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FATTY ACID COMPOSITION OF *Longissimus dorsi* MUSCLE OF SUFFOLK DOWN LAMBS FED ON DIFFERENT DRYLAND FORAGES

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Sheep production on dryland is based on natural pasture. The aim of this study was to evaluate the effect of dryland legume pastures on the fatty acid composition of lamb meat. The study included 21 Suffolk Down male lambs aged about 2-mo that were randomly assigned to three types of pastures: successional pasture, subterranean clover/Wimmera ryegrass (*Trifolium subterraneum* L./*Lolium rigidum* Gaudin), or red clover/Wimmera ryegrass (*T. pratense* L./*L. rigidum*) for 66 d, and slaughtered after. The fatty acid profile measured in the *Longissimus dorsi* muscle showed no significant differences as to the content of saturated (SFA), polyunsaturated fatty acids (PUFA), and the omega6/omega3 rate (ω6/ω3). There was a trend ($p > 0.06$) towards a higher monounsaturated fatty acid (MUFA) content in the meat of lambs fed on red clover when compared to the other pasture types. Although there were no significant differences among treatments, the results obtained with natural pasture cannot be extrapolated to natural dryland pastures due to their high legume content. It is concluded that using subterranean clover/Wimmera ryegrass would provide a similar meat quality when compared to results obtained from forage based on red clover/Wimmera ryegrass.

Key words: Lamb meat, muscle fat, legume pastures.

The Maule Region in Chile is characterized by its agricultural and animal production. It has a vast extension of dryland pastures which, under the framework of sustainable production, offer an interesting forage potential for sheep production. Experimental research shows that new forage species introduced into the country are able to improve pasture production in the region by increasing herbage mass (Avendaño *et al*., 2005and animal weight gains (Gallardo *et al*., 2008).

Enhancing the content of polyunsaturated fatty acids (PUFA) omega 3 in animal products is a good strategy to increase the contribution of these fatty acids in the human diet (Andersson *et al*., 2002). Special attention must be given to its precursor, α-linolenic acid (ALNA) that is present in forages. Although ALNA constitutes a highly limited source of fatty acids for humans, it is a good productive alternative due to its low susceptibility to biohydrogenation and lower costs when compared with PUFA present in fish oil (Givens *et al*., 2006).

In contrast to pork, ruminant meat is characterized by a lower proportion of essential fatty acids, such as linoleic acid (18:2 n-6) and ALNA (18:3 n-3), which can be due to the reducing role produced by ruminal bacteria on double bonds, thus saturating dietary fatty acids (Wood *et al*., 2008).

In non-ruminants, the diet profile of fatty acids is almost closely reflected in body fat, both on superficial and infiltrated fat (Gallardo, 2004). Nevertheless, that effectiveness in ruminants is reduced by ruminal hydrogenation. Certain forages with high PUFA omega 3 contents (such as red clover (*Trifolium pratense* L.) have been proven as able to protect themselves from this ruminal biohydrogenation (Lee *et al*., 2009a). This can be reflected in the fat of ruminants, and in their meat for human consumption, thus benefiting health by reducing the risk of chronic diseases, such as coronary diseases, cancer, obesity, and type 2 diabetes (Simopoulos, 2000).

Although the introduction of subterranean clover (*Trifolium subterraneum* L.) in Chile's dryland area has been under study for 5 yr, there is no information about its effects on meat composition. The hypothesis formulated that the use of dryland legume pastures would improve lamb meat fatty acid composition. The aim of this study was to evaluate the effect of grazing dryland legume pastures on lamb meat fatty acid composition in the Maule Region.

MATERIALS AND METHODS

The trial was carried out on the inner dryland of the Maule Region. Twenty-one Suffolk Down entire male lambs were used without differentiating the ewe parturition number (third parturition), lamb age (approximately 2 mo), and live weight (average weight 29.5 ± 3.35 kg). Lambs were identified by ear tags and randomly assigned

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along with their mothers (without weaning) to three treatments each with seven animals for a period of 66 d. Treatments consisted of continuous *ad-libitum* grazing (pasture height was never lower than 7 cm), successional pasture, post cultivation (a naturalized pasture planted with legumes in previous years that germinates when temperature and humidity conditions are suitable and occurs spontaneously), pasture mainly with subterranean clover/Wimmera ryegrass (*Trifolium subterraneum* L./ *Lolium rigidum* Gaudin), and pasture mainly with red clover/Wimmera ryegrass (*T. pratense* L./*L. rigidum*). Each pasture consisted of 5 ha; lambs had permanent water availability. Before starting the trial, all lambs were grazing on successional pasture. Once assigned to the treatments, one group continued grazing the same pasture, another group started to graze on the subterranean clover/ Wimmera ryegrass pasture, and another started to graze on the red clover/Wimmera ryegrass pasture.

The day the trial started (10 October 2009), forage samples from each pasture were taken with exclusion cages where five random samples per pasture were collected and the harvested material of each pasture was pooled in order to obtain one composite and representative sample for each treatment. This procedure was repeated on 11 November and 15 December 2009. Samples were sent to the Forage Laboratory of the Animal Production Department of the Universidad Austral de Chile, Valdivia to be analyzed. The botanic composition of each pasture for each sampling was obtained by manually separating the species and then dried in an oven at 60 ºC for 48 h.

Before beginning the trial, samples of *Longissimus dorsi* muscle were obtained from four lambs, randomly chosen from each treatment, through a biopsy procedure carried out on the right side of the loin between the 10th and 13th ribs. A trichotomy was performed in the described area before the biopsy. The biopsy procedure included a local anesthetic (4 mL lidocaine 2% by infiltration) and surgical instruments. After the anesthetic infiltration, the skin was dissected to reach the *L. dorsi* muscle. A muscle sample (triangular shape) of approximately 3 g was obtained from each lamb. Finally, the muscle and the skin were sutured, and an anti inflammatory (1.5 mL flunixin meglumine) and antibiotic (3 mL oxytetracycline 5%) were applied intramuscularly. Therefore, samples of the four lambs had to be grouped into only one initial sample per treatment (three initial samples), which were kept in Eppendorf tubes at -20 ºC for analyses.

Two days after the trial ended, lambs were slaughtered according to the standards of Chile's meat industry. Samples of approximately 80-g of *L. dorsi* muscle were taken from all lambs immediately after death and homogenized by interrupted grinding in a Moulinex device for 30 s and kept in hermetic bags at a temperature of -20 °C for further analyses.

The following productive variables were measured and then calculated: initial live weight, final live weight, weight immediately before slaughter (after transport and fasting), hot carcass weight, daily weight gain (DWG), and carcass dressing percentage (using weight before slaughter).

Chemical analyses were carried out for both the forage and muscle samples to determine dry matter content (DM), total ashes (TA), and crude protein (CP) (Bateman, 1970). Metabolizable energy (ME) was estimated by regression with a "D" value (Digestible organic matter/DM × 100) determinated *in vitro* (Tilley and Terry, 1963) according to Goering and Van Soest (1970). This trial also determined neutral detergent fiber (NDF) (Van Soest *et al*., 1991), acid detergent fiber (ADF), and crude fiber (AOAC, 1996). Muscle samples were grouped according to the treatment received, they were thawed and lyophilized before being chemically analyzed.

Fatty acid composition in both forage and muscle was measured at the Instituto de Investigaciones Agropecuarias (INIA Remehue) by extraction with a mixture of distilled water, methanol, and chloroform (Bligh and Dyer, 1959), and methylation with methanol KOH (Ichihara *et al*., 1996). The derivative was injected into a gas chromatographer at the following weights: initial muscle samples (2 g), final muscle samples (20 g), and pasture samples (50 g).

A completely randomized model was used, which was submitted to a one-way ANOVA with the Minitab Statistical Package, version 14. Measurement contrasting was carried out by the Tukey multiple comparison method for means ($p \leq 0.05$).

RESULTS AND DISCUSSION

Botanic composition of the successional pasture exhibited high species heterogeneity (Table 1) and was not a typical natural pasture of the dryland area (Avendaño *et al*., 2005; Muñoz *et al*., 2005) due to its high legume content in which red clover predominated by mid-spring and hualputra (*Medicago polimorpha* L.) by late spring. This needs to be explained because successional pasture (post cultivation) corresponds to a type of spontaneous pasture that is unpredictable with a large bank of legume seeds from planting in previous years. This explains its high content of red clover (in November), which does not correspond to a typical natural pasture of the dryland. Hualputra is a species that has been naturalized and is found in many soils and depends on soil moisture and the effect of rain.

When analyzing the chemical composition of pasture treatments (Table 2), red clover/Wimmera ryegrass pasture showed the highest CP ($p \leq 0.05$) mean content and a trend towards a lower dry matter (DM) and fiber content as compared to successional and subterranean clover pastures.

Pasture fatty acid composition (Table 3) was similar to that reported for forages in the literature (Demirel *et*

Table 1. Botanic composition of pastures during spring expressed as dry matter percentage.

Species (%DM)	Successional pasture			Subterranean clover			Red clover		
	October[1]	November	December	October	November	December	October	November	December
Agrostis tenuis Sibth.	52.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Medicago polymorpha L.	8.3	0.8	41.9	0.0	0.0	0.0	0.0	0.0	0.0
Cortaderia selloana[2]	10.5	14.3	22.7	0.0	2.1	0.0	0.0	0.0	1.2
Trifolium subterraneum L.	1.3	0.0	0.0	87.1	74.5	28.0	0.0	9.1	0.0
Trifolium repens L.	15.2	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trifolium pratense L.	1.9	54.6	1.6	0.0	0.0	0.0	81.7	50.7	50.5
Lolium rigidum Gaudin	9.5	0.0	17.9	12.9	23.5	64.5	18.3	40.3	19.6
Bromus hordeaceus L.	0.6	23.7	0.0	0.0	0.0	0.0	0.0	0.0	20.5
Others	0.1	1.9	16.0	0.0	0.0	7.5	0.0	0.0	8.2

[1]October (10 October 2009); November (11 November 2009); December (15 December 2009).
[2]Cortaderia selloana (Schult. & Schult. f.) Asch. & Graebn.

al., 2006; Aurousseau *et al*., 2007). The successional and red clover/Wimmera ryegrass pastures exhibited the highest PUFA numerical values, mainly owing to their high ALNA contents ($p \leq 0.05$). This could be explained by the high percentage of red clover found when the successional pasture botanical composition was analyzed. In the subterranean clover/Wimmera ryegrass pasture, higher SFA percentages ($p > 0.05$) would be related to the higher numerical content of mirystic and estearic acids. Red clover/Wimmera ryegrass pasture exhibited the lowest omega6/omega3 rate ($p \leq 0.05$) as given by its higher ALNA percentages. Lee *et al*. (2003) reported that both white and red clover have a higher PUFA content than a natural pasture mainly with *Lolium perenne*. This result does not coincide with the findings of this study because of the high legume content found in the natural pasture that resulted in a lower omega6/omega3 rate than in the subterranean clover/Wimmera ryegrass pasture.

Regarding lamb productive characteristics (Table 4), the means for daily weight gain, final weight, weight before slaughter, and hot carcass weight were all higher in lambs fed on the red clover/Wimmera ryegrass pasture ($p \leq 0.05$) as compared to the other treatments. Gallardo *et al*. (2008), working with Suffolk Down lambs in the same geographical area, reported weight gains of 399 g d^{-1} for lambs and 391 g d^{-1} for ewes consuming subterranean clover pastures, values somewhat higher than those reported for legume pastures in this trial (259 g d^{-1} for subterranean and 296 g d^{-1} for red clover), which could be explained by the shorter duration of the cited trial (15 d) and by the lower lamb age (Avendaño *et al*., 1994).

Chemical analysis of the *L. dorsi* muscle of lambs fed on different pastures (Table 5) showed significant differences only for CP and ether extract (EE) ($p \leq 0.01$). The higher mean protein values found in the red clover/Wimmera ryegrass pasture were not reflected in higher mean protein

Table 2. Chemical analysis of pastures grazed before the trial (Initial pasture) and for each treatment during the trial.

	Initial pasture	Successional pasture	Subterranean clover	Red clover	p-value
DM, %	21.7	25.8 ± 4.49	27.3 ± 20.07	20.7 ± 7.70	ns
TA, %	13.2	10.0 ± 2.79	9.5 ± 0.72	13.6 ± 4.65	ns
CP, %	17.3	14.1 ± 4.04b	13.6 ± 2.70b	22.3 ± 4.58a	0.056
ME, Mcal kg^{-1}	2.7	2.50 ± 0.32	2.51 ± 0.36	2.62 ± 0.16	ns
NDF, %	40.7	44.9 ± 5.71	40.2 ± 9.36	33.5 ± 3.73	ns
ADF, %	26.7	32.0 ± 6.51	32.8 ± 8.29	27.1 ± 1.86	ns

n = 3. ns: non significant; DM: dry matter; TA: total ashes; CP: crude protein; ME: metabolizable energy; NDF: neutral detergent fiber; ADF: acid detergent fiber.

Table 3. Fatty acid composition of pastures before the trial (Initial pasture) and for each treatment during the trial (summary table).

Fatty acids (%)	Initial pasture	Successional pasture	Subterranean clover	Red clover	p-value
C14:0 (myristic acid)	6.40	6.15 ± 3.22	12.03 ± 12.38	3.83 ± 2.53	ns
C16:0 (palmitic acid)	18.40	19.83 ± 5.05	19.78 ± 3.00	19.13 ± 2.23	ns
C18:0 (estearic acid)	2.80	3.67 ± 1.36	5.10 ± 2.11	4.60 ± 1.73	ns
C18:1N9C (oleic acid)	1.25	3.00 ± 1.16	5.45 ± 3.57	5.40 ± 6.26	ns
C18:2N6C (linoleic acid)	8.55	10.50 ± 3.37	13.58 ± 6.82	11.30 ± 5.93	ns
C18:3N3 (α linolenic acid)	24.55	32.32 ± 10.34b	23.75 ± 11.47b	42.80 ± 12.53a	0.037
SFA	36.15	32.98 ± 4.89	40.60 ± 15.77	29.43 ± 3.81	ns
PUFA	53.30	60.82 ± 9.01	52.22 ± 14.82	60.08 ± 11.97	ns
MUFA	10.55	5.47 ± 5.15	7.10 ± 5.56	10.42 ± 8.79	ns
ω6/ω3	1.17	0.71 ± 0.50b	1.39 ± 0.71a	0.37 ± 0.34c	0.013

n = 3. ns: non significant; SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; ω6/ω3: omega6/omega3 rate.

Table 4. Productive characteristics in lambs fed on three pasture types.

	Successional pasture[1]	Subterranean clover[1]	Red clover[1]	p-value
Initial weight, kg	28.4 ± 3.78	29.8 ± 3.82	30.1 ± 2.56	ns
Final weight, kg	43.0 ± 4.79b	46.9 ± 5.56ab	49.6 ± 1.97a	0.036
DWG, g d^{-1}	0.221 ± 0.04c	0.259 ± 0.03b	0.296 ± 0.05a	0.006
Weight before slaughter, kg	41.2 ± 4.01b	41.9 ± 4.12b	46.0 ± 2.99a	0.055
Hot carcass weight, kg	21.4 ± 2.24b	22.0 ± 2.27b	24.5 ± 1.19a	0.021
Hot carcass dressing, %[2]	0.52 ± 0.02	0.52 ± 0.02	0.53 ± 0.02	ns

[1]n = 7; [2]Based on weight before slaughter; DWG: daily weight gain, total period (66 d); ns: non significant.

Table 5. Chemical analysis of *Longissimus dorsi* muscle in lambs fed on three types of pastures.

	Successional pasture[1]	Subterranean clover[1]	Red clover[1]	p-value
DM, %	23.8 ± 0.00	24.5 ± 0.00	24.0 ± 0.00	ns
TA, %	1.1 ± 0.01	1.1 ± 0.01	1.1 ± 0.02	ns
CP, %	19.0 ± 0.06c	20.2 ± 0.06a	19.6 ± 0.13b	0.000
EE, %	2.8 ± 0.02a	2.3 ± 0.00c	2.4 ± 0.05b	0.000
CF, %	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.01	ns

[1]n = 7; DM: dry matter; TA: total ashes; CP: crude protein; EE: ether extract; CF: crude fiber; ns: non significant.

levels in the meat of lambs fed on this forage. Although final weight and weight gains were higher in lambs fed on red clover/Wimmera ryegrass, these animals showed intermediate values of EE in their meat, which were not related to higher carcass fat contents.

Fatty acid composition of *L. dorsi* muscle in the lambs from the different treatments (Table 6) was within the values reported by the literature for grazing sheep (Enser *et al*., 1996; Demirel *et al*., 2006). Notwithstanding that grazing systems increase ALNA concentrations in meat (Givens *et al*., 2006), ALNA quantities in the forages turned out to be much higher than those found in meat (except in the case of 18:2n6), which can be due to a higher ruminal biohydrogenation suffered by ALNA (85 to 100%) as compared to the biohydrogenation of 18:2n-6 (70 to 95%) (Doreau and Ferlay, 1994). Values reported for PUFA in this trial are higher than those reported by other authors (Aurousseau *et al*., 2007; Cañeque *et al*., 2007); this is probably due to the higher 18:2n-6 and 18:3n-3 levels observed in the present study (Enser *et al*., 1998; Fisher *et al*., 2000), which could be explained by the high clover content in all the pastures.

When comparing the initial muscle samples (obtained by biopsy) with the samples obtained at the end of the trial, a decrease in the percentage of SFA ($p \leq 0.05$) and an increase in PUFA ($p \leq 0.05$) were observed. This effect could be explained by the likely decrease in the lambs' milk consumption throughout the trial and the corresponding increase in forage consumption. Final meat samples also exhibited a higher ω6/ω3 rate ($p \leq 0.05$) given their greater numerical contents of C18:2n6 and C18:3n3.

In general, no significant differences among treatments were found when comparing muscle samples obtained at the end of the trial (except in certain individual fatty acids) with regards to SFA and PUFA contents. The ω6/ω3 rate was < 2.2 in all treatments, thus making the lamb meat highly recommendable for consumption (< 4) (Scollan *et al*., 2006). There was a trend for the meat of lambs fed on red clover/Wimmera ryegrass pasture ($p > 0.06$) to contain higher numerical percentages of MUFA as compared to the other two pastures, which could be explained by the higher oleic acid content (18:1n9) of this pasture. Lee *et al*. (2009b) fed dairy cull cows with grass silage and red clover silage, reporting significantly higher values of oleic acid in clover although this was not reflected in the meat. Contrary to what was expected, lamb meat from the successional pasture was not different from meat from pastures based on clover (subterranean or red). This can be due to the high content of red clover (mid-spring) and hualputra (late spring) found when analyzing the botanic composition of the successional pasture, thus making this pasture similar to the other two in terms of legume content.

It is known that the effect of grazing on meat fatty acid composition will depend on the forage species consumed (Lee *et al*., 2009a). The better results with red clover to increase muscle fatty acid composition reported so far would be linked to a decrease in ruminal biohydrogenation. This has been explained through the protective effects of the polyphenol oxidasa (PPO) enzyme (Lee *et al*., 2004) that allows a higher proportion of ALNA to escape from ruminal biohydrogenation and is reflected in meat composition. However, that effect was not observed in this trial. Although red clover/Wimmera ryegrass pasture lambs exhibited the highest percentage of ALNA in their meat, no significant differences were found in this trial when compared with the meat from the lambs grazing on successional or subterranean clover/Wimmera ryegrass pasture. Another important factor to be considered is the use of forage mixtures (as in this study) instead of monocultures. Lourenco *et al*. (2008) reported that lambs fed on a mixture of legumes and other forages would show more PUFA at muscle level than animals fed only on pastures (result observed in this trial given the high legume content in the successional pasture). For the same reason, there were no significant differences among treatments regarding CLA content in the meat.

CONCLUSIONS

The botanic composition of pastures grazed by the lambs varied as spring advanced and the successional pasture

Table 6. Main fatty acid composition of *Longissimus dorsi* muscle in lambs before the trial (Initial) and after grazing on three types of pastures.

Fatty acids (%)	Initial (n = 3)	Successional pasture (n = 7)	Subterranean clover (n = 7)	Red clover (n = 7)	p-value
C14:0 (myristic acid)	9.22 ± 0.84a	1.37 ± 0.98b	1.25 ± 1.10b	1.76 ± 1.46b	ns
C16:0 (palmitic acid)	29.25 ± 3.45a	18.33 ± 2.10c	18.57 ± 2.62c	21.17 ± 3.77b	0.024
C18:0 (estearic acid)	19.00 ± 4.20a	15.08 ± 1.00b	17.72 ± 2.24a	15.28 ± 4.64b	0.048
C18:1N9C (oleic acid)	31.14 ± 10.68a	22.81 ± 2.98b	23.72 ± 4.85b	27.04 ± 4.19a	0.023
CLA (conjugated linoleic acid)	nd	0.24 ± 0.63	0.17 ± 0.23	0.22 ± 0.21	ns
C18:2N6C (linoleic acid)	2.81 ± 0.94b	16.21 ± 3.08a	15.91 ± 4.98a	13.98 ± 5.34a	ns
C18:3N3(α linolenic acid)	1.99 ± 0.49b	4.92 ± 0.76a	5.33 ± 1.24a	4.31 ± 1.71a	ns
SFA	60.33 ± 8.70a	36.20 ± 3.53b	38.98 ± 4.83b	39.05 ± 6.40b	ns
PUFA	5.81 ± 2.11b	27.20 ± 4.14a	25.97 ± 70.24a	23.03 ± 7.83a	ns
MUFA	33.91 ± 10.85	28.44 ± 2.91	29.25 ± 4.72	31.86 ± 3.75	ns
ω6/ω3	0.94 ± 0.08b	1.81 ± 0.20a	2.05 ± 0.57a	1.87 ± 0.43a	ns

nd: not determined; ns: non significant among the three treatments, but significant when compared with the initial samples; SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; ω6/ω3: omega6/omega3 rate.

contained high levels of legumes, which resembled the contents of red clover and subterranean clover in the other grazing systems. Lamb productive performance in terms of weight gains and final live weight was higher in the red clover/Wimmera ryegrass pasture ($p \leq 0.05$) without producing an increase in chemical fat content of *L. dorsi*.

Muscle fatty acid composition did not differ significantly among treatments ($p > 0.05$) with regards to SFA and PUFA content, and the ω6/ω3 rate, the latter being very favorable for human consumption (< 4) in all treatments. The fact that the meat from lambs grazing successional pasture was not different from meat obtained from lambs grazing clover pastures can be due to the high legume content of this particular successional pasture, making it impossible to extrapolate results to other natural pastures of the dryland area. In summary, the use of subterranean clover would provide similar meat quality when compared to results obtained from forage based on red clover.

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Composición de ácidos grasos del músculo *Longissimus dorsi* de corderos Suffolk Down alimentados con diferentes forrajes. La producción ovina en el secano está basada en la pradera natural. El objetivo de este estudio fue evaluar el efecto de praderas de leguminosas de secano sobre la composición de ácidos grasos de la carne de cordero. El estudio incluyó 21 corderos machos Suffolk Down, de alrededor de 2 meses de edad, los que fueron asignados aleatoriamente en tres praderas: pradera sucesional, trébol subterráneo/ballica Wimmera (*Trifolium subterraneum* L./*Lolium rigidum* Gaudin) o trébol rosado/ballica Wimmera (*T. pratense* L./*L. rigidum*), por 66 d, siendo posteriormente sacrificados. El perfil de ácidos grasos, medido en el músculo *Longissimus dorsi* no mostró diferencias significativas en contenido de ácidos grasos saturados (SFA), poliinsaturados (PUFA), y razón omega 6/omega 3 (ω6/ω3). Hubo una tendencia ($p > 0,06$) hacia un mayor contenido de ácidos grasos monoinsaturados (MUFA) en la carne de corderos alimentados con trébol rosado, comparado con los otros tipos de praderas. Aunque no hubo diferencias significativas entre tratamientos, los resultados obtenidos con la pradera sucesional no pueden ser extrapolados a pasturas naturales en el secano, debido a sus altos contenidos de leguminosas. Se concluye que el uso de trébol subterráneo proveería una similar calidad de carne, al comparar con forrajes basados en trébol rosado.

Palabras clave: carne de cordero, grasa muscular, praderas de leguminosas.

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