The effect of supplemented chestnut tannin to grass silage either at ensiling or at feeding on lamb performance, carcass characteristics and meat quality

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This study was designed to investigate the effect of supplemented chestnut hydrolysable tannin (HT) both at ensiling and at feeding on lamb growth, carcass characteristic, and meat quality. Twenty tons of ryegrass (*Lolium perenne*) were used to produce silage. The ryegrass was treated at ensiling with one of three additives: 30 g kg^{-1} DM chestnut HT (GET), an inoculant as a positive control (GI), or water as a negative control (G). Another two treatments were made from ensiled grass by adding the 30 g kg^{-1} DM of chestnut HT to either positive (GI+T) or negative (GT) control. Forty Suffolk cross Mule lambs were used in this experiment and allocated to receive one of five experimental forage treatments with eight lambs per treatment. The diet consisted of two parts: concentrate and silage. Lambs were fed 215 g DM day^{-1} lamb of concentrate diet and *ad libitum* grass silage for seven weeks and then slaughtered. Back fat thickness tended to be lower ($p = 0.07$) for lambs fed the GT and GI+T treatments compared to lambs in the other experimental groups' (10.0, 10.1, 9.8, 10.0, and 9.8 mm for GET, G, GT, GI, and GI+T, respectively). Feeding lambs GET tended to reduce ($p = 0.06$) meat lightness ($L*$) compared to the other treatments. Ammonia nitrogen concentration in rumen fluid was reduced significantly ($p < 0.05$) when lambs consumed diets treated with tannin both at ensiling and at feeding (0.14, 0.19, 0.17, 0.17 and 0.14g l^{-1} GET G, GT, GI, and GI+T, respectively). The experimental treatments had no effect ($p > 0.05$) on voluntary feed intake (914, 916, 899, 928, or 914 g day^{-1} for GET, G, GT, GI, and GI+T, respectively) or lamb performance.

Key words: hydrolysable tannin, protein degradability, silage additives, inoculate

Introduction

Oilseed meal contains a high protein level (170–700 g kg^{-1} DM) and is considered the main protein source in animal nutrition (McKevith 2005, Taha 2015). Hence, the global oilseed cultivated area increased from approximately 195 million ha in 2001 to 260 million ha in 2020, with an increase in production volume from 326 to more than 610 million metric tonnes (t) in the same period (Statista 2021). However, although the cultivation area and production volume have increased, increasing the use of oilseed meals especially soybean meal (SBM) in non-ruminant animal nutrition (pig and poultry) has led to an increase in price (Jezierny et al. 2010). In the UK and other European countries, most SBM is imported due to unsuitable weather conditions for cultivating soya beans (Wilkins and Jones 2000). The European Commission (EC 2020) reported that rapeseed is the main oilseed (59% among all oilseeds) cultivated in the EU with a CP content of 400 g kg^{-1} DM after oil extraction (AFRC 1993). The price of SBM, rapeseed meal, sunflower meal, and linseed cack increased by approximately 260, 225, 220, and 75% from 2010 until 2022 (TESEO 2022), which has encouraged ruminant farmers to utilize alternative sources of home-grown protein (pasture and forages) in ruminant nutrition.

Protein requirements of ruminants could be supplied by pasture and forage using good management and cultivation systems (Wilkins and Jones 2000). Hart (2005) reported that a reduction in feed cost could be achieved by replacing SBM with grass and leguminous silage in ruminant nutrition (dairy cows) without compromising performance. However, forage protein is highly degradable in the rumen (700–800 g kg^{-1} DM; McDonald et al. 2011) and could result in an oversupply of dietary rumen degradable protein, without satisfying metabolizable protein requirements. Reducing the rumen protein degradability of ensiled crops could improve the efficiency of utilization of dietary protein and carbohydrates by improving the synchrony of nutrient supply in the rumen (Sinclair et al. 1993). Supplementing ruminant diets with plant secondary compounds such as tannins could reduce protein degradability in the rumen (Makkar 2003, Taha et al. 2014).

Tannins have been described as poly-phenolic compounds, that are found in different plant species and different plant components (Piluzza et al. 2014). In general, tannins can be divided into two main groups, condensed tannins (CT) and hydrolysable tannins (HT), although natural tannins may contain a combination of the two groups rather than existing single tannin species (Makkar 2003). Tannins (HT and/or CT) have been shown to create a reversible complex compound with different feed ingredients such as proteins (McSweeney et al. 2001) via the active sites available on different tannin types. As consequence, the rumen protein degradability would be reduced and the undegradable protein supply would be increased (Sinclair et al. 2009). It may be postulated that these effects may be due to the protein-tannins complex inhibiting microbial activity (Makkar 2003) plus a direct effect of tannins reducing protozoa and bacteria numbers (Aghamohamadi et al. 2014). An increased flow of dietary protein to the small intestine would result in increasing animal performance. However, tannin has often been described as an anti-nutritional compound that has a negative effect on nutrient utilisation, dry matter intake, and digestibility (Lamy et al. 2011). Schofield et al. (2001) and Makkar (2003) suggested that the anti-nutritional effects of tannins could depend on tannin concentration, source, type, animal species, physiological status of the animal, and feed composition. Deaville et al. (2010) reported that using relatively low levels of either chestnut or mimosa tannins (<50 g kg^{-1} DM) in ruminant nutrition might increase microbial protein synthesis in the rumen, improve animal digestion and performance.

It has been reported that the colour and flavour of red meat are affected by the oxidation process (Luciano et al. 2009). Oxidation of lipids would perhaps develop meat off-flavour and oxidation of myoglobin would result in discoloration of the meat (Gray et al. 1996). Luciano et al. (2009) reported that there is a possibility that both colour and lipid oxidation are linked together. Feeding ruminants anti-oxidant compounds such as vitamin E or polyphenols such as tannins have been shown to delay oxidation of lipid and discoloration (Baron et al. 2002). To our knowledge there is a lack of studies regarding using chestnut HT as a silage additive to improve lamb performance and meat quality. Therefore, the objectives of this experiment were to evaluate the effect of supplementing with chestnut HT (30g kg^{-1} DM) at ensiling or at feeding on lamb dry matter intake (DMI), liveweight gain, diet whole tract digestibility, carcass characteristics, and meat quality.

Materials and methods
Silage production and experimental design

Approximately 20 tons of ryegrass (perennial ryegrass mix sword [*Lolium perenne*]) forage (second cut) was used for silage making in this experiment. The ryegrass was mowed and wilted for 48 h. The wilted grass was then chopped (approximately 10 cm) using a mower machine (Fait 1580 DT 1984 Italy) and treated at ensiling with one of three additives; 30 g kg^{-1} DM chestnut HT (Thomas Ware & Sons Bristol, according to the manufacturer chestnut HT had an actual tannin content of 750 g kg^{-1} DM with a mix of the following tannins: castalagin, vescalagin, castalin, and vescalin in the proportion 530, 350, 35, and 85 g kg^{-1} DM, respectively), an inoculant (10^6 colonies forming unit, homofermentative *Lactobacillus plantarum* g^{-1}, produced at Silage Solution Ltd., Aberystwyth, UK) as a positive control, or water as a negative control. Additives were mixed with the forages individually using a mixer (Super 10 MIXMAX. HISPEC Ltd, Carlow, Republic of Ireland) with a 500 kg capacity. To ensure consistency, water was applied to all treatments at a rate of 1 l t^{-1} fresh weight. To facilitate accurate tannin supplementation at ensiling, forage DM was measured using a moisture analyser machine (METTLER TOLEDO-HB43-S Halogen, Columbus, OH, USA). Three silage clamps (one per treatment) were filled rapidly (approximately 6.5 t clamp^{-1}) and rolled using a tractor and then sealed with three layers of plastic sheet. Big square bales of straw were used to add weight to the clamps to prevent oxygen invasion, and the clamps were left for more than 100 days to ensile. A subsample from each of the treatment was taken and stored at -20 °C prior to further analysis. Another two treatments were made from ensiled grass by adding 30 g kg^{-1} DM of chestnut HT to either positive or negative control groups manually every day before feeding the lambs. Therefore, the experimental groups were ryegrass silage (negative control) (G), ryegrass forage treated with 30 g kg^{-1} chestnut HT at ensiling (GET), ryegrass silage treated with inoculum (positive control) (GI), G + supplemented (30 g kg^{-1} DM) chestnut HT after opening the silo (GT) and GI + supplemented (30 g kg^{-1} DM) chestnut HT after opening the silo (GI+T).

Animal and experimental diet formulation

All animal procedures used in the current experiment have been conducted according to the UK Animals (Scientific Procedures Act) 1986; amended in 2012 and authorized by the Harper Adams University Animal Welfare and Ethical Review Board (AWERB).

Forty Suffolk cross Mule lambs (20 wethers, an average liveweight of 29.5 kg ± 2.5, and 20 ewes average liveweight 29.2 kg ± 2.0) were used in this experiment. Lambs were blocked by liveweight and sex. Blocks were randomly allocated to receive one of the five experimental forage treatments with eight lambs per treatment. Lambs were kept on a wood shaving bed and fed in individual pens (2 m^2) with free access to clean water. The diet was formulated to meet the requirements of growing Suffolk cross Mule breed lambs (body weight 33 kg) gaining 0.2 kg day^{-1} according to AFRC (1993). The diet consisted of two parts: concentrate and silage. Lambs were fed 215 g DM day^{-1} lamb^{-1} concentrate diet in the morning meal at 09:00. Silage treatments were weighed out every day and offered (*ad libitum*) in two equal meals in the morning (at 09:00) and afternoon (at 16:00) at a rate of approximately 1.5 kg fresh for each meal. Refusals were weighed back twice a week. There were no concentrate refusals on any day for any lamb. Samples of each treatment (concentrate, silages, and refusals) were taken (Tuesday and Friday every week for six weeks) and stored frozen at −20 °C to await proximate analysis. Concentrate and silage samples were analysed chemically (in triplicate) according to AOAC (2000) for dry matter (DM), pH, ammonia-nitrogen (NH$_3$-N), organic matter (OM), crude protein (CP), natural detergent fibre (NDF), acid detergent fibre (ADF) and ether extract (EE). In addition, metabolizable energy (ME) and metabolizable protein (MP) of the silage samples were estimated using near-infrared reflectance spectroscopy (NIR) (RUMENCO, Staffordshire, UK), while for the concentrates the metabolizable energy and metabolizable protein were calculated according to McDonald et al. (2011). Proximate analysis results of the silages and concentrate are shown in Table 1. The lambs received the experimental diet treatments for seven weeks, one week as an adaptation period to introduce lambs to experimental diets, and six weeks as an experimental period, and then the lambs were slaughtered. Lambs were weighed at 11:00 on Tuesday of each week during the experimental period using portable calf scales (IAE Leek, Staffordshire, UK). The scale was calibrated prior to use using standard weights.

Table 1. Proximate analysis of concentrate diet and ryegrass silage treated with tannin at ensiling, inoculated with bacteria or water

Analysis	GET	GI	G	Concentrate
DM g kg^{-1}	244	247	245	880
pH	3.8	4.0	3.7	
NH$_3$N g kg^{-1} TN	18	21	22	
CP g kg^{-1} DM	168	165	166	180
OM g kg^{-1} DM	906	907	904	920
NDF g kg^{-1} DM	433	445	434	111
ADF g kg^{-1} DM	278	277	280	
EE g kg^{-1} DM	26	28	27	50
ME MJ kg^{-1} DM	11.4*	10.9*	11.9*	11.2**
MP g kg^{-1} DM	100*	99*	102*	110**

DM = dry matter; NH$_3$-N = ammonia nitrogen; CP = crude protein; OM = organic matter; NDF = neutral detergent fibre; ADF = acid detergent fibre; EE = ether extract; ME = metabolizable energy; MP = metabolizable protein; GET = supplemented tannin at ensiling; GI = ryegrass silage treated with inoculate; G = ryegrass silage treated with water; * estimated using NIR; ** estimated according to McDonald et al. (2011)

Blood samples were collected from each lamb four times during the experimental period on days 0, 15, 30, and 38 days. Blood samples (10 ml) were taken via jugular venepuncture at 11:30 (2.5 hours post feeding) on Monday once every two weeks, into lithium heparin vacutainers tubes (for total protein, urea, and BHB analysis) and into potassium oxalate vacutainers tubes (for glucose analysis) (Bioscience Int. Plc., Bridgend, UK). Blood samples were centrifuged at 3000 g for 15 min at 4 °C, and the plasma was transferred into 2 ml tubes and stored at −20 °C for further analysis. Frozen plasma samples were defrosted in the fridge and analysed as mentioned above. Diet DM digestibility was measured using acid insoluble ash (AIA) according to Van Keulen and Young (1977).

Lamb performance

Six weeks post experimental period, the lambs were weighed and kept in one group and fasted for 12 hours with free access to clean water. The next morning at 06:00 the lambs were sent via truck (30 mint driving) to a commercial abattoir (Approved Design Slaughterhouse. Walsall, West Midland, UK) for harvesting. The rumen of each lamb was removed (15 mints post slaughter) and 100 ml of rumen fluid was taken and filtered using two layers

of muslin into a 100 ml pot. Immediately the rumen pH was measured using a pH meter (HACH, H160, Loveland, USA), and the rumen fluid was stored at −20 °C for prior analysis for NH_3-N, total, and individual volatile fatty acids (VFA). Lamb carcasses were weighed twice, 30 min post slaughter as hot carcass weight and 24 h post slaughter as chilled carcass weight (carcasses were kept in a chiller at 4 °C for 24 h). Prior to hygiene inspection, the dimensional measurements of the carcasses (body length, barrel width, chest depth, and gigot depth and gigot width) were recorded according to Brown and Williams (1979). Carcasses were then halved longitudinally with a band saw and the left side was collected for carcass characteristics and meat parameters. The left half was then quartered between the penultimate and rib 12 using a knife. The subcutaneous fat thickness (at rib 12) was measured using a set of metal callipers, and the eye muscle area was obtained by tracing the eye muscle at rib 12 upon acetate paper; later the papers; were scanned and the muscle area measured using an Image Analyser Pro Plus 4.1 software (Media Cybernetics Inc., PA 15086, USA). The dressing percentage was calculated by dividing the hot carcass weight by the final live weight. A hundred grams of loin muscle (*longissimus dorsi*) of each lamb were stored at 4 °C for meat colour, fatty acid profile, and rancidity measurements.

Meat colour parameters of the chilled loin muscle (stored at 4 °C) were measured 3 days post slaughter. A Minolta colour meter (model CR-400, Konica Minolta Sensing Inc., Osaka, JAPAN) was used to measure meat colour co-ordinates including lightness (*L**), yellowness (*b**), and redness (*a**). The Minolta colour meter contained a head with 0.8 cm diameter, a measuring surface, and a diffused illumination viewing. The measurements were made using the D65 illuminate and 2° standard observed. The camera was first calibrated with a white calibration plate at the beginning of measurements. The lens of the camera was allowed to touch the surface of the meat during the measurement, and a double reading was made for each sample.

The extent of lipid oxidation in raw meat was calculated by measuring 2-thiobarbituric acid reactive substance (TBARS) according to the method described by Buege and Aust (1978).

Meat fatty acids (FA) were measured according to O'Fallon et al. (2007) using a Gas Chromatography apparatus (GC, Hewlett Packard HP 6890). Samples of *longissimus dorsi* muscle were freeze dried and milled through a 1 mm screen. Approximately 0.5 g of freeze-dried meat were weighed in Kimax test tubes and mixed with 1 ml of internal standard (4 mg of C13: 0 ml^{-1} of methanol), 0.7 ml KOH (10N), and 5.3 ml of methanol. The tubes were incubated in a 55 °C water bath for 90 min with manual shaking every 20 min for 5 seconds. The tubes were cooled at room temperature and 0.58 ml of H_2SO_4 (24N) was added and incubated in a 55 °C water bath for another 90 min with manual shaking every 20 min for 5 seconds to facilitate the formation of methyl esters of the liberated free fatty acids. Hexane (3ml) was added to the tubes and samples were vortexed after being cooled to room temperature, samples were centrifuged at 2500 rpm for 10 min and the solvent layer (containing the fatty acids methyl esters) was transferred to a GC vial using a glass pipette. Fatty acid methyl ester (1 µl) was used in split mode at a ratio of 100:1. The identification of FA was measured by comparing the retention time of FA methyl ester to known standards.

Statistical analyses

All measured parameters were analysed using an ANOVA procedure of GenStat (GenStat version 15, VSN International Ltd, UK). Chestnut HT supplemented at ensiling (GET) was compared to the mean of the other treatments' factorial 2 x 2 (tannin supplemented prior feeding × inoculum). Lamb average daily liveweight gain (ADG) was calculated using linear regression in Microsoft Excel. Feed efficiency was measured by dividing the total DMI by total gain (kg kg^{-1}).

Results

Effect of supplemented tannin lamb performance

Supplemented tannin (30g kg^{-1} DM) at ensiling (GET) had no effect ($p > 0.05$) on lamb performance including lamb growth, forage DMI, total DMI, diet DM digestibility, ADG, or feed efficiency compared to the other treatments (Table 2). The lambs' liveweight gain during the experiential period is shown in Figure 1.

Table 2. Effects of supplemented chestnut HT to ryegrass silage either at ensiling or at feeding on lambs dry matter intake, digestibility, total gain and feed efficiency

	Treatments					SED			Probability		
	GET	G	GT	GI	GI+T	GET	GT	GI	GET	GT	GI
F-DDMI g d^{-1}	699	701	684	713	699	19.8	21.7	21.7	0.93	0.38	0.43
T-DDMI g d^{-1}	914	916	899	928	914	19.8	21.7	21.7	0.93	0.38	0.43
E-DDMI g d^{-1} BW$^{0.75}$	50.3	51.4	49.8	50.4	51.4	1.25	1.37	1.37	0.72	0.26	0.81
F-MP g d^{-1}	72	70	70	71	72	1.8	2.0	2.0	0.81	0.16	0.27
T-MP g d^{-1}	96	94	93	94	94	1.8	2.0	2.0	0.81	0.16	0.27
DM Digestibility kg kg^{-1}	0.83	0.79	0.81	0.83	0.81	0.016	0.018	0.018	0.26	0.86	0.18
Initial weight kg	29.4	29.1	29.4	29.3	29.3	0.61	0.67	0.67	0.78	0.69	0.96
Final weight kg	37.5	36.7	36.9	37.9	37.9	1.09	1.19	1.19	0.89	0.92	0.24
ADG g d^{-1}	197	204	181	212	210	17.0	18.6	18.6	0.71	0.43	0.20
Total gain kg	8.1	7.6	7.5	8.7	8.6	0.78	0.86	0.86	0.96	0.86	0.10
FE kg DM kg^{-1} gain	4.6	6.3	5.2	4.5	4.7	0.99	1.09	1.09	0.58	0.60	0.21

HT = hydrolysable tannin; GET = Ryegrass silage treated with chestnut HT at ensiling; G = ryegrass silages, GT = ryegrass silage treated with chestnut HT at feeding; GI = ryegrass treated inoculated; GI+T = ryegrass silage with inoculate and chestnut HT at feeding; F-DDMI = daily forage dry matter intake; T-DDMI = total daily dry matter intake; E-DDI = total dry matter intake based on empty body weight; F-MP = forage metabolisable protein; T-MP ; total metabolisable protein; DM = dry matter; ADG = average daily gain; FE = feed efficiency
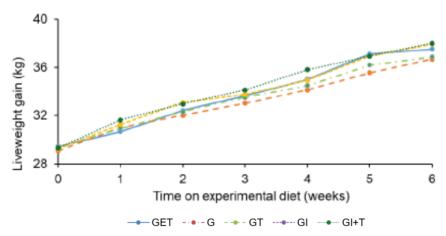

Fig. 1. Effects of supplemented chestnut HT either at ensiling or at feeding on lamb liveweight gain (kg), GET: ryegrass silage treated with chestnut HT at ensiling, G: ryegrass silages, GT: ryegrass silage treated with chestnut HT at feeding, GI: ryegrass treated with inoculated, GI+T: ryegrass silage treated with inoculate and chestnut HT at feeding. (SED, tannin at ensiling =1.06, tannin at feeding =0.98, inoculate =0.98).

Metabolic profile

Tannin supplementation either at ensiling or at feeding was found to increase blood plasma total protein during the whole experimental period (65, 56, 65, 59, or 57 for GET, G, GT, GI, or GI+T respectively SED = 5.57). The effect of supplemented tannin for increasing total protein of blood plasma was noticed at week four (67, 52, 65, 57, or 54 for GET, G, GT, GI, or GI+T respectively SED = 2.91) and week six (77, 61, 76, 66 or 62 for GET, G, GT, GI or GI+T respectively SED = 7.97) of the experimental period (Fig. 2). Feeding lamb's ryegrass silage treated with tannin either at ensiling or at feeding or inoculate did not have any significant ($p > 0.05$) effect on blood plasma urea concentration throughout the experiment (6.8, 6.9, 6.4, 6.7 or 6.6 for GET, G, GT, GI or GI+T, respectively SED = 0.73).
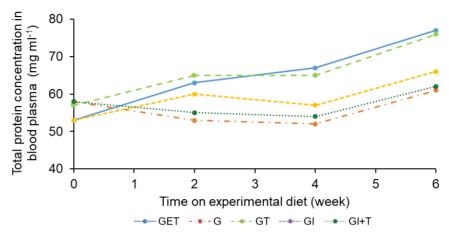
Fig. 2. Effects of supplemented chestnut HT either at ensiling or at feeding on blood plasma total protein concentration. GET: ryegrass silage treated with chestnut HT at ensiling, G: ryegrass silages, GT: ryegrass silage treated with chestnut HT at feeding, GI: ryegrass treated with inoculated, GI+T: ryegrass silage treated with inoculate and chestnut HT at feeding. (SED, tannin at ensiling =4.60, tannin at feeding =2.53, inoculate =2.53)

However, at week six a significant reduction in blood plasma urea concentration was found for lambs in the GT group compared to GET or G groups (6.4, 6.6, 5.6, 6.1, or 6.1 for GET, G, GT, GI, or GI+T, respectively, SED = 0.56) as shown in Figure 3. Tannin supplementation either at ensiling or at feeding had no effect ($p > 0.05$) on beta-hydroxybutyrate (BHB) or glucose concentration in blood samples.
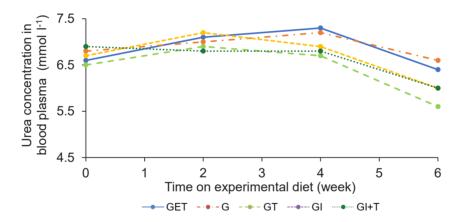

Fig. 3. Effects of supplemented chestnut HT either at ensiling or at feeding on blood plasma urea concentration, GET: ryegrass silage treated with chestnut HT at ensiling, G: ryegrass silages, GT: ryegrass silage treated with chestnut HT at feeding, GI: ryegrass treated with inoculated, GI+T: ryegrass silage treated with inoculate and chestnut HT at feeding. (SED, tannin at ensiling =0.58, tannin at feeding =0.41, inoculate =0.41)

Rumen fermentation

The results of the rumen fluid parameters are presented in Table 3. There was a tend ($p = 0.07$) for tannin supplementation at ensiling to reduce rumen pH compared to other treatments (6.6 and 6.7, 6.7, 6.7, and 6.8 for GET, G, GT, GI, and GI+T respectively), whereas supplemented tannin at feeding had no effect ($p > 0.05$) on rumen pH. Both GET and GI+T groups were found to reduce ($p = 0.03$) NH_3-N concentration compared to the other treatments (0.14, 0.19, 0.17, 0.17, and 0.14g l^{-1} for GET, G, GT, GI, and GI+T, respectively). In addition, treated ryegrass silage with tannin at feeding (GT) or inoculate GI was also found to reduce ($p = 0.03$) NH_3-N concentration compared to the negative control (0.17, 0.17, and 0.19 g l^{-1} for GT, GI, and G, respectively). The molar concentration or the proportion of the total or individual VFA of the rumen fluid was not affected by feeding lambs ryegrass silage treated with chestnut HT either at ensiling or at feeding (Table 3).

Table 3. Effects of supplemented chestnut HT to ryegrass silage either at ensiling or at feeding on lambs' rumen characteristics at slaughter

	Treatments					SED			Probability		
	GET	G	GT	GI	GI+T	GET	GT	GI	GET	GT	GI
pH	6.6	6.7	6.7	6.7	6.8	0.06	0.06	0.06	0.07	0.61	0.15
NH_3-N g l^{-1}	0.14	0.19	0.17	0.17	0.14	0.013	0.014	0.014	0.03	0.03	0.04
tVFA mmol l^{-1}	151.1	158.5	139.9	145.2	143.2	10.51	11.52	11.52	0.68	0.28	0.60
Acetic mmol l^{-1}	112.4	120.9	110.0	111.8	108.7	6.64	7.27	7.24	0.94	0.25	0.38
Butyric mmol l^{-1}	5.9	6.6	5.5	6.1	6.5	0.64	0.70	0.70	0.71	0.59	0.67
Propionic mmol l^{-1}	22.4	22.7	20.5	23.0	23.6	2.54	2.78	2.78	0.97	0.73	0.45
Isobutyric mmol l^{-1}	1.3	1.6	0.9	1.4	1.4	0.23	0.25	0.25	0.83	0.11	0.44
Valeric mmol l^{-1}	0.9	1.1	0.8	0.9	1.1	0.12	0.13	0.13	0.74	0.76	0.88
Isovaleric mmol l^{-1}	1.5	1.5	1.5	1.6	1.5	0.23	0.26	0.26	0.84	0.81	0.95

HT = hydrolysable tannin; GET = ryegrass silage treated with chestnut HT at ensiling; G = ryegrass silages; GT = ryegrass silage treated with chestnut HT at feeding; GI = ryegrass treated inoculated; GI+T = ryegrass silage with inoculate and chestnut HT at feeding; NH_3 = ammonia nitrogen; tVFA = total volatile fatty acid

Carcass parameters and meat quality

Table 4 shows that treating ryegrass silage with tannin or inoculate had no effect ($p > 0.05$) on all studied slaughter parameters. However, there was a tend ($p = 0.1$) for animal fed GET to have an increase in barrel width compared to other treatments (22.1, 21.9, 21.3, 21.5, or 21.6 for GET, G, GT, GI, or GI+T, respectively). Back fat thickness tended ($p = 0.07$) to be lower for lambs fed ryegrass treated with chestnut HT at feeding (10.0, 10.1, 9.8, 10.0, and 9.8 mm for GET, G, GT, GI, and GI+T respectively). Feeding lambs GET tended to reduce ($p = 0.06$) meat lightness (*L**) compared to the other treatments (44.0, 45.1, 45.3, 45.7, and 45.8 for GET, G, GT, GI, and GI+T respectively). While no significant ($p > 0.05$) difference was found in other meat colour parameters or meat rancidity (TBARS) (Table 4). Meat fatty acid profile did not change when lambs were fed ryegrass silages treated with tannin either at ensiling or at feeding compared to both control groups. Palmitic acid (C16:0) and Docosapentae-noic acid (C22:5) percentages were found to increase ($p = 0.07$ and 0.09 respectively) when tannin was added to grass silages at feeding (Table 5).

Table 4. Effects of supplemented chestnut HT to ryegrass silage either at ensiling or at feeding on slaughter trait, carcass characteristics, meat rancidity, and meat colour

	Treatments					SED			Probability		
	GET	G	GT	GI	GI+T	GET	GT	GI	GET	GT	GI
HCW kg	16.1	15.7	15.4	16.1	16	0.48	0.52	0.52	0.55	0.66	0.24
Dressing %	42.6	42.8	41.7	42.3	42.3	0.76	0.83	0.83	0.46	0.41	0.93
CCW kg	15.6	15.1	14.9	15.5	15.6	0.47	0.51	0.51	0.48	0.89	0.19
BFT cm	10.0	10.1	9.8	10.0	9.8	0.16	0.18	0.18	0.55	0.07	0.81
EMA cm^{-2}	16.7	15.9	13.7	15.4	15.4	1.40	1.54	1.54	0.26	0.37	0.64
BW cm	22.1	21.9	21.3	21.5	21.6	0.35	0.38	0.38	0.10	0.42	0.98
BL cm	54.0	54.1	54.8	54.4	54.4	0.57	0.62	0.62	0.47	0.54	0.90
BD cm	24.0	23.8	23.8	23.9	23.5	0.39	0.43	0.43	0.48	0.59	0.86
GW cm	21.8	21.6	21.5	21.3	21.4	0.39	0.43	0.43	0.43	0.98	0.47
Meat pH	5.60	5.66	5.64	5.65	5.62	0.027	0.030	0.030	0.14	0.26	0.57
L*	44.0	45.1	45.3	45.7	45.8	0.81	0.88	0.88	0.06	0.93	0.38
a*	14.1	14.4	15.0	13.8	14.1	0.57	0.63	0.63	0.74	0.38	0.17
b*	10.9	11.1	11.3	11.5	11.2	0.53	0.59	0.59	0.45	0.93	0.75
TBARS mgkg^{-1} meat FW	18.1	21.6	15.6	17.8	18.39	1.87	2.05	2.05	0.90	0.12	0.77

HT = hydrolysable tannin; GET = ryegrass silage treated with chestnut HT at ensiling; G = ryegrass silages; GT = ryegrass silage treated with chestnut HT at feeding; GI = ryegrass treated inoculated; GI+T = ryegrass silage with inoculate and chestnut HT at feeding; HCW = hot carcass weight; CCW = child carcass weight; BFT = back fat thickness; EMA = eye muscle area; BW = barrel width; BL = body length; BD = body depth; GW = gigot width; *L** = lightness; *a** = redness; *b** = yellowness; TABRS = lipid oxidation (2-thiobarbituric acid)

Table 5. Effects of supplemented chestnut HT to ryegrass silage either at ensiling or at feeding on fatty acids percentage of lamb meat

		Treatments					SED			Probability		
		GET	G	GT	GI	GI+T	GET	GT	GI	GET	GT	GI
Myristic	C14:0	2.4	2.4	2.2	2.5	2.7	0.25	0.27	0.27	0.84	0.96	0.23
Palmitic	C16:0	21.6	21.1	20.6	19.8	21.4	0.86	0.94	0.94	0.30	0.45	0.72
Palmitoleic	C16:1	1.4	1.4	1.4	1.8	1.3	0.17	0.18	0.18	0.66	0.07	0.36
Heptadecanoic	C17:0	1.1	0.9	0.9	2.3	0.9	0.60	0.66	0.66	0.76	0.21	0.18
Heptadecaenoic	C17:1	1.3	1.5	1.7	2.6	1.6	0.57	0.63	0.63	0.37	0.41	0.39
Stearic	C18:0	17.4	16.4	17.2	25	16.8	3.21	3.52	3.52	0.65	0.21	0.16
Oleic	C18:1	40.5	40.3	39.6	38.5	38.6	1.52	1.66	1.66	0.40	0.86	0.31
Linoleic	C18:2	5.7	6.5	6.6	6.7	6.3	0.66	0.72	0.72	0.33	0.80	0.91
Linolenic	C18:3	1.0	1.0	1.1	1.8	1.1	0.40	0.44	0.44	0.54	0.41	0.22
Archidic	C20:0	0.4	0.4	0.5	1.0	0.4	0.24	0.26	0.26	0.38	0.31	0.21
Arachidonic	C20:4	2.9	3.2	3.3	2.6	3.4	0.39	0.43	0.43	0.51	0.29	0.50
Eicospenataenoic	C20:5	0.6	0.7	0.7	0.6	0.8	0.13	0.14	0.14	0.21	0.38	0.82
Docosapentaenoic	C22:5	1.1	1.3	1.3	1.0	1.4	0.15	0.17	0.17	0.24	0.09	0.54

HT = hydrolysable tannin; GET: Ryegrass silage treated with chestnut HT at ensiling; G =ryegrass silages; GT = ryegrass silage treated with chestnut HT at feeding; GI = ryegrass treated inoculated; GI+T = ryegrass silage with inoculate and chestnut HT at feeding

Discussion
Animal Performance

Mean ryegrass silage intake in the current study was 50.2 g DM kg^{-1} BW$^{0.75}$d^{-1} which was higher than reported by Fitzgerald (1996), Speijers et al. (2005) and Kruege et al. (2010) who observed an intake of 26.2, 43.4 and 26.5 g DM kg^{-1} BW$^{0.75}$d^{-1}, respectively.

The high forage DMI could be due to the fact that lambs in the current study were fed 215 g d^{-1}head^{-1} of concentrated diet only during the experimental period, which was a relatively low amount for lambs with a live body weight of 29–37 kg, hence the lambs need more energy to meet their requirements. In addition, lambs used in this study were lighter than those used in the other studies, thus, DMI was higher when expressed as units of DMI to empty body weight (BW$^{0.75}$). In the current study, the average daily forage DMI were 699, 701, 684, 713, or 699 for GET, G, GT, GI, or GI+T groups respectively, which give an indication that the lambs fed grass silage treated with 30 g kg^{-1} DM chestnut HT either at ensiling or at feeding consumed approximately 21 g d^{-1} head^{-1} of chestnut HT which was equivalent to approximately 0.6 g kg $^{-1}$ liveweight. Although lambs ate a relatively high level of chestnut HT, there was no significant influence on forage DMI. Similar results were observed by De Oliveira et al. (2007) and Krueger et al. (2010) who found that feeding diets rich in tannins did not reduce forage palatability. Similarly, Toral et al. (2011) also found that supplementing 10 g of a mix of chestnut HT and quebracho CT kg^{-1} DM to lactating ewes' diet had no effect on DMI and animal performance. In contrast, Hervas et al. (2003) found that feed intake was completely stopped after 5–6 days when 3 g of quebracho CT kg^{-1} body weight (which is equivalent to 166 g kg^{-1} DM) was directly infused into the rumen via rumen cannula in four mature sheep. Hervas et al. (2003) suggested that high tannin levels could have a negative impact on the physiological status of the animal and can be harmful or toxic resulting in kidney and liver lesions and even animal death. Dschaak et al. (2011) reported that supplementing 30 g quebracho CT kg^{-1}DM to dairy cows' diet reduced DM, OM, CP, and NDF intake, with no effect on DM, OM, CP, and NDF digestibility. In contrast, an increase in DMI was noticed in goats (Puchala et al. 2005) and dairy cows (Sinclair et al. 2009) when fed forages rich in tannins.

The variation in the effect of tannin on DMI between different studies may perhaps be due to the variation between tannin sources, types, and levels that have been used. Makkar (2003) suggested the source and type of tannins would have more impact than tannin levels on DMI. Krueger et al. (2010) reported that some herbivorous mammals such as goats and deer produced special types of protein in their saliva called proline rich salivary protein which is highly reactive with dietary tannin to reduce tannin's effect.

Mueller-Harvey (2006) and Piluzza et al. (2014) suggested that the reaction between proline-rich salivary protein and tannin would be responsible for the astringent taste in the animal's mouth, thus the DMI could be reduced. Makkar (2003) explained that there are no such proteins (proline) in cattle and sheep saliva, hence no reaction

or astringent test would occur in cattle and sheep. Priolo et al. (2000) showed that tannin from different sources could be more astringent. For example, CT in croup pulp seems to provide a more astringent taste in the animal's mouth than other tannins, thus feeding ruminants small amounts of this type of tannin (>25 g kg^{-1} DM) would have a great reduction in DMI (approximately 37 %) (Priolo et al. 2000), whereas, supplementing 55.1 g chestnut HT kg^{-1} DM to lucerne silage did not affect DMI (Deaville et al. 2010). In the current study, the diet DM digestibility was measured using AIA with an average of 0.81 kg kg^{-1} with no effect ($p > 0.05$) of additional tannin (Table 2). These results agree with the results reported by Hart et al. (2012) who found that different tannin levels did not have a significant impact on DM digestibility when they fed lambs whole crop pea silage containing different levels of CT.

Deaville et al. (2010) also observed that there was no significant effect of supplementing 55.1 g of chestnut HT kg^{-1} DM to grass silage on DM and OM digestibility while an additional 55.6 g of mimosa CT kg^{-1} DM was found to reduce ($p < 0.01$) DM and OM digestibility. Makkar (2003) suggested that not all tannin protein complexes could dissociate in the abomasum, or complexes could be reversible in the small intestine depending on the tannin source, type, and concentration. Mueller-Harvey (2006) and Piluzza et al. (2014) reported that the fate of tannins in the digestive tract is still unclear especially post ruminally and it is uncertain whether tannin binds with endogenous protein or it rebinds with feed protein again. It was suggested that HT could be hydrolysed in acidic conditions (<4) while CT can resist acid hydrolysis. As a result, HT may be hydrolysed in the abomasum and not show a negative influence on digestibility (Smeriglio et al. 2017).

The average daily gain and feed efficiency of the lambs used in the current study was approximately 200.8 g d^{-1} and 5.06 kg kg^{-1}, respectively, with no significant difference between treatments, which could be due to that the lambs were fed the experimental treatments for a relatively short time (6 weeks). In addition, in the current study chestnut HT was used, and chestnut HT is characterised as being a less aggressive tannin compared to some other types such as quebracho or mimosa tannin (Deaville et al. 2010, Pellikaan et al. 2011). Makkar (2003) reported that the availability of tannin (low levels) could depend on type and tannin sources, which could modulate rumen fermentation and increase microbial protein synthesis as well as reduce protein degradability and increase amino acids supply to the lower gut. Increasing those two sources of amino acids supply to the small intestine could lead to an increase in animal performance in the form of producing higher milk, meat and wool, reducing CH_4, CO_2, and NH_3 emissions plus nitrogen excretion to the soil via urine, thus reducing the environmental pollution. The initial liveweight was approximate 29 kg and the final liveweight was approximately 37.5 kg, with an average daily liveweight gain of approximately 0.2 kg, thus the MP requirements for both castrated and ewe lambs in the current study were approximate 95 and 88 g d^{-1} respectively. Metabolizable protein supplied from dietary treatments used in the current study was approximately 97 g d^{-1} which covered the MP requirements requirements for castrated and ewe lambs, with no differences between experimental treatments, which may be one of the reasons that no differences in lamb's liveweight gain or performance were noticed.

Metabolic profile

The mean blood plasma analysis results for BHB, glucose, urea and total protein were 0.59, 4.09, 6.59 mmol l^{-1}, and 59.75 mg ml^{-1} respectively. The results showed that blood parameters were within the normal physiological condition of the lambs 2–3 h post morning feed as reported by Hamo (2019) and Seixas et al. (2021). Additional tannin at ensiling or at feeding was found to reduce plasma urea concentration by approximately 5 and 14 % respectively, compared with the positive control (GI) or negative control (G). Lewis (1957) reported that plasma urea N is highly correlated with rumen ammonia concentration, and as mentioned previously, in the current study tannin supplementation reduced ammonia concentration in the rumen. Therefore, these results concur with the reduced rumen NH_3 concentration observed when tannin was fed. The results showed that supplemental tannin was found to increase total protein in blood plasma in the last week of the experiment, which could indicate that tannin improved protein utilization in the animal body, but the experiment needed to be longer to establish a long-term trend. In contrast, additional tannin at either ensiling or at feeding did not affect BHB or glucose concentration in the blood. These results coincide with no effect of tannin on rumen VFA (Table 3).

Rumen fermentation

Tannin supplementation both at ensiling and at feeding was found to reduce ($p < 0.05$) NH_3-N concentration of rumen fluid with no significant ($p > 0.05$) effect on pH, total and individual VFA. Makkar (2003) reported that complexing tannin with protein could reduce protein hydrolysis in the rumen and hence reduce NH_3-N production during rumen fermentation. Similar results were reported by Min et al. (2003) who found that feeding sheep *Lotus corniculatus* forage containing 32 g kg^{-1} DM CT reduced rumen NH_3-N and soluble N concentration. Pellikaan et al.

(2011) noticed that mixing 100 g kg^{-1} DM of three types of CT (grape seed, quebracho, or green tea) or four types of HT (chestnut, myrobalan, tara, or valonia) with lucerne hay reduced the total VFA ($p = 0.007$), pH ($p < 0.001$) and NH$_3$ ($p < 0.001$). Krueger et al. (2010) observed that an additional 14.9 g of either chestnut HT or mimosa CT to concentrate diets fed to growing steers had no effect on rumen pH, NH$_3$-N concentration, total VFA nor the molar proportion of acetate and propionate acids. Hervas et al. (2003) also did not find any significant differences in rumen pH or NH$_3$-N concentration for cannulated sheep when they infused three levels of quebracho CT (0, 28, and 83 g kg^{-1} DM) directly into the rumen. Therefore, the effect of tannin on the rumen fermentation parameters seems to be related not only to the type but also to the source of the tannin used.

Meat colour and rancidity

In the current study, three groups of lambs were fed ryegrass silage treated with tannin either at ensiling or prior to feeding, however, tannin supplementation (both methods of inclusion) did not have any significant effect on meat colour or lipid oxidation. These results agreed with those reported by Luciano et al. (2009), who fed a group of 7 lambs' a concentrated diet supplemented with 40.3 g kg^{-1} DM quebracho CT and found that tannin did not affect fresh meat colour. However, Luciano et al. (2009) found that after the meat was stored for 14 days at a chilled temperature (4 °C), the meat colour and lipid oxidation was less in the lambs' group that were fed tannins (the meat kept its original colour and lipid oxidation) compared to the meat from lambs fed the control diet. Therefore, tannin can behave as an antioxidant compound during the storage period. However, in the current study, the effect of the storage period on meat colour and lipid oxidation has not been studied.

Conclusion

 The experimental treatments did not affect voluntary feed intake or lamb performance, which may have been related to a short experimental period (6 weeks). Also, the MP supply was sufficient for the requirements of growing lambs, with no differences in MP supply between all experimental treatments. Feeding ruminants with higher MP requirements (lactating ewes) forage silage treated with different levels of chestnut HT for longer periods (10–12 weeks) would be important, to understand whether tannin would have a negative effect on DMI, increased MP supply to the small intestine and thus enhance animal performance.

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