franco di Sotto, Pisa, Italy).

Table 2. Milk yield and composition from ewes fed 800 g / head / d of a concentrate containing 84 g of soybean oil / kg DM plus 0 (control diet) or 52.8 g / kg DM of a chestnut tannin extract (CHE diet) or 52.8 g / kg of DM of quebracho tannin extract (QUE diet).

Item		Diet				P value[1]		
		Control	CHE	QUE	SEM[4]	D	T	D x T
Milk yield	g / d	767	834	895	60.2	0.3293	0.9864	0.9985
Milk composition	g / 100 g							
Fat		7.08	7.45	7.22	0.303	0.7191	0.1606	0.0886
Lactose		4.77	4.72	4.88	0.068	0.3067	<0.0001	0.7247
Protein		5.69	5.94	5.83	0.170	0.6109	0.9527	0.0084
Casein		4.44	4.61	4.62	0.143	0.6148	0.9588	0.0084
Urea	mg / dl	14.92	17.58	17.64	2.340	0.6590	0.0009	0.5701
Total solids	g / d	0.13	0.15	0.16	0.020	0.6836	0.8930	0.2772
Casein Index[2]		78.01 b	77.57 b	79.21 a	0.375	0.0259	0.0817	0.8965
Linear Score[3]		4.89	3.82	3.64	0.727	0.4526	0.0016	0.0721
Clotting parameters								
r	min	14.33 b	15.11 b	18.08 a	0.519	<0.0001	0.0031	0.0795
k_{20}	"	1.44 b	1.69 a	1.85 a	0.112	0.0435	0.0010	0.1986
a_{30}	mm	41.40	44.16	42.70	3.337	0.8565	0.8111	0.4755

[1]Probability of significant effect due to experimental factors: diet (D), time (T), and their interaction (D X T);

[2]Casein Index: total casein / total protein *100.

[3]Linear Score = $\log_2$(SCC / 12,500) where SCC is Somatic Cell Count.

[4]Standard Error Mean

Table 3. Blood parameters from ewes fed 800 g / head / d of a concentrate containing 84 g of soybean oil / kg DM plus 0 (control diet) or 52.8 g / kg DM of a chestnut tannin extract (CHE diet) or 52.8 g / kg of DM of quebracho tannin extract (QUE diet).

					P value [3]		
Item[1]	**Control**	**CHE**	**QUE**	**SEM[2]**	**D**	**T**	**D X T**
P tot g / dl	8.21	8.27	7.85	0.340	0.6644	0.0011	0.8699
Urea mg / dl	44.14	43.19	48.37	2.144	0.2895	<0.0001	0.1484
ALB g / dl	3.79	3.73	3.73	0.056	0.6525	0.4641	0.1110
GLB g / dl	4.42	4.54	4.12	0.162	0.4952	< 0.0001	0.0256
γ-GT U / l	58.87	60.66	62.75	4.120	0.5347	< 0.0001	0.7359
SGPT U / l	18.50	19.39	22.78	2.632	0.5393	0.0117	0.2041
SGOT U / l	207.52	248.31	195.45	22.413	0.6049	0.0065	0.4033
GLU mg / dl	67.20	67.85	66.83	1.397	0.8865	<0.0001	0.7079
CHO mg /dl	83.25	83.75	86.29	6.890	0.9467	0.3081	0.0628
Creatinine mg / dl	0.73	0.72	0.79	0.026	0.1629	0.1438	0.6079
Triglycerides mg / dl	18.08	22.05	17.75	1.338	0.0935	0.0048	0.0614
Phosphorus mg/dl	4.58 a	4.29 b	4.52 a	0.492	0.0038	0.9297	0.9811

[1]P tot: total protein; ALB: albumine; GBL: globuline; γ-GT: γ-glutamil-transferase; SGPT: serum glutamic-pyruvic-transaminase; SGOT: serum glutamic-oxaloacetic-transaminase; GLU, glucose; CHO, cholesterol.

[2]Standard Error Mean.

[3]Probability of significant effect due to experimental factors: diet (D), time (T), and their interaction (D X T).

Table 4. Fatty acid composition of blood from sheep fed 800g /head and day of a concentrate containing 84 g of soybean oil / kg DM plus 0 (control diet) or 52.8 g /kg DM of chestnut tannin extract (CHE diet) or 52.8 g /kg DM of a quebracho extract (QUE diet)

	Diet				P value[2]		
Fatty acid g/100g FA	Control	CHE	QUE	SEM[1]	D	T	D x T
2:0	0.30	0.28	0.24	0.033	0.4672	<0.0001	0.7232
3:0	0.20	0.11	0.11	0.040	0.2482	0.9142	0.5382
4:0	0.24 a	0.17 b	0.17 b	0.014	0.0203	0.0577	0.4421
6:0	0.29	0.31	0.32	0.012	0.5267	0.4325	0.8541
8:0	0.37	0.58	0.22	0.140	0.0846	0.0324	0.0008
10:0	0.26	0.32	0.22	0.072	0.3399	0.1285	0.0112
12:0	0.08	0.08	0.12	0.032	0.6288	0.4123	0.1495
14:0	0.29	0.29	0.28	0.016	0.8388	0.0053	0.7158
cis-9 14:1	0.06	0.06	0.05	0.011	0.8682	0.5891	0.9334
anteiso 15:0	0.13	0.13	0.11	0.010	0.2768	0.1472	0.8038
15:0	0.36	0.32	0.36	0.020	0.2686	0.0885	0.9054
iso 16:0	0.11	0.11	0.12	0.017	0.9836	0.0326	0.9866
16:0	13.44	13.27	13.48	0.385	0.9191	0.0327	0.7097
cis-9 16:1	0.18	0.16	0.15	0.016	0.5863	0.0884	0.7434
iso 17:0	0.26	0.24	0.26	0.015	0.4674	0.3260	0.3963
anteiso 17:0	0.30	0.32	0.31	0.017	0.875	0.1539	0.1144
17:0	0.84	0.78	0.80	0.034	0.5045	0.0030	0.1730
cis-9 17:1	0.11	0.11	0.10	0.010	0.7918	0.3345	0.6518
18:0	24.02 a	21.14 b	22.25 b	0.610	0.0264	<0.0001	0.0419
trans-6,8 18:1	0.17	0.27	0.23	0.034	0.2182	0.0810	0.0906
trans-9 18:1	0.39	0.39	0.37	0.038	0.8109	0.3785	0.1943
trans-10 18:1	0.32	0.41	0.37	0.037	0.2334	0.4428	0.0820
trans-11 18:1	2.21 c	2.73 b	3.18 a	0.213	0.0309	0.1761	0.8676
trans-12 18:1	0.65	0.63	0.77	0.076	0.4023	0.8504	0.5560
cis-7 18:1	0.30	0.37	0.83	0.337	0.5214	0.6267	0.5443
cis-9 18:1	11.14	10.32	10.6	0.480	0.5584	0.0635	0.8051
cis-11 18:1	0.47	0.50	0.48	0.024	0.5968	0.1606	0.6554
cis-12 18:1	2.62	2.06	2.19	0.146	0.0626	0.0116	0.1968
cis-15 18:1	0.08	0.09	0.06	0.016	0.6386	0.5605	0.3394
cis-9, *cis*-12 18:2	24.36	23.34	25.55	0.622	0.0849	<0.0001	0.9687
cis-9, *trans*-11 18:2	0.48	0.54	0.59	0.044	0.2822	0.9649	0.3093
cis-9, *cis*-12, *cis*-15 18:3	1.99	1.74	1.93	0.146	0.5268	0.0028	0.1669
trans-11, *cis*-15 18:2	0.23	0.21	0.22	0.013	0.5636	0.3153	0.2733
Other fatty acids	12.75 b	17.62 a	12.96 b	0.423	0.0426	0.4251	0.3271
SFA[3]	41.49 a	38.45 c	39.37 b	0.361	0.0485	0.9524	0.7259
MUFA[4]	19.00 b	18.42 c	19.69 a	0.263	0.0425	0.5246	0.2546

PUFA[5]	27.06 b	25.83 c	28.29 a	0.594	0.0345	0.2543	0.3581
< 16:0[8]	16.31	16.19	15.95	0.352	0.3526	0.2485	0.5214
> 16:0[9]	71.12 a	66.35 b	71.24 a	0.523	0.0425	0.2541	0.5963

[1]Standard Error Mean.

[2]Probability of significant effect due to experimental factors: diet (D). time (T). and their interaction (D X T); means within a row with different letters differ (P < 0.05).

[3]SFA: saturated fatty acids

[4]MUFA: monounsaturated fatty acids

[5]PUFA: polyunsaturated fatty acids

[6]< 16:0 de novo fatty acids calculated according to Fievez et al. 2012.

[7]> 16:0 preformed fatty acids calculated according to Chilliard et al.. 2000 and Fievez et al. 2012

Table 5. Fatty acid composition of milk from sheep fed 800 g / head / d of a concentrate containing 84 g of soybean oil / kg DM plus 0 (control diet) or 52.8 g / kg DM of a chestnut tannin extract (CHE diet) or 52.8 g / kg of DM of quebracho tannin extract (QUE diet).

	Diet				P value[2]		
Fatty acid g/100g FA	Control	CHE	QUE	SEM[1]	D	T	D x T
4:0	4.363	4.397	3.882	0.234	0.2647	<0.0001	0.6455
6:0	1.311	1.343	1.161	0.067	0.1682	0.0031	0.9678
8:0	0.918	1.047	0.992	0.064	0.4605	0.1087	0.8375
10:0	2.595	3.087	2.849	0.237	0.3521	0.0074	0.9408
cis-9 10:1	0.15	0.15	0.15	0.003	0.2598	0.3586	0.4528
12:0	2.013	2.065	1.883	0.093	0.4074	0.2027	0.7435
cis-9 12:1	0.006	0.008	0.011	0.002	0.3530	0.159	0.2033
iso 13:0	0.042	0.027	0.031	0.008	0.5137	0.2531	0.4457
anteiso 13:0	0.010	0.010	0.020	0.005	0.5246	0.8254	0.1562
13:0	0.030	0.030	0.030	0.012	0.4852	0.2589	0.5914
iso 14:0	0.075	0.048	0.047	0.011	0.2030	0.8325	0.6128
14:0	6.963	7.123	6.821	0.141	0.3799	0.0020	0.7559

iso 15:0	0.155	0.156	0.150	0.006	0.7114	0.5916	0.7471
cis-9 14:1	0.126	0.115	0.124	0.007	0.6010	0.6495	0.8473
anteiso 15:0	0.288	0.276	0.291	0.013	0.6752	0.3354	0.5820
15:0	0.593	0.627	0.608	0.021	0.5327	0.0043	0.4372
iso 16:0	0.085	0.103	0.090	0.014	0.7377	0.0005	0.3660
16:0	16.611	19.290	16.590	0.947	0.1279	<0.0001	0.1649
cis-9 16:1	0.521	0.430	0.471	0.041	0.4017	0.0118	0.7001
iso 17:0	0.296	0.327	0.333	0.027	0.5385	0.3159	0.9608
anteiso 17:0	0.270	0.310	0.325	0.024	0.3164	0.3739	0.9443
17:0	0.472	0.468	0.481	0.018	0.9110	0.0173	0.9841
cis-9 17:1	0.13 b	0.13 b	0.15 a	0.006	0.0319	0.3419	0.4526
18:0	13.011	12.612	12.121	0.276	0.1149	0.0313	0.8718
trans-6,8 18:1	0.770	0.733	0.728	0.051	0.8534	0.1072	0.8936
trans-9 18:1	0.648	0.776	0.754	0.035	0.0662	0.0141	0.3605
trans-10 18:1	1.534	1.396	1.476	0.073	0.5105	0.3596	0.8554
trans-11 18:1	5.361	5.263	5.592	0.292	0.7440	0.0125	0.7759
trans-12 18:1	0.985	1.076	0.985	0.052	0.4616	0.7079	0.6560
cis-7 18:1	0.411	0.395	0.378	0.090	0.9702	<0.0001	0.7934
cis-9 18:1	19.506	19.612	18.790	0.578	0.5856	0.0664	0.2015
cis-11 18:1	0.526	0.515	0.516	0.031	0.9694	0.3217	0.9537
cis-12 18:1	0.487	0.487	0.479	0.032	0.9743	0.9078	0.9884
cis-15 18:1	1.118	1.213	1.064	0.043	0.0865	0.0390	0.5914
cis-9, cis-12 18:2	3.838	3.766	3.782	0.112	0.9162	0.0001	0.8258
cis-9, trans-11 18:2	2.196 c	2.293 b	2.716 a	0.081	0.0002	0.0003	0.1\972
cis-9, cis-12, cis-15 18:3	0.507	0.526	0.550	0.020	0.3776	<0.0001	0.1861
cis-9, trans-12, trans-15 18:3	0.023	0.024	0.043	0.013	0.5457	0.0574	0.7528
trans-9, cis-12, trans-15 18:3	0.034	0.043	0.033	0.005	0.3871	0.0017	0.1998
cis-9, cis-15 18:2	0.064 a	0.017 b	0.011 c	0.006	<0.0001	0.5544	0.5435
trans-11, cis-15 18:2	0.194	0.226	0.225	0.024	0.6470	0.0378	0.8339
trans-10, cis-12 18:2	0.01	0.01	0.01	0.005	0.9523	0.2596	0.1458
20:0	0.255	0.302	0.242	0.022	0.2245	0.8747	0.4103
20:4	0.022	0.022	0.025	0.005	0.9127	0.0655	0.7641
22:0	0.112	0.088	0.097	0.013	0.5314	0.3006	0.8853
SFA[3]	50.246	53.038	48.206	1.781	0.1173	<0.0001	0.7641
MUFA[4]	32.435	32.124	31.216	0.841	0.5863	0.0057	0.3198
PUFA[5]	6.922	6.922	7.365	0.208	0.2635	0.0008	0.6908
OIAR[6]	0.803 b	0.824 a	0.784 c	0.037	0.7879	0.0302	0.1638
DI [7]	0.017	0.016	0.017	0.002	0.1926	0.1159	0.2166
< 16:0[8]	36.440	39.636	35.166	1.508	0.1432	<0.0001	0.6570
> 16:0[9]	70.440	72.324	68.629	1.705	0.3565	<0.0001	0.2010

[1]Standard Error Mean.

[2]Probability of significant effect due to experimental factors: diet (D), time (T), and their interaction (D X T); means within a row with different letters differ ($P < 0.05$).

[3]SFA: saturated fatty acids.

[4]MUFA: monounsaturated fatty acids.

[5]PUFA: polyunsaturated fatty acids.

[6]Ratio odd-*iso* to odd-*anteiso* FA: (*iso* 15:0 + *iso* 17:0) / (*anteiso* 15:0 + *anteiso* 17:0).

[7]Desaturation index, DI = (*cis*-9 14:1 / 14:0 + *cis*-9 14:1).

[8]< 16:0 de novo fatty acids calculated according to Fiviez et al 2012.

[9]> 16:0 preformed fatty acids calculated according to Chilliard et al., 2000 and Fiviez et al., 2012.