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Use of faba bean as a replacer of soybean meal in diet of Fabrianese lambs

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Research

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CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

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ABSTRACT

Most of the lamb diets are based on soybean meal as the main protein source, often imported and derived from genetically modified varieties, which is not possible to use in organic meat production.

Use of faba bean to replace soybean meal as main protein source in the diet of Fabrianese lambs was investigated. Growth performance, dressing percentage, physical and chemical characteristics of *longissimus thoracis* and its fatty acid composition were investigated.

Twenty-four Fabrianese entire male lambs were weaned at 59±5 days of age and were divided into two homogeneous groups (n = 12), then fed for 78 days with two different experimental diets. One group received a concentrate including 242 g/kg of faba bean (FB), the other group was fed on concentrate including 160 g/kg of soybean meal (SBM).

Carcass quality, meat chemical composition and meat tenderness were not influenced by dietary treatment. The total amount of n-3 fatty acids was significantly ($P < 0.05$) higher in lambs fed with FB, with a consequent more favourable n-6/n-3 ratio. Conjugated Linoleic Acids (CLA) content was significantly ($P < 0.05$) higher in meat obtained from lambs fed FB diet.

FB can replace SBM in concentrate mixtures for fattening lambs without adverse effects in growth performances and in carcass quality, causing a significant improvement of meat acidic profile, particularly considering n-3 fatty acids and CLA content.

Key words: Faba beans, soybean meal, meat tenderness, fatty acids, CLA

INTRODUCTION

Most of the lamb diets in Europe are based on soybean meal as the main protein source, and cereal grains as carbohydrate source (Priolo et al. 2003). Soybean meal is widely used in livestock feeding, it is often imported and derived from genetically modified varieties, which is not possible to use in organic meat production (Council Regulation No. 834/2007; Commission Regulation No. 889/2008). The growing demand of European consumers for organic meat has led in the recent years to an increase in the number of organic livestock farms (Thøgersen 2010). Along with this increase comes a need to optimize meat production systems, especially feeding costs. Use of legume seeds to replace soybean meal as main protein source in lamb diets could help farmers in reducing feeding costs and produce organic meat (Wilkins and Jones 2000). Moreover, the ecological role of legume crops to reduce nitrogen depletion in soil is consistent with organic rules (Caballero 1999).

Faba bean (*Vicia faba* var. *minor*) is a legume seed largely diffused in Europe, its production is relatively cheap compared to the nutritional value (Lanza et al. 2001), protein content appears to be reasonably well balanced considering the presence of amino acids, with the exception of sulphur amino acids (Ortiz et al. 1993). Previous studies have shown that partial or total replacement of soybean in the diet with alternative legume seeds such as faba bean has no adverse effects on animal growth or carcass and meat quality (Purroy et al. 1992, Antongiovanni et al. 2002, Bonanno et al. 2012). Nevertheless, the faba bean-fed lambs had a lower lean deposition compared to the soybean-fed animals; besides, meat from lambs fed faba bean diets showed better results on sensory analysis, leading to a higher acceptability compared to the meat of animals offered the soybean diet (Vasta et al. 2008).

Following consumer requirements, animal feeding strategies are nowadays oriented either in decreasing saturated fatty acids and enhancing polyunsaturated fatty acids, especially of n-3 series, and conjugated linoleic acid (CLA) in meat production (Wood et al. 2008, Lanza et al. 2011). Despite the ability of the rumen microorganisms to partially hydrogenate dietary polyunsaturated fatty acids, increasing saturated fatty acids in animal tissue, diet could play an important role in changing intramuscular fatty acid composition (Wood and Enser 1997, Priolo et al. 2001).

The aim of this study was to investigate the effects of the use of faba bean as a replacer of soybean meal in diets on growth performance and on carcass and meat quality, with particular care to fatty acid composition, of 24 Fabrianese lambs slaughtered at 145 days of age.

MATERIALS AND METHODS

Animals and diets

Twenty-four male entire lambs of Fabrianese breed were used, born on the same farm located in the Marche Region, Italy (43° 07' latitude and 13° 05' longitude) within a 6-day period and reared on their mother's milk until weaning at 59±5 days of age, then stratified according to average live weight (kg 19.7±2.34). From the second week of age a starter commercial concentrate (20% Crude Protein) was offered to them together with grass hay (about 250 g/head/d). After weaning lambs were divided into two groups, 12 lambs per group, and housed by diet in two adjacent identical pens. From day 60 to 67 of age, lambs were gradually adapted to the experimental diet. The diets were based on polyphite hay and concentrate (30/70). In detail, the control group (SBM) received a concentrate that comprised mainly barley, wheat, dehydrated lucerne and soybean meal. The lambs fed with Faba bean (FB) received diets in which all soybean meal and part of the wheat were replaced by 24% of faba bean. Moreover, the concentrate fed to the FB group contained small quantities of gluten corn, to obtain isoproteic and isoenergetic diets. Water was accessible at all times, feed offered and refusals were recorded once daily. Samples of the offered feed were analysed for dry matter, ash, ether extract, crude protein, determined, respectively, according to the methods: 984.13, 920.39 and 942.05 of AOAC (1995). Structural carbohydrates such as neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991). The lambs were individually weighed at weekly intervals prior to feeding in order to calculate average daily body weight gain and feed

conversion ratio. The final live body weight was registered directly in the slaughter house after 12 h food deprivation (water was allowed).

Slaughter procedure, carcass evaluation and muscle sampling

Animals were slaughtered according to European Union Regulations (Council Directive 93/119 EEC) at an approved abattoir at 145 days of age, by throat cut after captive bolt stunning. The viscera were removed and their contents were weighed in order to determine empty body weight. Warm carcass weights were recorded immediately after slaughter, then carcasses were transferred to a cold room at a temperature of 3°C. Carcass dressing percentages were calculated in all the animals used in this experiment 1 h post mortem dividing carcass weight for final live body weight. Twenty-four h after slaughter, samples of *longissimus thoracis* muscle were collected from the right side of each carcass, removed at the level of 13th thoracic rib. Samples (70 g for each animal) were vacuum packaged and stored at -20°C until analysis.

Chemical and physical analysis

Meat pH was measured 1 h and 24 h after slaughter inserting a pH-meter probe (Portamess Knick mod. 910) into the muscle *longissimus thoracis* 2.5 cm below the dorsal surface adjacent to the thirteenth vertebra (Polidori et al. 2008). For chemical analysis, moisture, and fat were determined on muscle *longissimus thoracis* using the methods described by AOAC (1995), while protein content was calculated by difference (100 − water − fat − ash).

Cooking loss was evaluated according to Lanza et al. (2003). Samples of m. *longissimus thoracis* were weighed and held in plastic bags and immersed in a water bath set to 75°C temperature until internal temperature reached 75°C as monitored with a thermocouple. Then the bags were cooled under running tap water for 30 min and blotted dry with paper towels and reweighed. Cooking losses were determined, as percentages, from the difference between the weights.

Colour parameters were measured 24 h after slaughter on a fresh surface of muscle *longissimus thoracis* using a Minolta CR-200 colorimeter, with the Hunter-Lab method, in order to determine L* (lightness), a* (redness) and b* (yellowness). After placing the measuring lens on the meat surface, it was turned through 0, 45 and 90° (clockwise) to obtain three different reflectance measurements that were later averaged. Chroma (C*) was calculated as: $(a^{*2} + b^{*2})^{1/2}$, while Hue angle (H*) was calculated as $\tan^{-1}$ (b*/a*), according to Priolo et al. (2000).

Fatty acid composition was determined firstly extracting total lipids from the meat following the procedure described by Bligh and Dyer (1959). Later, for the preparation of fatty acids methyl esters the lipid sample (20 mg) was dissolved in 0.1 ml of tetrahydrofuran in a test tube and 10% methanolic hydrogen chloride (2 ml) were added (Sukhija and Palmquist 1988). The sample was sealed and heated at 100°C for 1 h. To each sample 2 ml of 1 M potassium carbonate solution was added. The fatty acids methyl esters were extracted with 2x2 ml of hexane and 1 ml was injected into a gas-chromatograph. Fatty acid analysis was performed on a Chrompack (model CP 9003, Agilent Technologies, California, USA) gas chromatograph with a flame ionization detector and a fused-silica capillary column, film thickness 0.2 mm, packed with CP Sil 88 (100 m x 0.25 mm i.d.). Helium was used as the carrier gas and the column temperature was held at 80°C for 4 min, and then increased at 10°C/min from 80°C to 140°C, then at 5°C/min from 140°C to a final temperature of 210°C (held for 14 min). Fatty acid identification was made by comparing gas chromatographic retention times with the anti-oxidant standard butylated hydroxytoluene (BHT).

Samples designated for shear force determination were removed from the carcasses four days post-mortem. Steaks (each 2.5 cm thick) were obtained from the mid-region of each sample, and roasted on a metal tray at an oven temperature of 180 °C to an internal temperature of 73 °C (monitored with thermocouples). Steaks were cooled to room temperature (25 °C) for 30 min. From each sample, eight cores (1.3 cm in diameter) were removed, and shear force determination was obtained using an Instron apparatus 4411 (Instron, High Wycombe, UK) with a Warner-Bratzler shear device and crosshead speed set at 200 mm/min (Polidori et al. 2015). Peak or maximum shear force

across the fibres was expressed in kg/cm^2.

Statistical analysis

Data were processed by ANOVA to compare the two diets (SAS 2001) using the statistical model:

$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$

where:

y_{ij} = experimental data; μ = overall mean; α_i = effect of diet (i = 1,2); ε_{ij} = error.

Significant differences between means were indicated when $P<0.05$.

RESULTS & DISCUSSION

Chemical composition of the diets

The ingredients and chemical composition of the diets are reported in Table 1; the two diets had similar crude protein and fat contents. Amino acid profile for FB and SBM has been described in detail by Lanza et al. (1999); in SBM there is an interesting content of histidine, leucine, lysine, tyrosine and valine that are not present in FB, while the content of the other amino acids can be considered well balanced. With regard to dietary fatty acid composition (Table 2), FB had higher contents of the two essential fatty acids, linoleic (C18:2 n-6) and linolenic (C18:3 n-3) compared to the SBM diet. Legumes have the potential to supply polyunsaturated fatty acid (PUFA), which can either bypass or be subject to biohydrogenation in the rumen (Turner et al. 2012). Dietary PUFA bypassing the rumen can reduce the proportion of hypercholesterolemic saturated fatty acid (SFA), namely 16:0, while increasing proportions of hypocholesterolemic monounsaturated (MUFA) and PUFA in ruminant meat (Williams 2000).

Table 1. Ingredients and chemical composition of the diets.

Ingredient (g/kg as fed)	Group SBM	Group FB
Barley	378	370
Lucerne dehydrated	177	168
Soybean meal 44	160	---
Wheat	199	122
Faba bean	---	242
Corn gluten	---	38
Sunflower meal	22	20
Cane molasses	35	15
Calcium carbonate	17	13
Sodium chloride	6	6
Vitamin mineral premix	6	6
Chemical composition		
Dry Matter (%)	86.8	86.7
Crude Protein (% DM)	19.6	19.3
Ether Extract (% DM)	2.32	2.28
Ash (% DM)	8.28	7.39
NDF (% DM)	26.5	25.0
ADF (% DM)	12.5	13.1
ADL (% DM)	2.87	3.53

Table 2. Fatty acid composition of the complete diets (g/100 g fatty acid methyl esters).

	SBM diet	FB diet
C12:0	0.58	0.55
C14:0	0.66	0.57
C16:0	12.36	11.00
C16:1	2.63	2.26
C18:0	2.91	2.27
C18:1 n-9	26.35	24.43
C18:2 n-6	52.03	54.58
C18:3 n-6	0.46	0.38
C18:3 n-3	2.02	3.96

Growth and slaughter performance

No significant differences in growth and slaughter performance were found among treatments (Table 3). Dry matter intake and feed conversion ratio were similar among the two groups, confirming the results obtained by Caballero et al. (1992) in a study in which soybean meal was replaced by faba beans at different levels. Average daily gain was 186.0 g/day in FB group and 198.8 g/day in SBM group, resulting in final weights respectively of 35.9 kg and 36.7 kg. Feed conversion ratio was similar between the two groups, but higher compared to those obtained by Lanza et al. (2003) in Barbaresca lambs slaughtered at the same final body weight, confirming the slow growth rate typical of Fabrianese lambs (Polidori et al. 2017). Warm carcass weight was 19.5 kg in FB group and 20.4 kg in SBM group; dressing percentage was 54.3% and 55.6%, respectively in FB and SBM group. These results are consistent with those obtained by Lanza et al. (2001) in a study where fattening lambs received different diets based on faba beans.

Table 3. Growth and slaughter performances.

Traits	SBM diet (n = 12)	FB diet (n = 12)	SEM	*P* - value
Live weight at weaning (kg)	19.9	19.5	1.98	n.s.
Live weight at 67 d (kg)	21.2	21.4	1.86	n.s.
Final live weight (kg)	36.7	35.9	1.46	n.s.
Average daily gain 67-145 (g/d)	198.8	186.0	0.023	n.s.
Dry matter intake 67-145 (g/d)	1132	1140		
Feed conversion ratio (g DM/g gain/d)	5.69	6.13		
Warm carcass weight (kg)	20.4	19.5	0.790	n.s.
Dressing percentage (%)	55.6	54.3	0.527	n.s.

n.s.: not significant
SEM: standard error of mean.

Meat chemical and physical characteristics

Protein source did not influence the physical and chemical parameters of muscle *longissimus thoracis* (Table 4). Chemical composition of muscle *longissimus dorsi* was not affected by diet, as determined in a previous study in which soybean meal was replaced with faba beans and peas in diets for Merinizzata italiana male lambs slaughtered at a similar age (Scerra et al. 2011).

Ultimate pH was not significant different between groups, slightly higher in SBM lambs (5.58 vs 5.52), within an accepted range of ultimate pH for meats; values higher than 5.6 can have a negative effect on lamb meat odour and flavour (Braggins 1996). Shear force values measured in Fabrianese meat samples were lower compared to those obtained in a study on Italian Merino male lambs slaughtered at the same age and recorded on the same muscle (Caparra et al. 2007). Colour parameters were not affected by diet and were similar to those obtained in other Mediterranean breeds (Sañudo et al. 1996, Lanza et al. 1999, Lestingi et al. 2015). Similar pH values and the same slaughter age played a major role in determining meat colour compared to diets effect. Cooking loss was not statistically different in meat obtained from lambs fed either FB or SBM. The similar carcass weight and the comparable protein content of the diets influenced this quality trait more than different protein source, as already stated by Kemp et al. (1976).

Shear force values measured on the cooked meat in this study were similar to those obtained in previous trials on lambs slaughtered at the same age (Scerra et al. 2001), within a range of acceptability by the consumers (Polidori et al. 2016).

Table 4. Effects of FB and SBM on muscle *longissimus dorsi* of Fabrianese lambs.

	FB (n = 12)	SBM (n = 12)	SEM	*P* - value
pH_1	6.63	6.71	0.13	n.s
pH_{24}	5.52	5.58	0.03	n.s.
Moisture (%)	74.8	75.2	5.86	n.s.
Fat (%)	3.91	4.04	6.65	n.s.
Protein (%)	19.8	19.3	3.31	n.s.
Ash (%)	1.49	1.46	0.36	n.s.
Cooking loss (%)	21.5	22.6	0.89	n.s.
WBSF (kg/cm^2)	3.85	4.32	0.47	n.s.
L* (lightness)	46.1	44.9	1.64	n.s.
a* (redness)	16.9	18.2	0.93	n.s.
b* (yellowness)	7.51	8.18	0.78	n.s.
C* (chroma)	9.62	10.5	0.86	n.s
H* (Hue angle)	23.6	22.6	1.75	n.s.

n.s.: not significant

SEM: standard error of mean.

WBSF: Warner-Bratzler Shear Force

Table 5. Fatty acids composition determined in muscle *longissimus dorsi* of lambs fed with different diets.

	Diet			
Fatty acid (g/100 g total fatty acids)	FB (n=14)	SBM (n=14)	SEM	*P*- value
C10:0	0.09	0.11	0.023	n.s.
C12:0	0.17	0.18	0.010	n.s.
C14:0	2.91	3.37	0.072	n.s.
C14:1 cis 9	0.71	0.63	0.089	n.s.
C15:0	0.30	0.37	0.654	n.s.
C16:0	23.34	24.82	0.352	n.s.
C16:1 cis 9	0.32	0.37	0.037	n.s.
C17:0	1.24	1.33	0.062	n.s.
C17:1 cis 9	0.67	0.64	0.078	n.s.
C17:1 trans 10	0.21	0.32	0.096	n.s.
C18:0	14.35	14.14	0.551	n.s.
C18:1 n-9	36.24	35.62	0.765	n.s.
C18:1 trans 11	0.16	0.10	0.097	n.s.
C18:2 n-6	8.14	8.21	0.576	n.s.
C18:2 cis 9, trans 11	1.54	0.85	0.067	0.040
C18:3 n-3	2.67	1.86	0.097	0.036
C20:0	0.11	0.14	0.063	n.s.
C20:1 cis 11	0.31	0.74	0.058	0.037
C20:3 n-3	0.06	0.10	0.034	n.s.
C20:4 n-6	3.06	2.82	0.207	n.s.
C20:5 n-3	1.54	1.41	0.054	n.s.
C22:0	0.03	0.02	0.032	n.s.
C22:4 n-6	0.18	0.21	0.041	n.s.
C22:5 n-3	1.12	1.23	0.075	n.s.
C22:6 n-3	0.49	0.41	0.017	n.s.
SFA	42.54	44.48	0.552	0.041
MUFA	38.62	38.42	0.637	n.s.
PUFA	18.80	17.10	0.479	0.048
PUFA/SFA	0.44	0.38	0.032	0.049
Σ n-3	5.88	5.01	0.093	0.046
Σ n-6	11.38	11.24	0.588	n.s.
Σ n-6/ Σ n-3	1.93	2.24	0.209	0.049

n.s.: not significant
SEM: standard error of mean.

Fatty acid composition

Results obtained for fatty acid profile in muscle *longissimus thoracis* taken from Fabrianese lambs are shown in Table 5. In both the two groups of lambs, the most abundant fatty acid was oleic acid (18:1 n-9), followed by palmitic acid (C16:0). Oleic acid contents in muscle were greater than those provided by the diets, probably because D^9-desaturase enzyme activity allows lambs to desaturate stearic acid (C18:0) to oleic acid (Lestingi et al. 2015). The levels of α-linolenic acid (C18:3 n-3) were significantly higher ($P < 0.05$) in meat from animals receiving FB diet compared to SBM diet. The higher C18:3 n-3 content in meat obtained from lambs fed FB diet compared to lambs fed SBM diet reflected the higher levels of this fatty acid found in faba bean meal compared to soybean meal, as previously demonstrated by other authors (Lanza et al. 2011, Turner et al. 2012).

Lambs fed with FB diet showed a significant ($P < 0.05$) higher amount of PUFA (18.24 g/100 g total fatty acids) compared to SBM diet (16.38 g/100 g total fatty acids), mainly due to C18:2 n-6 and C20:4 n-6 content (Table 5). A previous study (Lestingi et al. 2015) has already demonstrated that FB has a positive effect on the fatty acid composition, increasing the n-6 PUFA content, specifically of linoleic acid (C18:2 n-6). Eicosapentaenoic acid (C20:5; EPA) was slightly higher in FB meat than in SBM, while docosahexaenoic acid (C22:6 n-3; DHA) was determined in similar amount in both groups. The PUFA/SFA ratios were 0.36 and 0.42 for lambs obtained by SBM and FB lambs, respectively. These values were very similar to those determined by Garcia et al. (2008) in Argentinean lambs slaughtered at 30 kg of final body weight, and were lower than 0.45, which is considered appropriate for the human diet (Lorenzo et al. 2010).

Among conjugated linoleic acid isomers (CLA), a number of health benefits have been attributed to rumenic acid (cis 9, trans 11 C18:2), the major isomer present in ruminant meats (Garcia et al. 2008), including prevention of carcinogenesis, atherosclerosis and obesity (Belury, 2002). Rumenic acid content was significantly ($P < 0.05$) influenced by diet, with higher content (1.04 g/100 g total fatty acids) in lambs fed FB compared to SBM animals (0.85 g/100 g total fatty acids). A study performed with the aim of comparing different protein sources in diets of growing lambs (Bonanno et al. 2012) found an increase in the total amounts of CLA isomers in the intramuscular fat of lambs fed with faba bean compared to the other diets. Conversely, Lanza et al. (2011) did not find significant effects on CLA rumenic acid contents in meat of lambs receiving different protein sources, including FB ad SBM. CLA is produced naturally in ruminants from dietary linoleic acid; elevating dietary intakes of PUFA increase CLA content of meat (Mulvihill 2001), as confirmed in the present study.

As a consequence of higher levels of polyunsaturated fatty acids n-3 in meat obtained from lambs fed with FB, the n-6/n-3 ratio was significantly affected by diets ($P < 0.05$), showing a lower value in FB lambs (1.93) compared to SBM ones (2.24). The n-6/n-3 ratio is modified by the kind of food consumed; lambs eating grass display higher n-3 PUFA levels, while the proportion of n-6 PUFA increases in those fed concentrates (Garcia et al. 2008). The FB diet showed (Table 2) a higher content of C18:3 n-3 linolenic acid (3.96 g/100 g fatty acid methyl esters) compared to SBM diet (2.02 g/100 g fatty acids methyl esters); according to the results obtained in previous studies (Lanza et al. 2011, Scerra et al. 2011), the higher amount of linolenic acid in diet responsible for the higher content of this fatty acid in lamb meat.

CONCLUSION

Protein is a critical nutrient for young growing animals and is normally the most expensive component of diet. The high price of imported soybean, fluctuations in the price, distribution as genetically modified food, have increased the consumer interest in alternative sources of protein. The results of this study confirmed that FB can be used as an alternative source of protein without adverse impacts on lamb growth performance, feed conversion rate, carcass and meat quality. Use of FB can contribute to an appreciable reduction of feeding costs in fattening lambs, and in this experiment, performed for the first time on Fabrianese lambs, was achieved an interesting result, related to the lipid profile of lamb meat, with a significant increase of the CLA and n-3 fatty acids contents in

lambs receiving FB. In organic lamb meat production, SBM in the concentrate can be replaced with FB, that represents a cheap protein source without Genetically Modified Organisms. Further studies are necessary in other meat producing animals, such as beef, pork, etc., in order to compare the effects of FB in other species different from lambs; it will be very important to understand the digestive mechanisms of FB compared to SBM in ruminants and monogastric species, too.

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