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REGULAR ARTICLES

Tropical grass and legume pastures may alter lamb meat physical and chemical characteristics

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Abstract

The present study assessed the influence of the type of the tropical pastures on lamb body weight (BW) gain and meat quality. Fifty-four lambs were allocated to three grazing pastures: (1) AG — Aruana grass (*Panicum maximum* cv. IZ-5); (2) PP — pigeon pea legume (*Cajanus cajan* cv. Anão); and (3) CS — contiguous swards, half of the paddock with AG and half with PP. After 92 days of grazing, the lambs were slaughtered. Carcasses were evaluated and the longissimus muscle was collected to determine color, lipid profile, tocopherol concentrations, and lipid oxidation. Although the pastures present differences in the characteristics of nutritional quality, the animals did not show difference in BW gain. The results show that all forage presented similar concentration of alpha-tocopherol (137 ± 14.37 mg kg^{-1} of fresh matter), whereas total and condensed tannin contents were greater in PP, intermediate in CS, and the lowest in AG treatment ($P = 0.0001$). Meat α-tocopherol content was similar among treatments ($P = 0.1392$), with an average concentration close to the optimal level to reduce the meat oxidation. Meat from AG treatment had 45 and 25% lower n-6/n-3 ratio than meat from PP and CS treatments, respectively. The legume increases the unsaturated fatty acids and the grass can reduce the n6/n3 ratio. The level of condensed tannin concentration did show to have important effect on meat characteristics. Both tropical pastures studied can provide a high amount of alpha-tocopherol, generating a great potential to increase the concentration of this antioxidant in lamb's meat.

Keywords Fatty acids · Grazing · Green panic · Pigeon pea · Tannin · Tocopherol

Introduction

Many tropical environments have great potential to produce meat from grasses due to the high forage growth, influenced by plant physiology and climatic variables as temperature and light. However, tropical grasslands are characterized by low nutritional quality to ruminants, presenting low levels of protein and soluble carbohydrate, and high cell wall and lignin contents (Van Soest, 1994). In this environment, tropical legume can be an important source of nutrients to these animals (Castro-Montoya and Dickhoefer, 2020). Although there are numerous studies concerning ruminant performance and meat quality in pastures (Ponnampalam et al., 2017; Ripoll et al., 2013; Luciano et al., 2009 and Realini et al., 2004), there is a lack of information on the effect of tropical grasses and legumes, and their antioxidant compounds, on the body weight gain and meat quality of lambs.

Sheep production is very important in tropical and subtropical countries, as Australia, Brazil, China, South Africa, Spain, and Uruguay (Poli et al., 2020). There is interest by

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the industry to reduce the oxidative processes in meat, which can be accomplished by feeding diets rich in antioxidants, as pasture (Wood et al., 2008; Realini et al., 2004). Previous experiments in our lab (Tontini et al., 2019) show that tropical grass and legume forages have considerable concentration of alpha-tocopherol, and tropical legumes can also have appreciable amount of condensed tannins (CT).

Tropical grass and legumes can modify the meat quality of grazing animals due to their biochemical characteristics, especially by their antioxidant compositions (Tontini et al., 2019; Jaturasitha et al., 2009). Understanding the effects of tropical pastures, including antioxidant compounds such as condensed tannins and tocopherol, may be the key for the production of high-quality animal protein in tropical environments. They can potentially alter the fatty acid (FA) profile of ruminant meat, affecting human heath (Dewhurst et al., 2003). In addition, antioxidant contents can maintain meat color and reduce the susceptibility of lipid and pigment oxidation (Liu et al., 2011), modifying the meat shelf-life (Ponnampalam et al., 2017; Luciano et al., 2009). These antioxidant compounds can stabilize free radicals action produced during lipid oxidation, which can inhibit the alteration of the myoglobin heme-group structure, causing meat color loss (Li and Liu, 2012). Descalzo and Sancho (2008), Realini et al. (2004), and Lobon et al. (2017) found that meat from grazing ruminants had higher tocopherol concentration, resulting in greater resistance to oxidative processes and improvement in color stability, than meat from animals finished with concentrate.

Therefore, we examined the effects of tropical pasture type (grass, legume, and contiguous areas of both species) on lamb performance, carcass traits, meat lipid profile, and meat physical and chemical characteristics.

Material and methods

The experimental protocol was approved by the Ethics Committee on the Use of Animals of the Universidad Federal do Rio Grande do Sul (CEUA UFRGS—Project N° 27830).

Experimental site and procedures

The study was conducted at the Agricultural Experiment Station (EEA) of the Universidade Federal do Rio Grande do Sul (UFRGS), Eldorado do Sul, located 46 m above sea level in southern region of Brazil (29°13′26″ S e 53°40′45″W). The experiment was carried out during 92 days from January 11th until April 12th, 2016.

Fifty-four weaned Corriedale x Texel male, castrated lambs with an average BW of 20.4 ± 3.97 kg (3 to 4 month of age) were allocated to three types of pastures: (1) AG — monoculture of Aruana grass (*Panicum maximum* cv. IZ-5); (2) PP — monoculture of pigeon pea (*Cajanus cajan* cv. anão); and (3) CS — contiguous swards with half the paddock with AG and half with PP. The experiment was set out in a randomized complete block design with three replicates (Fig. 1). Paddocks of 0.2 ha constituted the experimental units. Six lambs were allocated to each paddock, three were drenched daily with 60 g of polyethylene glycol (PEG), and three were drenched with water. All animals were dosed orally twice daily (at 9:00 and 16:00 h) to determine the effect of condensed tannin (CT) (Makkar et al., 1995).

Lambs were weighed every 21 days following a 12-h water and feed fasting. Lambs grazed the paddocks continuously for 92 days, and all treatments had a 10% leaf lamina minimum allowance (10 kg leaf lamina dry matter (DM) per 100 kg of BW/day) adjusted every 21 days using the "put-and-take" technique (Mott and Lucas, 1952). According to this technique, there were two groups of animals, "testers" that grazed continuously and showed the effect of the treatments, and "grazers" that were used only to regulate leaf allowance. Pasture height was obtained every 21 days in each paddock by averaging plant height at 50 random points, using a sward stick as in Bircham (1981). In order to estimate forage mass, six 0.25-m^2 quadrats were hand-clipped

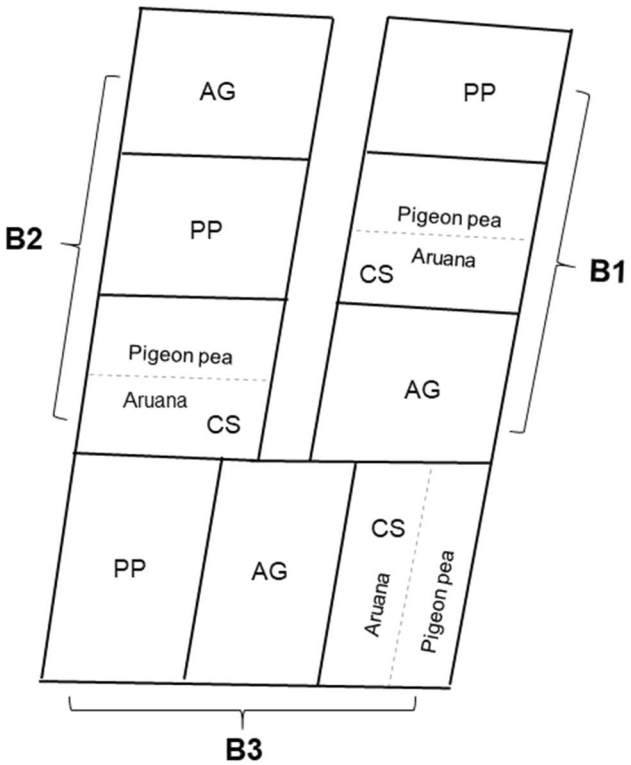

Fig. 1 Design of the experimental area with a randomized block design with three replications. Blocks: B1, B2, and B3. Treatments: AG, Aruana grass (*Panicum maximum* cv. IZ-5); PP, pigeon pea legume (*Cajanus cajan* cv. Anão); and CS, contiguous swards, half of the paddock with AG and half with PP

at ground level from each paddock: three representing the average pasture height and three random samples. Subsamples were collected to separate leaves from stems and sheaths. The rate of DM accumulation was obtained using three grazing exclusion cages per paddock allocated at an area representing the average pasture height, according to Klingman et al. (1943).

Intake and in situ *dry matter digestibility*

Total feeding intake was estimated every 21 days using Cr_2O_2. The tester lambs (54 lambs) were dosed at 9:00 h with 1.0 g of the Cr_2O_2 during 11 days. From the eighth day, fecal collection was performed according to the methodology described by Kozloski et al. (2006). The organic matter intake (OMI, % BW) was calculated by the formula: OMI = ((fecal production / (1-digestibility of the pasture))/(BW*100). Based on OMI value, alpha-tocopherol intake was calculated by (ATI) = ((OMI*100) / % DM)*(mg kg^{-1}, alpha-tocopherol in pastures).

The concentration of chromium in dry feces was determined by atomic absorption spectrophotometry (Kozloski et al., 1998). Fecal production (FP) was estimated according to Pond et al. (1989). In situ dry matter digestibility (ISDMD) was determined using a rumen fistulated bovine, according to Ørskov and McDonald (1979). The dried samples were weighed and incubated in polyester filter bags (porosity 41 μm) for 48 h.

Chemical composition of the pastures

To evaluate pasture nutritional value, samples were collected by the hand plucking technique described by Johnson (1978) at 21-day intervals. Samples were dried in a forced air oven at 55 °C until constant weight, and ground through a mill fitted with a 1-mm screen. Pasture chemical determinations included DM, ash, and crude protein (CP) content (AOAC, 1995); neutral detergent fiber (NDF) (Van Soest et al., 1991), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Goering and Van Soest, 1970); and ether extract (EE) (AOAC 1995; method number 920.39).

The forage α-tocopherol content in pasture was determined following the methodology of Val et al. (1994). Extraction was done with acetone and ascorbic acid, and the determination was performed using high-performance liquid chromatography (HPLC) (Waters Acquity UPLC CLASS), MeOH: H_2O 93:7 (v:v) as mobile phase and a NovaPak4 μm 30-cm column (Górnaś et al., 2014).

The tannin content was determined in freeze-dried pasture samples ground in a Wiley mill fitted with a 0.5-mm screen. The total tannin (TT), hydrolyzable (HT), and condensed (CT) contents were determined by adapting Grabber et al. (2013) and Makkar (2000) methods, and they were expressed as gram equivalent (eq-g) of leucocianidine kg^{-1} of DM. The following formula was used to determine the different tannin contents: eq-g leucocianidine (L)/kg DM = {absorbance * [10 * (dilution volume in ml) / (460 * sample weight)] / (DM, in kg)} * 10.

Carcass measurements and meat analysis

After 92 days of feeding regime, lambs were weighed and slaughtered in a commercial slaughterhouse, with average weight of 25.7 ± 4.36 kg. Lambs were subjected to 24-h fasting for feed and 12 h for water before slaughter. Lambs were electrically stunned and then exsanguinated and eviscerated. Immediately after, the carcasses were weighed to obtain hot carcass weight (HCW) and stored at 4 °C for 24 h and reweighed to obtain cold carcass weight (CCW). Carcass yield (CY) was estimated using the formula: CY = [(CCW/BW)*100].

Visual carcass conformation (CC) score, from 1 to 5 (very poor to excellent — according to the variation in the amount of muscle and fat in relation to the size of the skeleton — Osório et al., 1998), was attributed with a scale of 0.5, as well as, for the fatness degree (FD) (excessively lean to excessively fat — Osório et al., 1998). Subcutaneous fat thickness (SFT) was measured between the 12th and 13th ribs, using a caliper.

Subcutaneous fat color was assessed in the *longissimums* muscle, lumbar region, with a portable colorimeter Chroma Meter CR-400 (Minolta Camera Co., Ltda., Osaka, Japan), using illuminant D65, opening diameter of 50 to 53 mm, angle of observation of 10° and the CIE L*, a*, and b* color scale. Lightness (L*), redness (a*), and yellowness (b*) were recorded. Hue angle (h) was calculated as $h = \tan - 1\ (b^*/a^*) \times 57.29$, and chroma (C*) was calculated as $C^* = (a^*2 + B^*2)\ 1/2$. The *longissimus* muscle pH was measured at 24-h post mortem (pH24h), in the space between the 4th and 5th lumbar vertebra, using a pHmeter *Lutron* PH-208, equipped with a cutting blade tip.

Carcasses were split along the dorsal line, and the *longissimus thoracis* and *lumborum* muscles (LTL) from both halves were collected. On the right side, LTL, from the 6th to 10th thoracic vertebrae, was sampled, vacuum-packed, and stored at − 20 °C until dry matter (DM) content and ether extract (EE) content analyses were performed according to Osório et al. (1998). The left LTL, from the 6th to 12th thoracic vertebra, was sliced into four 2.5-cm-thick steaks. Two slices were used to determine color and water retention capacity (WRC) respectively, according to Osório et al. (1998). The remaining two slices were randomly assigned to two storage periods for lipid oxidation analysis (TBARS), 1 and 6 days of storage. In the first day of storage, the meat pH was assessed in all samples. Half of the samples were placed on trays, wrapped in oxygen-permeable polyvinyl chloride

film, and kept in the dark at 4 °C for 6 days. After their respective storage period, all samples were wrapped in aluminum foil and plastic, and stored at − 80 °C until TBARS analysis. Lipid oxidation was estimated using thiobarbituric acid reactive substances (TBARS) by the extraction method described by Pikul et al. (1989). Results were expressed as thiobarbituric acid reactive substances in mg malondialdehyde (MDA)/kg sample.

Shear force (SF) was evaluated by the maximum shear force in kgf/cm^2 in a Warner–Bratzler cell with a 1.016-mm blade coupled in a Texture Analyzer TA-500 (Lloyd Instruments), using NEXYGEN software. Maximum shear force was logged for each sub-sample in NEXYGEN software curve. The average of seven sub-samples of a steak collected from the carcass left side of the LTL lumbar region was calculated for the statistical analysis.

Two samples of 2.5-cm thickness were collected from LTL between the 12th and 13th thoracic vertebra of the carcass left side. These were vacuum packed and stored at − 80 °C in the dark for determination of tocopherol and FA profile. Tocopherol concentrations were determined using the method described in Bertolín et al. (2018). Intramuscular FA profile was analyzed according to Lee et al. (2012). Fatty acid methyl ester determination was performed using gas chromatography (SCION 436-GC; Bruker, Billerica, MA) equipped with a cyanopropyl capillary column (BR-2560, 100-m × 0.25 mm i.d. × 0.20-µm thickness; Bruker), flame ionization detector, and Compass CDS software (Bruker). Fatty acid identification was analyzed using the GLC-532, GLC-401, GLC-643, GLC-642, GLC-463, C18:1 t11, C19:0, C23:0 standard references (Nu-Chek-Prep Inc., Elysian, MN, USA) and the relative retention times according to what was observed in the bibliography (Alves et al., 2009, Lee et al., 2012; Yoshinaga, 2013; Bravo-Lamas et al., 2016). FA quantification was performed as described in UNE-EN 12,966–4 Official Method (2015). FAs were expressed as a percentage of the total amount of the identified FAs. Groups of FA based on the saturation level were estimated as saturated FAs (∑SFA), monounsaturated FAs (∑MUFA), polyunsaturated FAs (∑PUFA), PUFA C18:2 n-6, PUFA C18:3 n-3 and the ratio n-6/ n-3.

Statistical analyses

Each animal was used as experimental unit for the carcass and meat quality data, and the paddock was used as experimental unit for the pasture data. Data were analyzed using Mixed Procedure of SAS (version 9.4—SAS Institute Inc., Cary, NC, USA) with a significance level of 5%. Analysis of variance models included fixed effects for the type of pasture, block, PEG and the interaction type of pasture x PEG, and random effect for animal. The analysis revealed no significant effect of PEG on the type of pasture or in the interaction. Thus, the model was simplified as described by Pinheiro and Bates (2000) to exclude the PEG effect and interaction type of pasture x PEG. The final statistical model only considered the effects of type of pasture and, block as fixed effect, and animal as random effect.

After the normality test of the residues, covariance structures were tested to fit the models, based on Bayesian information criterion from which the structure component of variance was selected. When significance ($P \leq 0.05$) was indicated by analysis of variance, means separations were performed using the Tukey test at 5%. When $0.05 \leq P < 0.10$, the result was described and discussed as a considerable probability of the effect having occurred.

Results

Pasture characteristics and animal intake

There were no differences between treatments in relation to leaf lamina allowance (10 ± 1.33 kg DM. 100 kg^{-1} BW). The forages selected by the animals were similar in relation to ISDMD and EE contents of the different treatments ($P > 0.10$; Table 1). Herbage ash, NDF, and ADF contents were higher and OM and ADL contents were lower in AG than in PP ($P < 0.05$), whereas CS presented intermediate contents ($P > 0.10$; Table 1). The CP content showed a tendency ($P = 0.09$) to be higher in PP treatment than in AG, but similar to CS (Table 1).

There were no differences of alpha-tocopherol levels among pasture types. The mean pasture alpha-tocopherol content was 137 ± 14.37 mg^{-1} kg fresh matter (FM) (Table 1). Total tannin and CT levels were higher in PP, intermediated in CS, and lower in AG ($P = 0.0001$; Table 1). All treatments presented low HT content, ranging from 1.6 to 3.5 ± 0.37 eq-g of leucocianidine kg^{-1} of DM (Table 1). Pasture OMI and α-tocopherol intakes are shown in Table 1. Lambs grazing CS had 13% less OMI than those grazing the monoculture pastures ($P = 0.0167$). Lambs grazing AG and PP presented a 14% greater intake of alpha-tocopherol than the ones grazing CS ($P = 0.0144$).

Lamb performance, carcass, and meat quality characteristics

The different pasture types showed similar lamb average daily gain (57 ± 0.13 g/d; $P > 0.05$) and slaughter weight (25.7 ± 4.36 kg; $P > 0.05$). Most of the carcass physical characteristics also showed similar values between treatments (HCW, CCW, CY, FD, CC, and SFT; Table 2). Pasture type had no effect on subcutaneous fat and meat color (Table 2; $P > 0.05$); however, the type of pasture has a tendency ($P = 0.09$) to affect the redness characteristic (a*) (Table 2).

Table 1 Chemical composition of pasture and intake of lambs on different types of tropical pasture

Variables [1]	Type of pasture[2]			MSE[3]	Probability *P*-Pasture
	AG	PP	CS		
DM (%)	22.51	24.17	23.50	0.89	0.4878
OM (%)	88.35^{b}	90.51^{a}	88.92^{ab}	0.49	0.0433
Ash (%)	11.33^{a}	9.09^{b}	10.80^{ab}	0.38	0.0247
ISDMD (%)	58.24	58.85	53.76	2.60	0.4076
CP (%)	14.00^{b}	20.00^{a}	16.18^{ab}	1.47	0.0973
EE (%)	2.52	3.04	3.22	0.39	0.2900
NDF (%)	62.54^{a}	47.83^{b}	56.49^{ab}	2.27	0.0359
ADF (%)	31.55^{a}	27.70^{b}	29.99^{ab}	0.72	0.0482
ADL (%)	3.60^{b}	7.73^{a}	5.02^{ab}	0.54	0.0142
Alpha-Tocopherol	138.8	136.9	136.0	14.37	0.9819
TT	2.44^{c}	15.10^{a}	7.51^{b}	1.41	0.0001
HT	1.67	3.55	1.82	0.37	0.3054
CT	1.17^{c}	9.87^{a}	4.49^{b}	1.00	0.0001
OMI (% BW)	1.67^{a}	1.68^{a}	1.45^{b}	0.062	0.0167
ATI (mg kg^{-1} FM)	253.84^{a}	242.78^{a}	213.11^{b}	13.18	0.0144

[1]DM (%), dry matter content; OM (%), organic matter content; ISDMD (%), in situ dry matter digestibility; CP (%), crude protein; EE (%), ether extract; NDF (%), neutral detergent fiber; ADF (%), acid detergent fiber; ADL (%), acid detergent lignin; Alpha-tocopherol, mg kg^{-1} of fresh matter (FM); TT, total tannin (eq-g of leucocianidine kg^{-1} of DM); HT, hydrolyzed tannin (eq-g of leucocianidine kg^{-1} of DM); CT, condensed tannin (eq-g of leucocianidine kg^{-1} of DM); OMI (% BW), organic matter intake; ATI (mg kg^{-1} FM), alfa-tocopherol intake of fresh matter

[2]Type of pasture: AG, *Panicum maximum*; PP, *Cajanus cajan*; CS, contiguous swards of AG and PP

[3]*MSE*, mean square error

[a,b]Values within a row with different letters differ at $P \leq 0.05$ and tended to differ between $P > 0.05$ and $P \leq 0.10$

Meat from AG treatment had the lowest a* value. Meat pH 24 h, DM, EE, WRC, and SF were not affected by the pasture type (Table 2).

Tocopherol concentration and intramuscular fatty acid profile

Alpha-tocopherol concentration in meat was not affected by the pasture type (Table 3). Gamma-tocopherol tended ($P = 0.0598$) to be greater in AG treatment, intermediated in CS, and lower in PP. The total meat FA content (mg FAME/100 g meat) was not affected by the treatments ($P > 0.05$; Table 3), but some FA proportions (100*FAMEs/total FAMES) were affected by the pasture type. The meat from AG treatment had the highest concentration of C15:0, C16:1trans-9, C17:0, C18:0, C18:1*trans*-11, C18:2*trans*-9, *cis*-11, C24:0 and C24:1n9, and the meat from PP had the highest level of C18:2 n-6 (Table 3). In most comparisons between pastures, the meat from CS treatment showed intermediate concentrations (Table 3).

Pasture type affected ∑SFA ($P = 0.0243$), ∑ n-6 ($P = 0.0257$), and n-6/n-3 ratio ($P = 0.0021$). Meat from AG treatment contained greater SFA, lower ∑ n-6 and lower n-6/n-3 ratio than PP treatment, and meat from CS pasture had intermediate values (Table 3). There was a tendency ($P = 0.07$) of higher ΣPUFAs levels in the meat from lambs grazing PP and CS, than consuming AG.

Storage time study: pH and lipid oxidation

The type of pasture did not affect meat pH ($P = 0.8371$; pH averages of 5.37 ± 0.11 and 5.24 ± 0.15, respectively), regardless of the day. The meat oxidation was affected by the treatments only at day 6 ($P = 0.0460$). The TBARS results show at day 1 of storage mean values of 0.1, 0.12, and 0.09 ± 0.02 mg MDA kg^{-1} meat, for the treatments AG, PP, and CS, respectively ($P = 0.4742$). At day 6 of storage, lambs grazing AG produced meat with lower oxidation (0.30 ± 0.05 mg MDA kg^{-1} meat) than CS (0.70 ± 0.05 mg MDA kg^{-1} meat), while meat from PP treatment presented intermediate values (0.39 ± 0.05 mg MDA kg^{-1} meat).

Discussion

As mentioned by Van Soest (1994) about tropical pastures, the forage quality found in this study demonstrates that tropical legumes have higher protein and low cell wall contents, but it has higher concentration of lignin than tropical grasses. These characteristics led to a similar digestibility of the different treatments and a similar animal BW gain among the different types of tropical pastures.

Therefore, our study shows that if a producer's main objective is to increase meat production in an area, a tropical legume, as PP, does not bring greater benefits, in relation to a tropical grass, as AG. However, Hess et al. (2004) mention that the inclusion of legumes in the tropic regions might be an environmentally friendly way to improve animal productivity once the legume has the potential to incorporate nitrogen in the soil and decrease methane emission by the animals. In fact, there is a need for more research that compares tropical grass and legume pastures for sheep production, as recently discussed by Silva et al. (2020) and Poli et al. (2020).

On the other hand, the lack of differences in animal performance observed in this study makes the comparison of the FA profile among treatments more reliable, once the comparison was done among animals with similar growth rate and fat deposition (Cañeque et al., 2001). In addition, the control of the leaf allowance might have been important to generate a reliable comparison between treatments, since

Table 2 Tropical pasture type effect on carcass physicochemical characteristics and subcutaneous fat and meat color

Variables[1]	Type of pasture[2]			MSE[3]	Probability *P*-Pasture
	AG	PP	CS		
Carcass physic characteristics					
HCW (kg)	10.5	10.0	9.7	0.62	0.4251
CCW (kg)	10.2	9.8	9.5	0.61	0.4170
CY (%)	38.1	38.3	37.5	0.84	0.7764
FD	2.7	2.7	2.6	0.21	0.8587
CC	2.8	2.7	2.5	0.17	0.2339
SFT (mm)	2.3	2.0	1.6	0.37	0.3689
Subcutaneous fat color					
Luminosity	74.56	73.59	73.23	1.04	0.5822
Redness	6.71	8.16	6.92	0.61	0.3691
Yellowness	9.26	9.9	8.24	0.79	0.3363
Hue angle	55.28	51.29	48.9	2.78	0.3610
Chroma	11.69	12.98	10.94	0.85	0.3270
Meat color					
Luminosity	43.87	41.99	42.77	0.67	0.2626
Redness	23.67[b]	24.61[a]	24.04[ab]	0.24	0.0886
Yellowness	9.20	8.77	8.90	0.23	0.4720
Hue angle	21.23	19.59	19.98	0.43	0.1304
Chroma	25.41	26.14	25.99	0.27	0.2030
Longissimus muscle					
pH 24 h	5.66	5.87	5.85	0.09	0.3726
DM (%)	24.25	23.99	23.71	0.67	0.5013
EE (%)	2.10	1.94	1.80	0.24	0.5142
WRC (g kg^{-1})	625.9	633.9	646.6	7.50	0.2564
SF (kg $fcm^{2\ -1}$)	3.06	3.09	3.29	0.21	0.7502

[1]HCW, hot carcass weight; CCW, cold carcass weight; CY, carcass yield; FD, fatness degree; CC, carcass conformation; SFT, subcutaneous fat thickness; DM, dry matter content; EE, ethereal extract; WRC, meat water retention capacity; SF, shear force

[2]Type of pasture: AG, *Panicum maximum*; PP, *Cajanus cajan*; CS, contiguous swards of AG and PP

[3]MSE, mean square error

[a,b]Values within a row with different letters tended to differ between $P > 0.05$ and $P \leq 0.10$

the presence of stem not eaten by the animals could have misled the estimated forage available to the animals, as discussed by Muir et al. (1998).

Our study shows the high concentration of tocopherol in tropical forages (137 ± 14.37 mg kg^{-1} FM), and this finding represents the first report of its potential effect on lamb meat. Both tropical forages, grass and legume, present noticeable greater contents of tocopherol than it has been reported in temperate forages (Blanco et al., 2019; Ponnampalam et al., 2012; Tramontano et al., 1993; Booth, 1964). In fact, tropical pastures show to be important sources of vitamin E for grazing animals. Montossi and Sañudo (2007), comparing meat from Uruguay and from different European countries, observed a greater amount of tocopherol in Uruguayan's meat. This difference was reported as a result of the feeding system on pasture in Uruguay and indoor feed in European countries. The sheep in Uruguay are mainly produced under grazing native and improved grasslands, where an important part of botanic composition is C4 grasses. Our study confirms this result once the content of tocopherol in meat was similar to what was observed by Montossi and Sañudo (2007). The improvement of tropical pasture management for sheep has high potential to improve not only the amount of meat produced, but also its quality.

There were no differences of alpha-tocopherol content in meat between pasture treatments ($P > 0.05$). The average content was 4.18 mg kg^{-1} of fresh meat, which is close to the optimal level to reduce meat oxidation (5.3 mg kg^{-1} of muscle; López-Bote et al., 2001). This result shows that the level of tocopherol either in tropical grass or legume can provide enough tocopherol to act as antioxidant in the muscle. Although there was greater amount of gamma tocopherol in the meat from AG treatment, the concentration was small, and, according to Bieri and McKenna (1981), the

Table 3 Pasture type effect on lamb meat tocopherol content and fatty acid profile (% FAMEs/total FAMES)

Variables	Type of pasture[11]			MSE[12]	Probability *P*-Pasture
	AG	PP	CS		
α-Tocopherol[1]	3.95	4.62	3.98	0.23	0.1392
γ-Tocopherol[1]	0.16[a]	0.13[b]	0.15[a]	0.006	0.0598
FA mg/100 g [2]	156.59	152.94	152.37	6.02	0.8857
C11:0 [3]	0.40	0.25	0.43	0.03	0.1017
C12:0[3]	0.21	0.16	0.22	0.02	0.2975
C13:0 [3]	0.42	0.31	0.31	0.05	0.3908
C14:0 [3]	1.72	1.49	1.80	0.16	0.5118
C15:0[3]	0.48[a]	0.16[c]	0.28 [b]	0.02	0.0006
C16:0[3]	19.97	19.02	18.96	0.53	0.4024
C16:1*trans*-9[3]	0.45[a]	0.29[b]	0.44 [a]	0.01	0.0027
C16:1*cis*- 9[3]	1.22	0.98	1.10	0.06	0.1498
C17:0[3]	0.69[a]	0.51[b]	0.58[ab]	0.02	0.0216
C17:1*cis*- 9[3]	1.41	1.43	1.54	0.04	0.3169
C18:0 [3]	19.65[a]	16.77[b]	17.72[b]	0.38	0.0140
C18:1*trans*-11[3]	1.19 [a]	0.80 [b]	1.02 [ab]	0.06	0.0275
ΣC18:1[3]–[4]	26.64	24.72	24.04	1.12	0.3040
C18:2 n-6 [3]	5.41 [b]	8.03 [a]	6.83 [ab]	0.36	0.0154
C18:2*cis*-9,*trans*-11[3]	0.58	0.45	0.53	0.05	0.2605
C18:2*trans*-9,*cis*-11[3]	0.04 [a]	0.01 [b]	0.02 [b]	0.003	0.0155
C18:2*trans*-10,*cis*-12[3]	0.06	0.06	0.06	0.002	0.7424
C18:3 n-3[3]	2.50	2.25	2.46	0.12	0.3399
C20:0 [3]	0.14	0.11	0.13	0.01	0.1461
C20:3n9[3]	0.27	0.42	0.36	0.002	0.1010
C22:0[3]	0.04[b]	0.07[a]	0.06[a]	0.006	0.0566
C24:0[3]	0.04[a]	0.01[b]	0.01[b]	0.01	0.0301
C24:1n9[3]	0.05[a]	0.02[b]	0.03[b]	0.01	0.0114
ΣSFA[3]-[5]	52.45[a]	50.47[b]	51.64[ab]	0.27	0.0243
ΣMUFA[3]-[6]	30.35	28.12	27.81	1.13	0.2939
ΣPUFA[3]-[7]	16.20[b]	20.44[a]	19.51[a]	1.00	0.0732
ΣCLA[3]-[8]	0.68	0.52	0.64	0.04	0.1748
Σn-6 [3]–[9]	8.32[b]	12.03[a]	10.74[ab]	0.59	0.0257
Σn-3 [3]–[10]	6.92	7.46	7.77	0.47	0.4201
n-6/n-3 [3]	1.12[c]	1.62[a]	1.40[b]	0.04	0.0021

[1] mg kg^{-1} of fresh meat

[2]Fatty acid (mg/100 g)

[3] % FAMEs /FAMES total

[4](C18:1trans-11; C18:1cis-9; C18:1trans-15; C18:1cis-11; C18:1cis-12; C18:1cis-13, C18:1trans-16 and C18:1cis-15)

[5](C24:0; C22:0; C20:0; C18:0; i-C18:0; DMA-C18:0; C17:0; C16:0; a-C18:0; i-C16:0; DMA-C16:0; C15:0; a-C15:0; i-C15:0; C14:0; i-C14:0; C13:0; a-C13:0; C12:0; C11:0 e C10:0)

[6](C24:1n9; C22:1; C20:1n9; C18:1cis-15; C18:1trans-16; C18:1cis-13; C18:1cis-12; C18:1cis-11; C18:1trans-15; C18:1cis-9; C18:1trans-11; C17:1cis-9; C16:1cis-9; C16:1cis-7; C16:1cis-7; C16:1trans-9; C15:1; C14:1cis-9 e C12:1)

[7](C22:6 n3; C22:5n3; C22:4n6; C20:5n3; C22:3n3; C20:4n6; C20:3n6; C20:3n9; C20:2n6; C18:2rans-10, cis-12CLA; C18:2trans-9, cis-11CLA; C18:2cis-9,trans-11 CLA; C18:3 n3; C18:3 n6; C18:2 n6 e C18:2 n6 trans-9,12)

[8](C18:2cis,trans-11; C18:2trans-9,cis-11; C18:2trans-10,cis-12)

[9](C22:4 n6; C20:4n6; C20:3n6; C20:2n6; C18:3n6; C18:2n6; C18:2n6trans-9,12)

[10](C22:6n3; C22:5n3; C20:5n3; C22:3n3; C18:3n3)

[11]Type of pasture: AG, Panicum maximum; PP, Cajanus cajan; CS, contiguous swards of AG and PP

[12]MSE, mean square error

[a,b]Values within a row with different letters differ at $P \leq 0.05$ and tended to differ at $P > 0.05$ and $P \leq 0.10$

effect of gamma tocopherol on meat oxidation is relatively small, about 10% of the alfa-tocopherol.

The deposition of alpha-tocopherol in the meat of other species is also reported. Study carried out by Realini et al. (2004) using Hereford steers on temperate pasture found alpha-tocopherol concentration of 3.91 μg g^{-1}. Arnold et al. (1993) proposed 3.55 μg g^{-1} as the target concentration to have a significant impact on reducing pigment and lipid oxidation in beef. In addition to meat, alpha-tocopherol also plays an important role in the maintenance and oxidative stability of milk and dairy products. Martini et al. (2021) evaluated a herd of ewes from Sardinia with dairy aptitude on natural mountain pasture, and observed an alpha-tocopherol content of 3.24 mg 100 g^{-1} of fat in milk and 1.47 mg 100 g^{-1} of fat in cheese. This demonstrates the ability of alpha-tocopherol present in pasture to be deposited in the animal product or by-product, making it a food rich in bioactive compounds that are important for human health. However, the metabolization of tocopherol from the sheep diet might be affected by the breed type, as observed by Asadian et al. (1995), which warrant further investigation.

It is not possible to conclude from this study that tocopherol intake can affect carcass physical characteristics because there were no significant differences on tocopherol intake between treatments. However, the lack of tocopherol effect on carcass was described by other studies. Kasapidou et al. (2012) and Turner et al. (2002), working with increasing doses of tocopherol in the diet, did not find correlation between levels of tocopherol in the diet and carcass physical characteristics.

Although there is a potential effect of alfa-tocopherol on fat and meat color, as found by Ripoll et al. (2013) and Lobon et al. (2017), our study demonstrates that there are no differences in meat and fat color between tropical grass and legume pastures. In the same way, water retention capacity and tenderness (shear force) were also not affected by the pasture type. The only difference in meat characteristics between treatments was found in the meat from lambs that grazed PP. They tended to present higher a* value (redness). This result may be related to greater CT content in

PP. Tannins can alter ruminal microorganism dynamics, mainly those associated with the biosynthesis of vitamin B12, which is related to heme-pigment synthesis (Priolo and Vasta, 2007) that can alter the meat redness. However, further studies are needed to better isolate the effect of tannins on meat quality, once the difference found in this study was relatively small and we did not find effect of PEG that was daily dosed.

Type of pasture did not affect the amount of muscle and fat content but they changed the fatty acid profile. Lambs grazing a tropical legume (PP) had meat with less ∑SFA and greater C18:2 n-6, causing a higher ratio of n-6/n-3 and greater ΣPUFA content in relation to AG. This result can be a consequence of the plant FA composition. Makmur et al. (2019) analyzed the fatty acids of a tropical grass and different legumes and, although the concentration of the lipids in the pasture is relatively small, they found a greater amount of PUFA in tropical legumes. In the same way, different studies (Ponnampalam et al., 2017; Turner et al., 2014; Vasta et al., 2009; Lourenço et al., 2008) report the importance of the legume as a potential source to increase the unsaturated FA in the meat. The higher PUFA content demonstrates that tropical legumes can produce better meat quality than grasses for human health. This benefit can be obtained through not only with a monoculture of legume, but also when there are contiguous areas of legume and grass, agreeing with Howes et al. (2015) that explain that legumes can be used to mitigate the negative effect of FA saturation.

The higher PUFA in meat from animals that grazed PP than AG might also partially be explained by the fact that the legume has CT and it may prevent ruminal biohydrogenation (Vasta et al., 2009). The biohydrogenation in the rumen can be indicated by C18:1trans-11 content in lamb meat. C18:1trans-11 is an important biohydrogenation intermediate in the rumen (Lourenço et al., 2007), and the low concentrate of this FA might suggest that there is an inhibition of biohydrogenation when animals are fed with legumes.

In addition, our study shows greater control of lipid oxidation in the meat of the lambs that grazed tropical grass. Meat from AG lambs showed lower lipid oxidation at day 6 of storage, which may be directly related to the lower PUFA content. Wood et al., (2008), reviewing several studies, explain that PUFAs are more prone to be oxidized than SFA. In addition to the type of FA, they mention that tocopherol is essential to protect the PUFA from oxidation and the high levels in the pasture are very important. In the same way, Realini et al. (2004) and Vatansever et al. (2000), comparing animals fed either with concentrate or forage, emphasize that vitamin E present in the pasture plays an important role in protecting the meat against oxidation, mainly the meat from grazing ruminants that have high concentration of PUFAs.

The action of alpha-tocopherol goes beyond its antioxidant capacity in meat. Vitamin E is part of the antioxidant defense system in humans; according to EFSA (2017), the ideal daily consumption for men is 13 mg day^{-1} and for women is 11 mg day^{-1}. Studies show that diets based on foods rich in vitamin E can also help fight Alzheimer's disease (Grundman, 2000), in preventing photo oxidative damage (Stahl and Sies, 2003). In addition, vitamin E acts on human physiology, capturing and releasing energy (Paixão and Stamford, 2004), and reducing heart diseases. These results show the great beneficial and value-adding potential that the production of meat from ruminants on pasture can obtain and which is very little explored. The composition and fat profile of ruminant meat are also important characteristics that affect human health. From a nutritional point of view, the World Health Organization (WHO) indicates that the consumption of omega6/omega 3 should be at a ratio lower than 4 (four), and the value found in this study is lower than 2 (two). This low omega6/omega 3 ratio contributes to reducing the risk of coronary heart disease (Freitas et al., 2014).

We can conclude that tropical grass and legumes can potentially alter the fatty acid profile values. The legume increases the unsaturated FAs and the grass can reduce the n6/n3 ratio. The higher concentration of unsaturated FAs makes the meat more prone to oxidation. However, tropical grass and legume pastures can equally provide a notable amount of alpha-tocopherol to grazing animals that can help to protect the lamb's meat from oxidation.

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Author contribution VSH, CHECP, JFT, and TD conceived and designed research. VSH, JFT, and NMF conducted experiments. MJ, JRBP, REFM, ENN, AGFS, ER, and VM assisted in laboratory analysis. VSH analyzed data and wrote the manuscript. VSH, CHECP, JFT, TD, and MJ have worked on corrections to the manuscript. All authors read and approved the manuscript.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Human and animal rights The experimental protocol involving finishing and slaughtering lambs was approved by the Ethics Committee on the Use of Animals of the Universidade Federal do Rio Grande do Sul (CEUA-UFRGS), Project No: 27830.

Conflict of interest The authors declare no competing interests.

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