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Original Article

Use of alternative protein sources for finishing lambs. 2. Effects on chemical and physical characteristics and fatty acid composition of meat

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Summary. This study investigated the use of different protein sources (soybean, faba bean, sweet lupin and pea) in lamb feed and their influence on the chemical and physical characteristics of the meat and on the acid composition of intramuscular fat. The meat of lambs fed on faba bean contained a greater percentage of fat than those fed on lupin ($P < 0.05$) and soybean ($P < 0.01$). The meat of lambs fed on faba bean had a greater ($P < 0.01$) proportion of PUFA and a higher PUFA/SFA ratio than the meat of all other groups. The thrombogenicity index and nutritive value of meat fat were higher for lambs fed with faba bean and pea. The use of lupin in feed determined the highest ($P < 0.01$) SFA percentage, the lowest PUFA incidence and the worst thrombogenicity index. In conclusion, the protein sources studied did not influence the physical characteristics of the meat. However, the acid composition improved with the use of pea, and especially of faba bean.

Key words: lambs, legume seeds, meat quality, fatty acids

«Impiego di fonti proteiche alternative per agnelli all'ingrasso. 2. Caratteristiche chimico-fisiche e composizione acidica della carne»

Riassunto. È stata valutata l'influenza della fonte proteica della razione (soia, favino, lupino e pisello proteico) sulle caratteristiche chimico-fisiche della carne e sul profilo acidico dei lipidi intramuscolari di agnelli in finissaggio. La carne degli agnelli alimentati con favino ha evidenziato una maggiore percentuale di lipidi rispetto a quella dei soggetti riceventi il lupino ($P < 0.05$) e la soia ($P < 0.01$) L'alimentazione con il favino, rispetto a tutti gli altri gruppi ha condizionato, una maggiore ($P < 0.01$) proporzione di PUFA e un maggiore rapporto PUFA/SFA. L'indice di trombogenicità e il valore nutritivo dei lipidi sono risultati migliori negli agnelli riceventi il favino e il pisello proteico. Il lupino ha determinato la maggiore ($P < 0.01$) percentuale di SFA, la minore incidenza di PUFA e il peggiore indice di trombogenicità. In conclusione, le fonti proteiche allo studio non hanno influenzato le caratteristiche fisiche della carne, mentre, il profilo acidico è stato migliorato dalla presenza, nella razione, del pisello proteico e soprattutto del favino.

Parole chiave: agnelli, semi di leguminose, qualità della carne, acidi grassi

Introduction

In recent decades, consumers have increasingly favoured high quality foods with positive health effects. Red meat consumption has fallen, since consumers see it as a potential cause of cancers and cardiovascular diseases (1-3), due to its high saturated fatty acids (SFA) and cholesterol contents. Nutritionists advise reducing the consumption of SFAs in favour of polyunsaturated fatty acids (PUFA) to achieve an optimum PUFA/SFA ratio (4). The relationship between omega-6 and omega-3 PUFAs is particularly important, since many of the fatty acids in these series are essential, and can only be obtained from food (5-7). Consumer acceptance of meat derives from its nutritional value and perception of its quality (8, 9), therefore other aspects of evaluation are important, such as sensorial and chemical-physical characteristics. These mostly concern flavour, which is connected to fatty acid composition (10, 11), colour (12), which is influenced by pH (13, 14), and tenderness (15, 16).
It must be emphasised that sheep are of great economical importance in Mediterranean livestock farming. Since sales of lamb have also fallen, possible strategies for obtaining meat that consumers perceive as "healthy" involve modifying the carcass of the animal *in vivo*. Carcass composition is conditioned by different factors, such as breed (17-19), slaughter weight (17, 19, 20), sex (20-22) and feed type (10, 23).
In recent years, research has focused on ruminant feeds containing single protein sources such as lupin, faba bean and pea, as alternatives to GM soybean. Different authors have reported that use of these legumes in feed has not had negative effects on productive performances (24-28), while contrasting results have been obtained regarding their effects on the acid composition of meat fat (24, 29, 30).
The aim of the present work was to compare diets containing the protein sources mentioned above, evaluating their effects on the chemical and physical characteristics of meat, and on the acid profile of intramuscular fat.

Material and methods

Experimental design and animal management

The study involved 32 male Gentile di Puglia lambs. These were weaned at 42 days, sub-divided into 4 homogeneous groups of 8 and then fed on isoenergetic and isoproteic pellets containing: a) soya (control); b) faba; c) lupin; d) protein pea. For the experimental design and animal management, see the previous note (31).
The lambs were slaughtered at the end of the 7th week of the study (according to veterinary police rules: D.P.R. 320/54), at an average weight of 20-23 Kg.

Physical analysis

The physical characteristics of meat (32) were defined using samples of *Longissimus dorsi* (*LD*) and *Semimembranosus* (*SM*) muscles, analysed to determine: a) pH at slaughter (pH_0) and after refrigeration for 24 h (pH_{24}), using a Eutech Instruments pH-meter mod. pH 110; b) shear resistance according to Warner Bratzler Shear (WBS) using an Instron 5544; c) colour (L* = lightness, a* = redness index, b* = yellowness index) using a HunterLab Miniscan™ XE Spectrophotometer (Model 4500/L, 45/0 LAV, 3.20 cm diameter aperture, 10° standard observer, focusing at 25 mm, illuminant D65/10, from Hunter Associates Laboratory, Inc. Reston, Virginia, USA) to take 3 readings at different points for each sample; the Hue angle ($H^* = \tan^{-1}(b^*/a^*)$) and Chroma (C^* = square root of $a^{*2}+b^{*2}$) were also calculated.

Chemical analysis

The chemical composition was analysed (32) by homogenising representative sub samples of *LD* to determine the fatty acid composition. The fats were then extracted from the samples according to the method suggested by Folch et al. (33) using 2:1 chloroform/methanol solution (v/v). The fatty acids were then methylated with a solution (12 % v/v) of BF3/methanol (34), and analysed by gas chromatography (Chromopack CP 9000) using a capillary column (70% Cyanopropyl Polysilphenylene-siloxane BPX 70 by SGE Analytical Science, length = 50 m, internal diameter = 0.22 mm, film thickness = 0.25 μm). The temperature was 135 °C for 7 minutes, then increased by 4 °C/minute up to 210 °C.

The food risk factor of the meat was determined by calculating the atherogenicity (I.A.) and thrombogenicity (I.T.) indexes (35) and the nutritive value (36). Representative samples of the feeds were collected weekly and mixed in order to obtain a single final sample for each diet; this was then analysed to determine the fatty acid composition, as has already been described for the meat. The single fatty acids were then expressed as a percentage of the total methylated fatty acids (Table 1).

Statistical analysis

Variance analysis of the data was performed using the GLM procedure of the SAS application package (37).

Table 1. Fatty acid composition of the complete diet (g/100 g of fatty acid metylesters)

Fatty acid	Common name	Diet			
		Soy bean	Faba bean	Lupin	Pea
C16:0	Palmitic acid	14.40	16.00	16.10	12.70
C18:0	Stearic acid	2.30	1.48	1.96	2.60
C18:1	Oleic acid	20.30	21.60	20.94	19.75
C18:2	Linoleic acid	48.00	45.90	44.86	48.45
C18:3	Linolenic acid	12.20	11.90	12.95	14.30

The averages estimated were compared using Student's *t* test. The statistical model considered the effect of diet (D) and muscle (M) and the interaction of diet x muscle (D x M).

Results and discussion

Physical characteristics of the meat

The pH_0 values were in agreement with those reported elsewhere (38, 39), varying between 6.71 for *SM* of lambs fed with lupin and 6.86 for *LD* of those fed with pea, and did not present any significant differences between muscles or diets (Table 2).

The pH_{24} values ranged between 5.63 and 5.87, without any significant differences attributable to diet or to muscle; the lack of a difference between the muscles agrees with Tschirhart-Hoelscher et al. (40). In general, the pH levels we recorded are within the range for other breeds (19, 41-44) and different geographical areas (45), indicating both the absence of pre-slaughter stress (46-48) and the normal function of the aerobic glycogen metabolism as observed by Immonem et al. (49) in cattle. The correlation has been reported between pH and meat colour (14, 50, 51), and considering that changes in meat colour are accompanied by final pH levels over 5.8 (49), our data demonstrate that feeding with field bean tends to give better results for both muscles.

WBS data were not statistically different between

Table 2. pH and tenderness of *Longississimus dorsi* and *Semimembranosus* muscle

Parameters	Muscle (M)	Diet (D) Soy bean	Faba bean	Lupin	Pea	SD[1] (DF= 38)	F Value D	M	D x M
pH_0	LD	6.79	6.69	6.74	6.86	0.175	2.46	0.01	0.54
	SM	6.72	6.79	6.71	6.82				
pH_{24}	LD	5.85	5.71	5.81	5.83	0.245	1.84	0.40	0.87
	SM	5.85	5.63	5.70	5.87				
ΔpH_{0-24}	LD	0.94	0.98	0.93	1.04	0.273	1.01	0.48	1.18
	SM	0.87	1.15	1.00	0.95				
WBS (kg/cm^2)	LD	2.70	2.54	2.48	2.17	0.702	0.10	0.80	0.22
	SM	2.70	2.62	2.59	2.65				

[1]standard deviation of mean.

muscles (Table 2), although the highest value was recorded for the lambs fed with soybean, probably because their carcasses contained a lower quantity of intramuscular fat than those of the other groups (1.90 *vs* 2.05 - 2.45%). It has been reported that the intramuscular fat separates and dilutes the perimisial fibres, disorganising the structure of the intramuscular connective tissue and increasing resistance (52). The literature contains contrasting data on the relationship between WBS and pH. Devine et al. (46) reported increases in tenderness with final pH values between 5.4 - 6.0, while Hoffman et al. (53), in agreement with our data (r = - 0.14; P > 0.05), reported a negative, but not significant, relationship. On the other hand, Safari et al. (54), found no relationship between pH and WBS in lambs of six different genotypes.

With regard to meat colour (Table 3), diet had no influence on lightness or on the yellowness and redness indexes. The values we recorded are within the range reported by other authors (25, 26, 55, 56) for other genotypes. Nor did we find significant differences between the muscles; this partly agrees with Tschirhart-Hoelscher et al. (40), who reported, however, that the lightness of *Longissimus thoracis* was significantly greater.

Chemical composition of the meat

The moisture and protein levels recorded did not determine any differences attributable to diet, whereas the fat content was markedly greater in the meat of lambs fed with faba bean (2.45%) than in those fed with lupin (2.05%; P < 0,05) or soybean (1.90%, P < 0.01); the ash percentage was greater for the groups fed with soybean and pea than for those fed with faba bean (1.52 - 1.53 *vs* 1.34%; P < 0.05) (Table 4). Lanza et al. (29) and Scerra et al. (30) report data on lambs fed with faba bean and pea as replacements for soybean, and did not detect any significant difference in the parameters under consideration; however, the higher

Table 3. Colorimetric characteristics of *Longissimus dorsi* and *Semimembranosus* muscle

Parameters		Diet (D)					F Value		
		Soy bean	Faba bean	Lupin	Pea	SD[1] (DF= 54)	D	M	D x M
L*	LD	40.08	39.60	40.75	39.46	1.226	3.73	1.03	0.35
	SM	40.90	39.95	40.81	39.50				
a*	LD	9.21	9.54	8.71	9.44	0.563	0.97	3.79	2.62
	SM	8.86	8.84	9.08	8.94				
b*	LD	8.06	8.37	8.47	8.54	0.550	0.47	1.19	1.28
	SM	8.37	8.04	8.15	8.25				

[1]standard deviation of mean.

Table 4. Meat chemical composition (% on as its basis)

Parameters	Diet					F Value
	Soy bean	Faba bean	Lupin	Pea	SD[1] (DF= 25)	
Moisture	73.29	73.37	73.45	73.10	1.154	0.12
Protein	22.24	21.85	21.91	21.96	0.851	0.27
Lipids	1.90^{B}	2.45^{Aa}	2.05^{b}	2.17	0.343	3.39*
Ash	1.52^{a}	1.34^{b}	1.46	1.53^{a}	0.143	2.73
Cooking loss	21.23	20.54	20.44	20.26	4.582	0.05

[1]standard deviation of mean. On row: $^{A, B}$: P < 0.01; $^{a, b}$: P < 0.05; *: P<0.05.

lipid percentage found in the present study may have been influenced by the heavier slaughtering weight of the lambs fed with faba and pea, as confirmed by the data from our previous research (31).

The influence of slaughtering weight on total (intramuscular, intermuscular and subcutaneous) fat has been documented. In general, fat content increases with age and weight at slaughter in Merino lambs (57) and other breeds (44, 58). Moreover, Andrews and Ørskov (59) suggest that differences in fat content within the same breed or cross-breeds are more evident at certain stages of growth than at others, and may vary according to growth rates or feeding systems.

Cooking loss varied between 20.26 and 21.23 % and was not influenced by diet (Table 4), although it tended to be greater in the soybean group; this was probably due to the higher final pH level recorded for this meat. Some studies have observed a positive correlation between final pH levels and cooking loss (60, 61).

Fatty acid composition of the meat

The fatty acid data are reported in Table 5. In agreement with other studies (58, 62, 63), oleic acid (C18:1 n-9 *cis* 9) was the fatty acid found in the greatest quantity, followed in decreasing order by palmitic (C16:0) and stearic (C18:0) acids.

The SFA percentage was significantly ($P < 0.01$) lower in the faba bean and pea groups than in the soybean or lupin groups; in addition, the values recorded for the latter two groups also differed markedly from one another ($P < 0.01$), with higher values for the lupin group.

For all groups, C16:0 (18.13 - 24.63%) was the SFA found in the greatest quantities. Moreover, the lamb from the lupin group contained higher percentages of C16:0, C14:0 and C18:0 than the meats from the other groups, probably due to differences in the rumen fermentation of these lambs (64), which may have increased propionic acid production, leading to increased deposition of medium- and long-chain fatty acids in fat tissue (65).

The pea group meat presented a greater percentage of MUFA than the faba bean group meat ($P < 0.05$), and a higher percentage than the meat of the lupin and soybean groups ($P < 0.01$). There were no significant differences between diets regarding palmitoleic (C16:1) and oleic (C18:1n-9 *cis* 9) acids, while the lupin group meat contained a lower proportion of vaccenic acid (C18:1 n-9 *trans* 11) than the meat of the pea ($P < 0.01$) and faba bean or soybean groups ($P < 0.05$). This is probably due to the formation of a different quantity of vaccenic acid by rumen biohydrogenation, and to the activity of reductase, which transforms vaccenic into stearic acid (66); in fact, the lupin group meat presented the highest levels of stearic acid, indicating that there was probably a higher level of reductase activity.

The highest PUFA level was found in the faba bean group meat, and the lowest in the lupin group (11.94 *vs* 3.50; $P < 0.01$). However, the PUFA percentage in the meat fat of all groups was lower than that of the feed, an indication that feed PUFAs had been partly hydrogenised in the rumen (67), although to different extents, in agreement with Bas and Morand-Fher (68) and Chilliard et al. (66), according to whom single feed components can have different effects on rumen biohydrogenation.

Regarding PUFA n-6, the proportion of linoleic acid (C18:2 *cis* 9,12) was greater ($P < 0.01$) in the faba bean group than in the other groups, and was accompanied by lower proportions of oleic, palmitic and stearic acids, as observed also by Wright et al. (69) in lambs whose feed was supplemented with sunflower seeds. It may be possible that this higher percentage of linoleic acid derives from a greater quantity of this acid escaping attack by rumen microflora, in close relation to the intrinsic characteristics of the feed consumed (66, 68).

The proportion of rumenic acid (C18:2 n-6 *cis* 9 *trans* 11CLA) was significantly ($P < 0.05$) greater for the faba bean group than for the lupin group. Aurousseau et al. (70) reported that the CLA content of meat is influenced by diet, growth rate and genotype, while De Smet et al. (71) recorded that CLA increased as total fat increased. Our experiment confirms this; the meat of lambs fed with faba bean contained more fat (Table 4), and as reported in a previous note (31), this group had the highest growth rate and fattiest carcasses.

The faba bean group contained a greater percentage ($P < 0.01$) of arachidonic acid (C20:4n-6 *cis* 5, 8,11, 14) than the others; this is probably due to the greater quantity of linoleic acid recorded for the field bean group, since arachidonic acid is the final product of the

Table 5. Fatty acid composition (g/100 g of fatty acid metylesters) and health indexes of *Longissimus dorsi* muscle

Parameters	Diet					F Value
	Soy bean	Faba bean	Lupin	Pea	SD[1] (DF= 20)	
C12:0 lauric	0.38^{B}	0.47^{a}	0.60^{A}	0.32^{Bb}	0.116	6.94^{**}
C14:0 myristic	3.33^{B}	3.99	5.03^{A}	3.09^{B}	0.919	12.58^{**}
C16:0 palmitic	22.41^{Ba}	18.13^{C}	24.63^{A}	19.68^{BCb}	1.999	26.62^{**}
C18:0 stearic	14.23	12.64^{B}	15.84^{A}	13.06^{B}	1.645	7.06^{**}
Total SFA	42.27^{C}	37.04^{B}	48.56^{A}	38.23^{B}	2.337	86.08^{**}
C16:1 palmitoleic	1.54	1.48	1.73	1.62	0.294	2.93
C18:1n-9 trans11 vaccenic	4.87^{a}	5.78^{a}	3.35^{Bb}	7.44^{A}	1.751	6.18^{*}
C18:1n-9 cis 9 oleic	36.42	35.66	36.23	38.55	3.310	3.22
Total MUFA	45.70^{B}	46.09^{b}	44.05^{B}	50.70^{Aa}	3.038	27.90^{**}
C18:2 n-6 cis 9, 12 linoleic	3.24^{B}	6.55^{A}	2.35^{Bb}	4.18^{Ba}	1.224	12.66^{**}
C18:2 n-6 cis 9 trans11 rumenic	0.25	0.32^{a}	0.12^{b}	0.18	0.118	2.97
C18:3 n-3 cis 9,12,15 linolenic	0.48	0.55	0.55	0.48	0.092	1.49
C20:4 n-6 cis 5,8,11,14 arachidonic	0.21^{B}	1.13^{A}	0.01^{B}	0.04^{B}	0.480	6.51^{**}
Total n-6	4.75^{Ba}	9.36^{A}	2.73^{Bb}	4.96^{Ba}	1.479	32.41^{**}
Total n-3	0.97^{B}	2.58^{Aa}	0.77^{BC}	1.66^{b}	0.762	10.55^{**}
Total PUFA	5.72^{B}	11.94^{A}	3.50^{Bb}	6.62^{Ba}	2.137	26.00^{**}
PUFA/SFA	0.14^{BC}	0.32^{A}	0.07^{C}	0.18^{B}	0.060	27.91^{**}
n-6/n-3	5.64^{Aa}	4.01^{b}	3.81^{b}	3.31^{B}	1.192	1.96
Atherogenic index	0.70^{Ba}	0.59^{B}	0.96^{A}	0.57^{Bb}	0.087	48.67^{**}
Thrombogenic index	1.42^{B}	0.98^{C}	1.76^{A}	1.09^{BC}	0.150	68.86^{**}
Nutritive value	2.26^{b}	2.66^{a}	2.14^{Bb}	2.66^{Aa}	0.306	10.40^{**}

[1]standard deviation of mean. On row: $^{A, B, C}$: $P < 0.01$; $^{a, b, c}$: $P < 0.05$; **: $P < 0.01$; *: $P < 0.05$.

transformation of linoleic acid, the substrate for the activity of enzymes such as Δ^6 and Δ^5 desaturase and elongase (72).

The PUFA/SFA ratio was significantly ($P < 0.01$) higher for the faba bean group meat and lower for the lupin group meat; however, our data contrast with those of Nürberg et al. (73), who report that this ratio decreases as meat fattiness increases.

The soybean group presented a higher n-6/n-3 ratio (5.64) than faba bean or lupin ($P < 0.05$) and higher ratio than pea ($P < 0.01$). This ratio is important for human health, since it represents the risk factor for tumours and coronary diseases (6), and should not exceed 4. Our data, therefore, have shown that soybean has a negative effect on this ratio, although lupin has an even more negative effect, with significantly higher atherogenicity and thrombogenicity indexes and lower nutritive value of meat lipids. For all groups, we found nutritive values of the meat lipids (stearic acid + oleic acid/palmitic acid) within the range (2 - 3) reported by other authors (74, 75). The nutritive value expresses the variations in the fatty acid content and enables an evaluation of the effects of lipid consumption on human health, since palmitic acid (C16:0) determines increases in circulating cholesterol, stearic acid (C18:0) has no such effects, and oleic acid (C18:1-n-9*cis*) reduces it (36). The faba bean and pea group meats gave the highest nutritive values, which were significantly different from the soybean group (P<0.05), and especially from the lupin group (P<0.01), indicating that faba and protein pea feeds give the healthiest meats.

Conclusions

The results of this study indicate that:

- feed did not influence the physical characteristics of the meat;
- the meat of the lambs fed with faba bean contained more fat and less ash;
- the use of pea, and especially of faba bean, improves the healthiness indexes of the intramuscular fat, unlike lupin.

Further studies will be able to determine the optimum percentages of these protein sources to include in feeds, and to evaluate the effects of their use in association and/or after technological seed processing.

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