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Production and Quality of Meat From Lambs Fed Fresh Sulla Forage (*Sulla coronaria* (L.) Medik)

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Dipartimento Scienze Agrarie, Alimentari e Forestali (SAAF), Università degli Studi di Palermo (UNIPA), 90128 Palermo, Italy

* Correspondence: marialetizia.ponte@unipa.it

Simple Summary: Sulla is a tanniferous legume species recognized for its excellent agronomic traits and positive effects in the feeding of small ruminants. Due to its palatability and interesting content in protein, α-linolenic acid and condensed tannins (CT), fresh sulla forage (FSF) showed to improve the physiological status and productivity of lactating sheep and goats, as well as the technological, nutritional and health properties of their dairy products. However, the effects of FSF on meat production has been limitedly investigated, especially in terms of fatty acid (FA) profile of meat fat. In this investigation, FSF in the lambs' diet is compared with a tannin-free dry forage, as alfalfa hay, by evaluating lambs' intake, growth performance, carcass and meat characteristics. The higher FSF intake, balanced by a lower concentrate consumption, did not improve lambs' growth and carcass weight, whereas contributed to reduce the carcass and meat adiposity, and did not interfere with the sensory properties of meat. The increase in trans-vaccenic and rumenic acids, both beneficial for consumers' health, confirmed the role of CT in limiting the ruminal biohydrogenation of ingested unsaturated FA and improving the FA profile of meat fat.

Abstract: This study aimed to evaluate the effects of fresh sulla forage (FSF) in the diet on intake, growth, carcass traits, and meat quality of lambs. Twenty-four male lambs of Comisana breed (age 80±8 days; live weight 17.8±3.2 kg) were assigned to 2 groups receiving, until slaughter (age 128±8 days), FSF (crude protein (CP) 174 g/kg dry matter (DM), condensed tannins (CT) 15.6 g/kg DM) or alfalfa hay pellets (AHP, CP 181 g/kg DM), and a faba beans and barley mixture offered ad libitum. The greater forage intake of FSF lambs (451 vs 306 g DM/day; $p<0.0001$) corresponded to a lower incidence of concentrate in the diet (51.3 vs 64.2 % DM; $p<0.0001$) and in a not significant increase in final live weight (27.3 vs 26.0 kg), leading to an equal feed conversion ratio (4.90) between diets. The FSF diet tended to limit perirenal and pelvic fat (1.62 vs 2.04 %; $p=0.0673$) and reduce the incidence of adipose tissue in the hind leg (7.65 vs 9.13 %; $p=0.0120$). The meat from FSF lambs showed a more intense yellow colour (7.90 vs 6.38; $p=0.0273$) and a lower intramuscular fat content (47.0 vs 61.0 g/kg; $p=0.0186$). The diet influenced meat fatty acid (FA) profile, confirming the effect of CT from FSF in limiting the ruminal biohydrogenation of ingested unsaturated FA. Indeed, the FSF diet increased the beneficial rumenic acid (1.58 vs 1.02 % FA; $p=0.0197$) and trans-vaccenic acid (1.95 vs 0.69 % FA; $p<0.0001$). In triangle tests, no sensory difference related to diets was perceived in meat. The results highlight the effects of FSF in reducing carcass and meat fatness and improving the intramuscular FA profile.

Keywords: fresh sulla forage; condensed tannins; feed intake; lamb growth; lamb meat; meat fatty acid profile; meat sensory evaluation

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1. Introduction

In recent years, consumers have become increasingly health-conscious and are looking for food products that not only meet their hedonistic expectations related to taste but are also nutritionally balanced. It is well-established that various aspects of meat and milk quality, including colour, flavor, and fatty acid (FA) composition greatly depend on livestock animals diet [1–3]. Several scientific studies showed that lambs fed fresh forage provide meat with superior nutritional properties than lambs confined indoors and fed dry diets based on stored forages and concentrates [2,4–6]. In addition, livestock farming systems based mainly on grazing are shown, in most cases, to be economically more competitive than grain-based systems. Nevertheless, in grazing farming systems, the high protein to carbohydrate ratio of pasture forages in early phenological phases can limit microbial protein synthesis in the rumen, with consequent nitrogen losses [7]. Moreover, an intensive protein degradation in the rumen leads to the formation of volatile indolic compounds derived from tryptophan, particularly skatole (3-methylindole) that, accumulated in fat tissues, became responsible of an intense and undesirable "pastoral" flavor in meat, highly perceived by consumers [8–11].

Improvements in sensory quality of lamb meat exploiting fresh forage at pasture could be achieved with appropriate feeding strategies, able to ensure the production of meat with appreciable organoleptic traits and health properties. In this regard, tanniferous legume species have acquired interest in ruminants feeding and nutrition, due to their ability to improve sustainability, animal health and quality of dairy products and meat [11–15]. This growing interest in forage legumes is linked to their moderate content (<6% dry matter (DM)) in condensed tannins (CT), phenolic compounds also called proanthocyanidins, known for their beneficial properties. Among these latter, CT show the ability to reduce the degradation of dietary proteins, to which they bind, limit the methanogenesis in the rumen, thus improving the efficiency of diet utilization and decreasing nitrogen and methane emissions in the environment [14,16–18], and control the gastrointestinal nematode parasitism in sheep, associated to the reduction of nematode and fecal excretion of their eggs [13,19]. Moreover, tannins showed to reduce skatole concentration in the fat of lambs and attenuate the sheep meat odour, as observed by Priolo et al. (2009) [8] using quebracho tannins as dietary supplement. As other polyphenolic compounds, also CT represent natural molecules with antioxidant activity, being able to improve the health status of animals and the oxidative stability of their products [20]. The antioxidant protection of CT showed to be able to preserve meat from oxidation of lipid and myoglobin, this latter directly involved in meat colour stability during storage [21]. Furthermore, CT intervene in modulating rumen biohydrogenation of dietary polyunsaturated fatty acids (PUFA) that, transferred in the animal tissues, contribute to improving the nutritional and health properties of the derived products [14,15,22–24]. In this regard, it is well recognized that PUFA intake in the human diet helps to reduce cardiovascular diseases and, among PUFA, rumenic acid (RA, C18:2 c9t11), the main isomer of conjugated linoleic acids (CLA), plays a protective role against carcinogenesis [25,26].

Among the tanniferous legume species, sulla (*Sulla coronaria* (L.) Medik) is the most largely widespread in semi-arid environments of Mediterranean basin, where it is grown as a 2-year forage crop to be exploited for grazing and hay production [27]. Sulla phytochemicals include terpenes and, among phenolic compounds, flavonoids and especially CT, this latter present mainly in leaves and flowers [28]. In several investigations involving small ruminants, fresh sulla forage (FSF) was proven to be highly palatable, and positively affect dairy production, especially increasing milk yield and casein content [29–32], and enhancing FA profile of dairy products [33,34]. Moreover, a role of CT intake from FSF in improving the plasma oxidative status was found in dairy goats [35,36] and ewes [32].

Although the general effects of pasture feeding on meat production and quality are well-known, there are relatively few specific studies examining the impact of FSF on lamb meat quality. In lambs fed FSF, Burke et al., (2004) [37] observed a higher growth rate and a reduced rumen proteolysis than in those fed other fresh forage resources. When comparing diets based on FSF or annual rygrass, also Bonanno et al. (2011) [2] reported favourable effects of feeding FSF on lambs growth and carcass

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yield, attributed to greater intake and better efficiency of dietary protein utilization, whereas no effect emerged on physical, chemical and sensory traits of meat.

Priolo et al. (2005) [38] found similar growth performance between lambs fed with only FSF or oats hay and concentrate, whereas observed an improved FA profile in meat from FSF-fed lambs, which resulted lower in saturated fatty acids (SFA) and higher in α-linolenic acid (ALA, C18:3 n-3) and RA. Apart this last finding, the effects of FSF on FA profile of lamb meat still remains limitedly investigated, and need further insights by comparisons with other dry forage resources.

This study aimed to assess the impact of FSF containing CT in the diet of lambs from 80 to 128 days of age, evaluating their feed intake, growth rate, carcass traits and meat quality, with particular regard to its FA profile, in comparison with lambs fed a diet based on CT-free alfalfa hay pellets.

2. Materials and Methods

2.1. Lambs and Experimental Diets

This research was conducted in winter (January-February) in a farm located in the province of Agrigento (Sicily, Italy).

At weaning, 24 male lambs of Comisana breed aged 65±8 days (mean±sd), with an average body weight of 16.8±3.1 kg (mean±sd), were assigned to 2 homogeneous groups according to age and body weight; then each group was divided into 2 subgroups of 6 lambs, and each subgroup was housed in a wheat straw–bedded pen within a semi-open shelter.

During the experimental period, the 2 groups received *ad libitum* a different legume forage: fresh sulla forage to the FSF group and alfalfa hay pellets to the AHP group. Sulla forage (variety Sparacia) was harvested daily by hand from a second-year sward that had not been previously grazed, and distributed to lambs two times in a day. All lambs received *ad libitum* a concentrate mixture of faba bean (*Vicia faba* var. minor L.) and barley mixture (76:24 on as fed basis), in form of coarsely ground meal. Forages and concentrate were daily supplied in amounts to ensure that refusals were higher than 20%.

After a 15-day phase of lamb's adaptation to the housing conditions and the experimental diets, the experiment lasted 48 days, until slaughter occurred at a lamb's average age of 128±8 day.

The experiment protocol had the approval of the Animal Welfare Body of the University of Palermo (Prot. 203099-13/12/2023), who ruled as not applicable the requirements established by the Italian Legislative Decree n. 26/2014 on the protection of animals used for scientific purposes, implementing Directive 2010/63/EU.

2.2. Measurements and Analyses

Data recording and feeds sampling started after the 15-day adaptation phase. The offered and refused feeds were weighed daily and sampled weekly for each pen, to calculate weekly feed intake and estimate the related chemical composition of ingested FSF.

Samples of offered and refused feeds were analyzed following AOAC (2012) [39] procedures for DM (method 967.03), crude protein (CP, N×6.25) (method 2001.11), ether extract (method 920.39), and ash (method 942.05). Neutral detergent fiber (NDF) and acid detergent fiber (ADF), both treated with heat-stable amylase and inclusive of residual ash, and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991) [40]. Non-fiber carbohydrate (NFC) content was calculated by difference, using the formula NFC = 100 – (% CP + % ether extract + % ash + % NDF). The net energy for gain (Mcal/kg DM) of forages and concentrate was estimated according to INRA (2018) [41].

Extracts from lyophilized samples of FSF were prepared in duplicate, as described by Gannuscio et al. (2022) [32], and analyzed for CT using the butanol-HCl-Fe^{+3} assay and delphinidin as reference standard [42]; the absorbance of extracts was read at 550 nm in duplicate using a HACH DR3900 spectrophotometer (Hach, Loveland, CO, USA), and results were expressed as delphinidin equivalent (g DE/kg DM).

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The FA composition of samples (50 mg) of lyophilized offered FSF, alfalfa hay and concentrate components was determined using the one-step extraction and transesterification procedure reported by Sukhija and Palmquist (1988) [43]. Each FA was identified as described for meat FA analyses, whereas the total and individual FA quantification was obtained using C23:0 (Sigma-Aldrich, Milan, Italy) as internal standard at a concentration of 0.4 mg/g lyophilized sample, and expressed as g/kg DM.

Lambs' body weight was monitored weekly and recorded at start (day 1) and end (day 48) of experiment. Before slaughter, the lambs were fasted for 12 hours, then they were transported to the slaughterhouse and weighed. The gastrointestinal content was removed and weighed to calculate the empty body weight. The hot carcasses were weighed and maintained at ambient temperature (>10 °C) for 6 hours, then chilled to 4 °C and weighed again at 24 hours after slaughter. Perirenal and pelvic fat, hind leg, and *Longissimus dorsi* (LD) muscle were removed from the right half of the carcass and their weights were recorded. Hing leg was dissected to weigh its tissue components (lean meat, fat, and bone).

The pH of LD muscle was measured using a Hanna HI 9025 pH meter (Hanna Instruments Inc., MI, USA) equipped with a penetrating probe. The colour of perirenal fat and the LD section cut between the last thoracic and first lumbar vertebrae was assessed after 1 hour of blooming in duplicate by a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan) using the illuminant C; after standardization by a white plate, lightness (L*, range values from 0 = black to 100 = white), redness (a*, range values from red = +a* to green = -a*) and yellowness (b*, range values from yellow = +b* to blue = -b*) were measured according to the CIE L*a*b* system [44]. Chroma (colour intensity or saturation; 0 = gray) was calculated as $(a^{*2} + b^{*2})^{0.5}$, whereas hue angle (colour tone; 0° = purple red) was calculated as arctangent(b*/a*) [45].

Then the LD samples were vacuum-packed and frozen at -20°C for later analyses. For thawing, the LD meat was kept at 4 °C for 24 hours and weighed to determine the thawing loss. Then LD samples were wrapped in polyethylene bags, heated at 75 °C for 40 min in a water bath, and reweighed to calculate the cooking loss. To estimate tenderness, the Warner-Bratzler shear force (WBSF, kg/cm^2) was measured on four cylinders (12.7 mm) excised from each cooked sample of LD meat with an Instron 5564 tester (Instron, Trezzano sul Naviglio, Milano, Italy).

Moisture, fat, and ash content were determined in LD meat according to AOAC (2012) [39] methods; protein percentage was calculated by difference (100 – % water – % fat – % ash), in accordance with Bonanno et al. (2011) [2].

The sensory evaluation of LD meat cooked on an electric grill was performed by triangle discriminant tests [46], involving a panel of 16 untrained volunteers, selected for their habitual consumption of lamb meat, in two separate sessions. In each test, three meat portions of approximately 40 g, two of which belong to the same dietary treatment, were offered hot for tasting to each panelist, who was asked to identify the different sample and indicate the degree of difference perceived. Each panelist compared meat from lambs fed FSF and AHP in both triangular combinations (FSF/FSF/AHP and AHP/AHP/FSF).

2.3. Intramuscular Fatty acid Analysis

Extraction of lipids from 1 g of lyophilized LD meat and preparation of FA methyl esters (FAME) were performed following the method of O'Fallon et al. (2007) [47], as described by Di Grigoli et al. (2019) [48]. Briefly, 1 g of sample was hydrolyzed with KOH (0.7 mL) in methanol (5.3 mL), and methylated directly by H_2SO_4 catalysis. The FAME were recovered in 1.5 mL hexane, and 1 µL of each sample was injected using an auto-sampler into an HP 6890 GC system equipped with a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). Separation of FAME was achieved using a CP-Sil 88 capillary column (100 m long, 0.25 mm internal diameter, 0.25 µm film thickness; Chrompack, Middelburg, The Netherlands). Gas chromatography conditions and identification were as follows: the initial oven temperature was set at 150 °C for 5 min, then increased at a rate of 1 °C/min to 160 °C held for 11 min, and finally increased at a rate of 7 °C/min to a final temperature of 230 °C,

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maintained for 9 min; helium was used as the carrier gas at a flow rate of 1.5 mL/min; the injector temperature was maintained at 200 °C, while the detector temperature was set at 250°C.

Each FA was identified by comparing its retention times to those of a FAME hexane mix solution C4-C24 (Supelco, Bellafonte, PA, USA). A commercial standard was used to identify the RA (Sigma-Aldrich, Milan, Italy). Individual FA were reported as g/100 g of the total identified FAME. The value of meat fat was calculated as health promoting index = (n-3 PUFA + n-6 PUFA + monounsaturated fatty acids (MUFA)) / (C12:0 + 4 × C14:0 + C16:0) [49].

2.4. Statistical Analysis

Data were statistically analyzed using the SAS 9.2 software [50]. The weekly data related to lambs' feed intake, with pen as experimental unit, were analysed according to a MIXED model including the fixed effect of diet (FSF and AHP) and the random effect of pen, this latter regarded as error term. The parameters referred to lambs' growth, carcass and meat traits, with the individual lamb as experimental unit, were analysed by the GLM (General Linear Model) procedure using a oneway model considering the effect of diet. The significance of the differences due to the diet in sensory triangle tests was evaluated using the reference tables from Amerine et al. (1965) [50].

3. Results and Discussion

3.1. Feeds Composition, Growth and Intake

Table 1 shows the chemical composition of dietary components offered to the two experimental groups of lambs, as well as that of ingested FSF. Compared to alfalfa hay, the offered FSF was characterized by a higher energy level, mainly due to the lower content of fibrous fractions, and showed a double concentration of ALA. A similar composition of the offered FSF was observed in spring by Bonanno et al. (2011) [2] when compared the growth performance of lambs fed with sulla or ryegrass green forage. The improvement of ingested FSF in comparison with offered FSF, especially with regard to CP and CT, both increased by 2 percentage points, is attributable to the lambs' preference for the more palatable parts of sulla plant, such as leaves and flowers; indeed these parts are more rich in protein and phenolic compounds than stems [28].

Table 1. Chemical composition and fatty acid profile of offered diet components and ingested fresh sulla forage (g/kg DM).

	Offered diet components					Ingested
	Faba bean	Barley	Concentrate[1]	Alfalfa hay pellets	Fresh sulla forage	fresh sulla forage
DM, %	868	906	877	910	148	145
Crude protein (CP)	246	105	212	181	174	194
Ether extract	15.5	21.1	16.9	27.4	28.9	32.3
Ash	34.0	28.8	32.7	96.4	122	124
NFC	560	671	587	230	273	275
NDF	144	174	151	465	402	374
ADF	111	86.2	105	375	350	328
ADL	7.10	11.9	8.26	82.5	77.2	75.5
Net energy for gain, Mcal/kg DM	2.03	2.02	2.03	1.04	1.37	1.43
Condensed tannins			-	-	15.6	17.7
C12:0	0.02	0.08	0.03	0.11	0.14	
C14:0	0.03	0.32	0.10	0.35	0.25	
C16:0	1.76	3.28	2.14	4.00	3.73	
C18:0	0.33	0.52	0.37	1.07	0.73	
C18:1 *c9*, OA	2.45	3.37	2.68	1.86	0.90	
C18:2 *c6c12* n-6, LA	7.97	8.74	8.16	5.62	2.56	

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C18:3 *c9c12c15* n-3, ALA	0.58	1.03	0.69	8.36	16.15

[1]Faba bean and barley mixture (0.76:0.24 on as fed basis). DM = dry matter. NFC = non-fiber carbohydrate = 100 – (% NDF + % CP + % ether extract + % ash) NDF = neutral detergent fiber, inclusive of residual ash. ADF = acid detergent fiber, inclusive of residual ash.

ADL = acid detergent lignin. OA = oleic acid. LA = linoleic acid. ALA = α-linolenic acid.

The effects of diet on lambs growth and feed intake are shown in Table 2. Although the lambs fed FSF showed a slightly higher growth rate and final body weight than AHP lambs, the differences did not reach significant levels, thus the growth performance have to be assumed comparable between lambs fed the different diets. The average weight gain of lambs fed FSF was lower by 30 g daily than that recorded by Bonanno et al. (2011) [2] for Comisana lambs received the same diets; the greater growth of those lambs could be attributed to the warmer season in which that experiment was carried out (spring vs winter), and to the older age of growing lambs (100-150 vs 80-128 days). Priolo et al. (2005) [38] observed a slower growth in lambs of the same breed and age fed an exclusively sulla-based diet.

On the contrary, significant differences between groups emerged for the lambs' DM intake (825 vs 924 g/day per head for AHP and FSF, respectively; p=0.0402), as well as for the daily intake of most nutrients and net energy, always higher for the FSF group. These results mainly depended on the higher forage intake of the more palatable FSF in comparison with alfalfa hay (451 vs 306 g/day per head; p<0.0001), as also emerged when Bonanno et al. (2011) [2] compared sulla and ryegrass, both as fresh forage. It has been noted that the higher FSF consumption was balanced by a tendency reduction in concentrate intake (p=0.0934), that led to a significant lower incidence of concentrate in the diet (p<0.0001) and equal feed conversion ratio between the groups (4.90), implying a profitable reduction of feeding cost linked to the concentrate integration.

Table 2. Effects of diet on lamb growth and daily feed intake.

	Diet		SEM	p-value
	Alfalfa hay pellets AHP	**Fresh sulla forage FSF**		
Initial body weight, kg	17.6	18.0	0.93	0.7556
Final body weight, kg	26.0	27.3	0.87	0.2867
Average weight gain 80-128 days, g/day	176	195	10.7	0.2103
Feed conversion ratio	4.90	4.90	0.29	0.9872
Daily concentrate intake, g DM/head	520	473	19.4	0.0934
Daily concentrate intake, % diet DM intake	64.2	51.3	1.02	<0.0001
Daily forage intake, g/head				
DM	306	451	17.2	<0.0001
Crude protein (CP)	55.4	86.6	3.14	<0.0001
Ether extract (EE)	8.38	14.4	0.47	<0.0001
Ash	29.5	56.0	2.00	<0.0001
NDF	142	172	8.17	0.0123
ADF	115	149	6.53	0.0003
ADL	25.2	33.9	1.44	<0.0001
NFC	70.4	122	3.88	<0.0001
Condensed tannins		8.05		
Net energy for gain, Mcal/head	0.32	0.64	0.021	<0.0001
Daily diet intake, g/head				
DM	825	924	33.5	0.0402
Crude protein (CP)	166	187	6.63	0.0252
Ether extract (EE)	17.2	22.4	0.74	<0.0001
Ash	46.5	71.4	2.44	<0.0001
NDF	221	243	10.4	0.1297
ADF	169	199	8.06	0.0098
ADL	29.5	37.8	1.56	0.0003

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NFC	376	400	14.1	0.2196
Net energy for gain, Mcal/head	1.37	1.60	0.055	0.0038

DM = dry matter. NDF = neutral detergent fiber, inclusive of residual ash. ADF = acid detergent fiber, inclusive of residual ash. ADL = acid detergent lignin. NFC = non-fiber carbohydrate = 100 – (% CP + % EE + % ash + % NDF). SEM = standard error of mean.

3.2. Carcass and Meat Traits

Table 3 shows the parameters recorded at slaughter. The effect of diet emerged at tendency level (p=0.0673) for the percentage of perirenal and pelvic fat, and especially for the incidence of fat tissue on hind leg, which were both lower in the carcasses of lambs fed FSF. The minor presence of adipose depots in the FSF carcasses can be attributed to the lower intake of starch with the concentrate, associated with the effect of CT in reducing starch degradability in the rumen, and thus the production of propionic acid involved in lipogenesis [52]. The lower fatness of carcass can be also related to the combined effects of the higher intake of protein occurred with FSF diet, and the protection of dietary protein exercised by CT that, improving the efficiency of protein utilization [14], could have promoted the muscle development rather than the fat deposition. Indeed CT, being able to bind dietary proteins, protect them from degradation in the rumen [16–18,53], favour their escape in the intestinal tract to be digested, thus increasing the amino acids availability for protein synthesis in muscle tissue and reducing the energy deposited as fat. However, no difference in fat level was observed by Priolo et al. (2005) [38] in carcasses of equal weight from lamb fed exclusive FSF or oat hay with concentrate, whereas Bonanno et al. (2011) [2] reported a higher fatness in the heavier carcasses obtained from lambs fed FSF than in lighter ones from lambs receiving fresh ryegrass.

Table 3. Effects of diet on carcass traits of lambs.

	Diet		SEM	p-value
	Alfalfa hay pellets AHP	**Fresh sulla forage FSF**		
Final body weight (BW) after 12 h-fasting, kg	24.4	25.4	0.84	0.4154
Empty body weight (EBW), kg	21.7	22.9	0.75	0.2890
Hot carcass, kg	11.5	12.0	0.45	0.4970
Carcass at 24 h, kg	11.1	11.5	0.45	0.4894
Hot carcass yield, % BW	47.1	46.9	0.49	0.7248
Hot carcass yield, % EBW	53.0	52.1	0.45	0.1684
Carcass yield at 24 h, % BW	45.4	45.3	0.50	0.8168
Carcass yield at 24 h, % EBW	51.1	50.3	0.48	0.2504
Half carcass (HC), kg	5.48	5.72	0.22	0.4678
Perirenal and pelvic fat, % HC	2.04	1.62	0.16	0.0673
Hind leg (HL), kg	1.44	1.43	0.048	0.9036
Meat, % HL	67.4	68.5	0.71	0.2640
Fat, % HL	9.13	7.65	0.38	0.0120
Bone, % HL	23.5	23.8	0.72	0.7491
HL meat/bone ratio	2.87	2.94	0.10	0.6501

SEM = standard error of mean.

Table 4 reports the physicochemical characteristics of *Longissimus dorsi* (LD) muscle as a function of the diet. Meat from lambs fed FSF showed higher values of yellowness (b*) and hue angle than that of the AHP group, indicating a more intense yellow tone, presumably due to the transfer of carotenoids pigments, present in higher amount in green forage than in hay [54]. Indeed, analogous increases of yellowness (b*) and hue angle were observed by Priolo et al. (2005) [38] comparing meat from lambs fed FSF or oat hay with concentrate, whereas no significant difference emerged between

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lambs fed sulla or ryegrass, both as fresh forage [2]. However, the diet did not affect the colour of perirenal fat. Moreover, intramuscular fat resulted significantly lower in FSF meat, in line with the reduced carcass adiposity. Therefore, feeding hay induced precocious fat infiltration, whereas feeding green forage containing CT delayed the maturation allowing to obtain more lean meat, in line with the consumers interest in healthier and environmentally friendly products obtained in extensive systems. However, the higher fatness of meat from lambs fed AHP did not correspond to a higher tenderness, since the WBSF was not influenced by the diet.

In triangle tests, panelists did not detect sensory differences between meat from the two dietary treatments at significant level, since the incidence of their correct answers in identifying the different meat sample was equal to 7/16, and the degree of perceived differences were indicated as weak. This result suggests that feeding FSF did not determine alteration of meat flavour attributable to skatole produced in the rumen and accumulated in the fat. However, Priolo et al. (2005) [38] found no effect of CT from FSF provided as exclusive diet in reducing skatole concentration in the perirenal fat of lambs, presumably linked to the higher intake of green forage occurred in that study.

Table 4. Effects of diet on physicochemical characteristics of lamb meat.

	Diet		SEM	p-value
	Alfalfa hay pellets	**Fresh sulla forage**		
	AHP	**FSF**		
***Longissimus dorsi* (LD) muscle**				
pH at 24 h	6.12	6.22	0.088	0.4019
Lightness, L*	46.2	46.5	0.77	0.7849
Redness, a*	18.9	19.9	0.60	0.2539
Yellowness, b*	6.38	7.90	0.46	0.0273
Chroma	20.0	21.5	0.70	0.1540
Hue angle	18.4	21.4	0.83	0.0175
Thawing loss, %	5.64	6.42	0.86	0.5274
Cooking loss, %	26.7	26.6	0.48	0.8256
Total loss, %	30.9	31.3	0.98	0.7861
WBSF after cooking, kg/cm^2	3.18	3.03	0.079	0.2111
Moisture, g/kg	739	746	4.23	0.2379
Protein, g/kg	189	196	2.42	0.0720
Fat, g/kg	61.0	47.0	5.92	0.0186
Ash, g/kg	10.5	10.8	0.15	0.2086
Perirenal fat				
Lightness, L*	79.2	78.2	0.49	0.1957
Redness, a*	8.39	8.22	0.31	0.7079
Yellowness, b*	8.49	9.34	0.41	0.1568
Chroma	11.9	12.5	0.44	0.3770
Hue angle	45.5	48.2	1.28	0.1406

WBSF = Warner-Bratzler shear force. SEM = standard error of mean.

3.3. Intramuscular Fatty Acid Composition

Table 5 shows the effect of diet on FA composition of LD muscle. Among the prevalent SFA, the FSF diet was responsible of the reduction of myristic acid (C14:0) and stearic acid (C18:0), whereas lauric acid (C12:0) and palmitic acid (C16:0), as well as the total SFA, were detected at levels comparable with those of AHP diet. As known, dietary guidelines recommend reducing the consumption of saturated fats to avoid the increase of serum cholesterol and, consequently, prevent the risk of cardiovascular diseases [26]. In this regard, the hypercholesterolemic effect is retained relevant only for myristic acid (C14:0), lower for lauric acid (C12:0) and palmitic acid (C16:0), whereas stearic acid (C18:0) seems to be characterized by a cholesterol-lowering action [55–58]. On this basis, the reduction of myristic acid (C14:0) represents a beneficial effect attributable to the FSF diet. However, recent meta-analysis highlighted how high saturated fat intake can contribute to reducing

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the risk of stroke [58] and found no relationship linking the intake of whole-fat dairy products with an increased risk of cardiovascular diseases [59].

The intramuscular fat of meat from lambs fed FSF was characterized by a higher content in oleic acid (OLA, C18:1 c9), which was the predominant FA in both groups, and trans-vaccenic acid (TVA, C18:1 t11), that together contributed to increase the total (MUFA) in the fat of FSF meat, as also found by Priolo et al. (2005) [38] in lambs fed with FSF. The increase of OLA can be related to the concomitant reduction of stearic acid with FSF diet; indeed, stearic acid, derived mainly by the activity of ruminal microbiota in biohydrogenating dietary LA and ALA, can be converted to OLA by action of Δ9-desaturase in the animal tissues, as reported by Priolo et al. (2005) [38]. Instead, the increase of TVA with FSF diet can be referred to the ability of CT in limiting in the rumen the complete biohydrogenation of dietary PUFA to stearic acid [23].

Table 5. Effects of diet on fatty acid (FA) composition (g/100 g identified FAME).

	Diet		SEM	p-value
	Alfalfa hay pellets AHP	**Fresh sulla forage FSF**		
C10:0	0.34	0.29	0.046	0.4872
C12:0	0.65	0.40	0.17	0.3237
C14:0	2.51	2.02	0.16	0.0410
C14:1 c9	0.25	0.11	0.033	0.0063
C15:0	0.28	0.18	0.042	0.0971
C16:0	20.2	19.2	0.75	0.3440
C16:1 c9	2.70	2.71	0.21	0.9838
C17:0	2.76	1.77	0.48	0.1610
C17:1 c10	2.30	1.87	0.23	0.1936
C18:0	11.2	9.9	0.20	0.0003
C18:1 t11, TVA	0.69	1.95	0.13	<0.0001
C18:1 c9, OLA	33.0	35.5	1.01	0.0482
C18:2 c9c12 n-6, LA	7.53	7.50	0.40	0.9615
C18:3 n-6, GLA	0.68	0.32	0.15	0.0967
C18:3 n-3, ALA	1.45	1.88	0.17	0.0901
CLA C18:2 c9t11, RA	1.02	1.58	0.16	0.0197
C20:2 n-6	0.88	0.57	0.16	0.1848
C22:0	0.69	1.76	0.20	0.0013
C20:3 n-6	0.98	1.04	0.12	0.7207
C20:4 n-6	3.66	3.62	0.28	0.9209
C24:0	1.85	2.49	0.31	0.1577
C20:5 n-3, EPA	0.75	0.20	0.12	0.0037
C24:1 c15 n-9	0.89	0.55	0.16	0.1702
C22:5 n-3, DPA	1.16	1.21	0.29	0.8973
C22:6 n-3, DHA	1.60	1.38	0.24	0.5103
Saturated fatty acids (SFA)	40.5	38.0	1.18	0.1622
Monounsaturated fatty acids (MUFA)	39.8	42.7	0.95	0.0488
Polyunsaturated fatty acids (PUFA)	19.7	19.3	1.27	0.8185
Unsaturated fatty acids (UFA)	59.5	62.0	1.18	0.1622
SFA/UFA	0.69	0.62	0.033	0.1287
Σ n-6 PUFA	13.7	13.0	0.79	0.5485
Σ n-3 PUFA	4.97	4.67	0.66	0.7551
n-6/n-3	3.11	3.62	0.56	0.5311
Health promoting index[1]	1.96	2.22	0.13	0.1705

TVA = *trans* vaccenic acid. OLA = oleic acid. LA = linoleic acid. GLA = γ-linolenic acid. ALA = α-linolenic acid. CLA = conjugated linoleic acid. RA = rumenic acid. EPA = eicosapentaenoic acid. DPA = docosapentaenoic acid. DHA = docosahexaenoic acid. [1]Health promoting index = (n-3 PUFA + n-6 PUFA + MUFA) / (C12:0 + 4 × C14:0 + C16:0) [49]. SEM = standard error of mean.

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In particular, CT seem to have contributed to limiting slightly the biohydrogenation of ALA, which was highly represented in FSF (16 g/kg DM, Table 1), until to TVA, and inhibiting more greatly the successive step from TVA to stearic acid; this condition also supports the reduction of stearic acid and the increase of TVA recorded with the FSF diet. As known, also TVA is subjected to the action of Δ9-desaturase in the animal tissues to obtain RA, of which TVA represents the precursor. Although Δ9-desaturase is active on different medium- and long-chain FA, the main substrates are stearic acid desaturated to OLA, and TVA, whose conversion to RA represents the main origin of this bioactive CLA in products derived from ruminants [60]. Thus, the effect of CT in enhancing TVA and the conversion of TVA to RA explains the higher level of RA detected in FSF than in AHP meat.

These results confirm the role of CT present in FSF in inhibiting the rumen biohydrogenation of dietary LA and ALA, and favouring the reduction of stearic C18:0, the accumulation of TVA and, consequently, the successive conversion of TVA to RA in animal tissues [14]. Thus, these results also confirm the effect of feeding FSF, rich in ALA as well as in CT, in enhancing the levels of TVA and RA in the lamb meat. It is known that RA, the main isomer of CLA, can be synthesized in the rumen by isomerization of LA, but most of it is formed by desaturation of TVA in the tissues of ruminants. The health benefits of RA are well-known, including its antitumor activity and its active role in the prevention of atherosclerosis by reducing plasma cholesterol and triglyceride levels, thus protecting against cardiovascular diseases [25,26,48,61–63]. Among the factors influencing the RA content in ruminant products, feeding plays one of the main roles, as the diet provides the precursors (LA and ALA) from which the RA derives. In this regard, a fresh forage diet with a high PUFA content is known to be responsible for the enrichment of ruminant products with RA [64,65].

The diet did not affect the amount of some n-3 or n-6 FA, with the exception of eicosapentaenoic acid (EPA, C20:5 n3), which was higher in AHP diets. Thus, the total n-3 and n-6 FA, as well as the total SFA and PUFA and their ratios, including the health promoting index, were not modified by the feeding system. Otherwise, Priolo et al. (2005) [38] found a lower level of SFA, other than higher content in ALA and RA, in meat fat of lambs fed FSF than in those fed oat hay and concentrate. Probably, in that study, the FA profile of dietary components contributed to increase the differences.

4. Conclusions

The results emerged in this investigation contributed to evaluating the effects of a diet based on fresh sulla forage containing condensed tannins on lamb meat production and quality, with particular regard to meat FA profile, by comparison with a tannin-free diet based on a dry forage as alfalfa hay.

The fresh sulla-based diet promoted the lambs voluntary forage intake that, balanced by a reduction of concentrate ingestion, did not allow improvements in terms of growth and carcass weight and yield, but led to an equal feed conversion, implying lower feed costs.

Nevertheless, sulla forage induced a more intense yellow colour in meat and contributed to reduce the adiposity of the carcasses and the intramuscular fat content.

Feeding sulla forage improved meat FA profile by reducing the hypercolesterolemic myristic acid (C12:0), and increasing the rumenic acid (CLA C18:2 c9t11) and its precursor trans vaccenic acid (C18:1 t11), both these latter recognized for their health benefits, thus confirming the role of condensed tannins in inhibiting the biohydrogenation of unsaturated FA in the rumen. Finally, no sensory difference or unpleasant attribute was perceived in the meat due to the diet.

Overall, these results confirm the positive effects of fresh sulla forage in reducing carcass and meat fatness and improving the health properties of intramuscular fat.

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Abbreviations

The following abbreviations are used in this manuscript:

ADF	acid detergent fiber
ADL	acid detergent lignin
AHP	alfalfa hay pellets
ALA	α-linolenic acid
BW	body weight
CLA	conjugated linoleic acid
CP	crude protein
CT	condensed tannins
DE	delphinidin equivalent
DM	dry matter
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EBW	empty body weight
EPA	eicosapentaenoic acid
FA	fatty acids
FAME	fatty acid methyl esters
FSF	fresh sulla forage
GLA	γ-linolenic acid
GLM	general linear model
HC	half carcass
HL	hind leg
LA	linoleic acid
LD	*Longissimus dorsi*
MUFA	monounsaturated fatty acids
NFC	non-fiber carbohydrate
NDF	neutral detergent fiber
OLA	oleic acid
PUFA	polyunsaturated fatty acids
RA	rumenic acid
SEM	standard error of mean
SFA	saturated fatty acids
TVA	*trans* vaccenic acid
UFA	unsaturated fatty acids
WBSF	Warner-Bratzler shear force

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