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Fatty acid composition and conjugated linoleic acid of meat from lambs fed diets containing moist-heat treated legume grains

J. Seifdavati[1*] and R. Seyedsharifi[1]

[1]*Department of Animal Science, University of Mohaghegh Ardabili, Ardabil, 56971, Iran*
**Corresponding E-mail: jseifdavati@yahoo.com*

ABSTRACT

This experiment was carried out to study on nutritional manipulation of the saturated fatty acid composition of lamb meat. 42 lambs diets of the seven groups differed in the protein source used in the concentrate, soybean meal group (SBM), bitter vetch group (BV), common vetch group (CV) and chickling vetch group (CLV). The linolenic acid was higher in meat from CV lambs than in meat from SBM, BV and CLV animals. In meat from CV lambs, CLA was higher than in meat from lambs of the other groups. Lambs fed Whole legume seeds-hay diets, inclusion of CV grain, as dietary oil seed with hay during fattening led to an increase in CLA content from 1.15 (SBM diet) to 2.23(CV diet) g/100 g fatty acid methyl esters. The use of legume seeds such as bitter vetch, common vetch and chickling vetch in lamb diets positively affected intramuscular fatty acid composition.

Keywords: Intramuscular fatty acid composition; Legume seeds; Meat Lamb; Rumenic acid.

INTRODUCTION

In recent years, the use limitations of animal by product, such as meat meals due to government regulations and consumer demand have been led to an increase in the use of plant protein sources. Soybean meal is the main plant protein source used in animal feeding and it is largely imported from Brazil, has recently been questioned. In addition, in animal feeding, so that efforts to reduce the costs. Therefore, an important objective of world farmers has been to increase the use of plant protein sources, preferably from local feedstuffs. Few studies of using locally legume grains in lamb nutrition have been studied. Several reports seemed to suggest that their use had no negative impact on meat quality such as fatty acid composition [1-3]. Locally produced legumes as alternative protein sources in the diets of ruminants, commonly used in worldwide, peas, field beans, types of vetch and rapeseed. Types of vetch, such as, bitter vetch (*Vicia ervilia*.L), common vetch (*Vicia sativa*.L) and chickling vetch (*Lathyrus sativus*.L) grains are the legume seeds available in the west north area of Iran and are comparatively cheap despite its relatively high nutritional value. One of the strategies of increasing functional food availability to increase polyunsaturated fatty acids, especially the ω-3 series, conjugated linoleic acid (c9,t11-CLA) level and reduce saturated fatty acids in animal products [2, 4]. Although there is a vast amount of literature available about the CLA content of milk, a few research trials focusing the CLA content of meat is limited. The CLA isomers appear to be concentrated in intra-muscular and subcutaneous fat of meat ruminants and the concentration of c9, t11-CLA being greater than the concentration of t10, c12-CLA in all tissues, but the proportion of the latter CLA isomer is greater in subcutaneous fat [5]. Of the many isomers identified, the cis-9, trans-11 CLA isomer (rumenic acid) accounts for up to 80-90% of the total CLA in ruminant products [6]. However, the amount of the CLAs found in milk and meat are small, relative to the recommended daily intake for appreciation of health benefits in humans, which is 3500 mg/d [5]. There is little data available on the effects of feeding types of vetch grains on lamb intramuscular fatty acid composition. The objective of the present study was to evaluate the effect of totally replacing dietary soybean meal

by bitter vetch (BV), common vetch (VC) and chickling vetch (CLV) grains in the concentrate fed to lambs on the intramuscular fatty acid composition of their meat.

MATERIALS AND METHODS

Experimental design, animals and diets

The feeding trial was conducted at an experimental farm of a region in northwestern Iran. The experiment was conducted on forty-two crossbred (Gizel×Merino and Moghani×Merino) ewe lambs, 60±5 days old, were divided into seven treatment groups balanced according to their weights, with 6 animals per treatment and were stratified according to weight (kg 18-25, live weight), housed in individual pens and randomly assigned to one of seven dietary treatments [treated and untreated CV, 6 animals; treated and untreated BV, 6 animals; treated and untreated CLV, 6 animals; SBM as control, 6 animals]. The lambs, after 10 days of adaptation to the experimental diet, fed for a further 84 days. All the diets were included maize, barley, lucerne hay, salt, di-calcium phosphate and mineral-vitamin premix. Seven isoenergetic and isonitrogenous experimental diets (legume grains) were formulated each one with 30% of the total protein from one of the experimental legume grains (Table1). Legume grains diets were included in total replacement of soybean meal and in partial replacement of maize and barley. SBM diet was considered as the control diet and included soybean meal as the main protein source. All the ingredients were ground and compounded into lucerne hay as TMR feed. The diets were formulated to meet requirements for the ewe lambs [7].

Lambs were slaughtered commercially at about 145 days of age (60 days of weaning, 10 days adaptation and 75 days of experimental period). Twenty-four hours after slaughter carcasses were split in left and right sides and from the right side, samples of *longissimus dorsi* muscle were collected at the level of the 13th thoracic rib, minced and vacuum-packed (50 g for each animal). Samples were frozen and transported on dry ice and stored at −80 °C until moisture, crude fat, protein and ash measurement according to AOAC procedures [8].

Fatty acid determination

Samples FA composition of *longissimus dorsi* muscle was determined after chloroform-methanol extraction of total lipids [9]. Briefly, a 5 g homogenised *longissimus dorsi* samples were blended with chloroform/methanol (2:1, v/v) twice, filtered, placed in a separator funnel and mixed with saline solution (0.88% KCl). After separation into two phases, the aqueous methanol fraction was discarded and the chloroform lipid fraction was washed with distilled water/methanol (1:1, v/v). After a further filtration and evaporation by means of a rotary evaporator, lipid extracts were transferred to test tubes for subsequent gas chromatographic analysis. Duplicates of 100 mg of lipid were methylated using 1 ml of hexane and 0.05 ml of 2 N methanolic KOH [10]. Separation of FAME were also performed on a gas chromatograph [11] model HP 6890 coupled with a 5973 mass spectrometer detector and HP 7683 Series Injector [11]. 1 µl of the final extract was injected using pulsed splitless mode (140 kPa, 0.4 min, 250 °C).HP-5MS 30 m, 0.25 mm, 0.25 µm columns was operated at a temperature program 2.1 min at 50 °C and a subsequent increase to 200 °C at 10 °C/min and the final temperature of 200 °C to 270°C at 15 °C/min, was held for 12min with helium as a carrier gas (99.9998 %; flow rate: 0.9 ml/min; a split ratio of 50:1; inlet temperature: 250 °C; SIAD, Bergamo, Italy). FAME was identified by comparing their mass-spectral data to the mass-spectral database in the library Wiley 7.0 [11]. Fatty acids were expressed as percent of total methylated fatty acids.

Statistical analysis

Data were analysed by a factorial variance analysis to study the effect of autoclaving on the intramuscular fatty acid composition of lamb meat each of the treatment groups. The model was:

$$Y_{ijk} = \mu + \text{feedstuff}_i + \text{treatment}_j + (\text{feedstuff} \times \text{treatment})_{ij} + e_{ijk} \quad (1)$$

Where main effects, kind of feedstuff in diets (2 d.f.), autoclaving treatment (1 d.f.) and the interaction between them (2 d.f.) were compared with the residual error (5 d.f.). When there were significant differences for studied effects, differences between mean values were determined using the least significant difference test. All statistical analyses were performed by the General Linear Model (GLM) procedure using the statistical package SAS after adjusting on covariate effect (initial body weight of lambs) with covariance analysis [12].

RESULTS AND DISCUSSION

Chemical composition of the experimental diets

The ingredients, fatty acid composition and proximate analyses of the diets are presented in Table 1. The seven diets had similar crude protein contents. With regard to dietary composition (Table 1), BV and SBM diets had higher C16:0 and C18:0 contents compared to the CV and CLV diets. The CV and CLV diets had higher contents of the two essential fatty acids, linoleic (C18:2) and linolenic (C18:3) compared to the BV and SBM diets. However, the total percentage of C18:2 + C18:3 fatty acids was ranged for the seven diets from 56.6 g to 57.05g /100 g of fatty acid methyl esters. The fatty acid composition of *longissimus dorsi* muscle partially reflected the dietary fatty acid composition. Ruminants do not deposit tissue FA in proportion to dietary lipid composition, as do non-ruminant animals such as pigs, poultry, farmed fish and horses, because rumen microorganisms hydrolyse the glycerides and then hydrogenate the dietary polyunsaturated fatty acid (PUFA) [13] and [14]. Thus, ruminant milk or meat has higher SFA/ PUFA than non-ruminant animals.

Table1. Ingredient, chemical composition (g/kg dry matter) and fatty acid composition (g/100 g fatty acid methylesters) of the experimental diets containing soybean meal (SBM), and raw or moist-heat treated bitter vetch (BV), common vetch (CV) and chickling vetch (CLV) grains

Ingredient	Diets [A]			
	SBM	**Raw or Heated CLV**	**Raw or Heated CV**	**Raw or Heated BV**
Alfalfa hay	417	417	416	416
Barley grain	443	421	423	424
Maize grain	58	53	52	51
Soybeans meal(SBM)	69	–	–	–
Chickling vetch (CLV)	–	97	–	–
Common vetch (CV)	–	–	96	–
Bitter vetch (BV)	–	–	–	96
UREA	–	7	5	9
Di Calcium Phosphate	5	5	5	6
Salt	8	8	8	8
Vitamin, mineral premix [B]	5	5	5	5
Chemical composition				
Dry matter/kg fresh matter	858	857	856	855
Crude protein	163	160	162	163
Rumen Degradable protein (RDP)	107	107	108	107
Rumen Undegradable protein RUP	57	53	54	55
Metabolisable Protein	104	104	104	104
Ether extract	28	28	29	29
Neutral detergent fibre	366	390	397	400
Acid detergent fibre	228	230	234	238
Gross energy, (MJ/kg DM)	18.5	18.1	18.1	18.0
Metabolisable Energy,(MJ/kg DM)	13.1	13.1	13.1	13.1
Fatty acid composition				
C14:0	0.65	0.5	0.64	0.52
C16:0	17.83	15.54	17.18	17.45
C18:0	3.76	3.38	3.62	3.68
C18:1 n-9	21.15	20.55	21.51	21.32
C18:2 n-6	43.58	45.49	44.15	43.96
C18:3 n-3	13.03	14.54	12.90	13.07

[A]CLV, CV and BV diets with 30% of the total protein supplied by raw or autoclaved CLV, CV and BV grains.

[B] Vitamin, mineral pre-mix (g/kg): 220 bicalcic phosphate, 220 magnesium oxide, 20 zinc sulphate, 20 ferric sulphate, 8 manganese sulphate,7.5 copper sulphate, 385 sodium bicarbonate, 0.45 of a mixture of vitamins A and and D_3, 0.8 vitamin E 50%, 0.45 copper and, (mg/kg): 0.45 sodium selenite, 1.5 potassium iodide, 1.5 cobalt sulphate.

Intramuscular fatty acid composition

Table 2 provides the fatty acid profile of *longissimus dorsi* muscle. Among the saturated fatty acids, there was a significant difference in C16:0 contents, it was higher ($P<0.001$) in meat from SBM and BV lambs than in meat from CLV and CV lambs. Also C18:0 was higher ($P<0.01$) in meat from CLV lambs compared to meat from the rest of lamb groups. Among the BV and SBM groups the level of palmitic acid (C16:0) was higher ($P<0.01$) in meat from SBM lambs. The fatty acid most abundant in the meat from all legume groups, as the protein source in the

concentrate, was cis-9 C18:1 (oleic acid), the amounts of which were significantly (P<0.01) different between treatments. Similar results for this fatty acid were observed by Lanza et al., (2003b), Wood et al., (2008) and Scerra et al., (2011) for other legume grains as the protein source in the dietary concentrate. Overall, the linolenic acid (C18:3 ω-3), was higher (P<0.01) in meat from CV lambs than in meat from SBM, BV and CLV animals.

Table2. Fatty acid (g/100 g fatty acid methylesters) and chemical composition of lamb meat (longissimus dorsi muscle) fed diets containing soybean meal (SBM), and raw or moist-heat treated bitter vetch (BV), common vetch (CV) and chickling vetch (CLV) grains

	Experimental Diets [A]							SEM	P-value
	SBM	Raw legume grains			Autoclaved legume grains				
		CLV	CV	BV	CLV	CV	BV		
Fatty acid composition									
C14:0	2.46	3.17	3.40	3.54	3.65	4.22	3.7	0.85	0.09
C16:0	23.43^{a}	13.22^{c}	13.37^{c}	21.28^{a}	13.49^{c}	17.73^{ab}	21.18^{a}	1.75	<.001
C18:0	15.27^{b}	13.66^{bc}	10.06^{c}	11.88^{bc}	18.14^{a}	11.32^{bc}	12.99^{bc}	1.94	0.001
C18:1 n-9	34.78^{a}	31.3^{b}	32.5^{b}	34.09^{ab}	35.5^{a}	33.34^{ab}	34.54^{a}	0.86	<.001
C18:2 n-6	11.81^{c}	15.23^{a}	13.61^{b}	14.59^{ab}	15.72^{a}	14.17^{b}	14.69^{b}	0.58	<.001
C18:3 n-3	0.09^{b}	0.09^{b}	0.11^{a}	0.06^{b}	0.08^{b}	0.10^{a}	0.03^{b}	0.03	0.01
CLA(9cis11transC18.2)	1.15^{b}	1.41^{ab}	2.23^{a}	1.94^{a}	1.66^{ab}	1.91^{a}	1.97^{a}	0.25	<.001
Chemical composition									
Moisture (%)	75.66	74.88	75.34	75.45	75.74	75.78	75.57	1.05	0.93
Ash (%)	1.23	1.37	1.32	1.31	1.27	1.32	1.27	0.05	0.10
Crude fat (%)	2.48^{b}	2.61^{b}	4.97^{a}	4.82^{a}	3.06^{b}	3.96^{a}	2.48^{b}	1.44	0.01
Crude protein (%)	20.59^{a}	21.14^{a}	18.38^{b}	18.53^{b}	19.94^{a}	18.96^{b}	18.68^{b}	1.65	0.03

[A]CLV, CV and BV diets with 30% of the total protein supplied by raw or autoclaved CLV, CV and BV grains. SEM, standard error of the mean; Different lowercase letters within a same row indicate significant differences among diets indicate significant differences (P< 0.05).

The levels of reduced palmitic (C16:0) and stearic (C18:0) acids in meat from lambs of the CLV and CV groups, respectively, compared to the other groups represent (P<0.01) potential both in decreasing the harmful effects on health because they may be directly responsible for increasing total and low density lipoproteins (LDL) cholesterol in plasma and enhancing risks for human health [4, 14]. In lamb and mutton, the proportions of these two fatty acids are more similar. There is little variation between cuts in the proportion of fatty acids. An alternative strategy to improve the human health attributes of sheep meat is to decrease tissue levels of 18:0 by increasing the activity of stearoyl-CoA desaturase (SCD), although the response is often relatively small [15]. Sheep meat is characterized as being high in saturated fatty acids and low in PUFA, attributes that are regarded as being disadvantageous within the human diet [15]. Despite early hypothesis [16] on the effects of dietary fats on human health, there were complex problems and ambiguities on the implications of saturated FA in elevated blood cholesterol leading to coronary heart disease. A meta-analysis from Hunter et al., (2010) of the evidence since 2000 resulted in one systematic review that covered all selected primary studies. This review was paying attention on the effect of stearic acid on cardiovascular disease (CVD) risks when replaced with for SFA, trans fatty acids (TFA), monounsaturated fatty acid (MUFA), PUFA or carbohydrates (CHO) and provided the evidence to deal with this question [17]. One goal of animal nutrition research is feasibility study nutritional manipulation of the fatty acid composition of sheep meat [14, 15] to reduce the concentration of these FA in meat from lambs fed legume grains and to raise the concentration of the hypocholesterolemic FA (C18:1, C18:2, C18:3). Moreover palmitic acid was higher (P<0.01) in meat from SBM and BV lambs than in meat from other lambs. The higher levels of these fatty acids in meat from BV and SBM lambs compared to CLV and CV lambs partially reflected the levels of these two fatty acids in the diets. The level of C16:1 was lower (P<0.01) in meat from the raw CLV group than in meat from the other groups. The amounts of intramuscular C18:1 for all diets was higher than the proportions in the dietary fatty acids. Fortunately, animal cells can synthesize oleic acid and its derivatives from stearic acid. Oleic acid is created by the dehydrogenation (desaturation) of stearic acid. On the contrary human, animals can be easily desaturated stearic acid (C18:0) to oleic acid by means of a Δ9-desaturase enzyme [18]. However, C18:2 was much lower in the muscle fat than in the diets, indicating that the dietary C18:2 was partially hydrogenated in the rumen [19]. The higher amount of linoleic acid (C18:2 n-6) (P<0.001) in meat from CLV lambs than in meat from BV,CV and SBM lambs was probably related to the higher level of this fatty acid in the CLV diet compared to the others. Moreover linoleic acid was higher (P<0.01) in meat from the BV and CV groups than in meat from the SBM group. The endogenous biosynthesis of this fatty acid in muscle from linolenic acid is well-known [20]. Linolenic acid (C18:3 ω-3), the precursor of long chain n-3 fatty acids that have a wide range of biological effects and which are believed to be beneficial for human

health [21-23]. The higher level of this fatty acid in the diets and also the lower level of this fatty acid in the muscle fat than in the diet, indicating that the dietary C18:3 was partially hydrogenated in the rumen [19]. In meat from CV lambs, rumenic acid (cis-9 trans-11 C18:2), more commonly known as CLA, was higher (P<0.01) than in meat from lambs of the other groups. The lambs of the CLV group showed higher levels (P<0.01) of the Linolenic acid (C18:3 ω-3), compared to the lambs of the SBM and BV groups. However, there was not the difference between the groups in terms of the CLA proportion of meat from lambs fed diets containing moist-heat treated and raw legume grains except the SBM group(P<0.01). Despite the apparent negative impact of ruminal metabolism on muscle FA content, the process of biohydrogenation is often incomplete and accompanied several of the intermediaries that can have positive effects on human health. One of the positive effects rumenic acid production (cis-9 trans-11 C18:2), known as conjugated linoleic acid (CLA), is the major geometric and positional isomer of linoleic acid. Increasing interest in CLA is attributed to its potential health benefits such as anticarcinogenic, antiatherogenic, antidiabetic and inhibition of excessive body fat accumulation in animal effects [14, 18, 24]. In addition, McGuire and McGuire (2000) gave address in its review the potential health effects of CLA in humans [25]. For the increased occurrence these effects in humans, raising the content of CLA in meat may be useful to human health as a result of nutritional manipulations in ruminant diets. In this research the level of rumenic acid was higher (P<0.001) in meat from CV animals than in meat from lambs of SBM groups, also numerically higher than all other vetches. This probably correlates with the higher level of linolenic acid in CV diets. As mentioned above, in non-ruminants, the diet profile of fatty acids is almost closely reflected in body fat, both on apparent and permeate fat [26]. Nevertheless, that effectiveness in ruminants is reduced by ruminal hydrogenation. This ruminal conversion of dietary unsaturated FA is normally not complete [27], and several studies [28-30] have shown that dietary FA composition differences can result in differences in tissue FA composition. One intermediary product of rumen biohydrogenation is CLA which is produced by rumen microorganisms from dietary linoleic acid. In animal models, CLA has been reported to prevent cancer, diabetes and atherogenesis [31-34], and is only found in ruminant animal products. In this experiment the content of CLA (cis-9 trans-11 C18:2) in lamb meat was two–three fold higher than that reported in the reviewed literature for lambs that were fed pasture and/or oilseeds [14, 35] and similar to that reported by Lanza et al., (2006) and Valvo et al., (2005) in lambs that were fed only maternal milk and as well as, reported by Tilak et al.,(2005) in lambs that were fed intact oilseed [36, 37]. Large variations in the CLA content are not only reported between animal species but also within muscles of the same species. In general, the CLA content of meat from ruminants is higher than products from non-ruminants [38]. The observed differences between the results this experiment with found other investigators can be explained with various influencing factors such as pasture compared with feedlot-finished, nature of diet in feedlot, whether the diet contained oil or oilseed, the fatty acid composition of the oil, proportion of grain and silage compared with hay, seasonal variations, animal genetics, grain type and production practices [5, 39]. Also in current study, no significantly the method of grain processing in the CLA content of meat from lambs fed diets containing moist-heat treated legume grains can be resulted from factors inherent in grain type. These results have been inconsistent with the findings of others [14]. They indicated in their report that meat from the extruded soybean-fed group had higher levels of conjugated linoleic acid compared with the other treatments. It is possible to modify the fatty acid composition of beef meat by including roasted soybean in the diet and processing exposes the oil to rumen biohydrogenation, but possibly not to the same extent as the oil from the raw soybean. So that, in contrast this found and consistent with our finding, a study of Mohammed et al., (2010) clearly demonstrated that the milk content and profile of t-18:1 and CLA isomers were more strongly influenced by the source of grain than by the method of grain processing indicating that factors inherent in grain type were responsible for the observed differences and these factors could not be modified by the routine processing methods used in farms such as anti-nutrient factors [40]. Tannins content CV grain are found as means to accumulate trans-vaccenic acid in the rumen both in vitro and *in vivo* because of their capability to inhibit bacteria from vaccenic acid to stearic acid conversion and as a result led to increase the CLA production [40]. In this study, feedlot lambs fed whole legume seeds-hay diets, especially inclusion of CV grain, as dietary oil seed with hay during both the growth and finishing phases led to an increase in CLA content from 1.15 (SBM diet) to 2.23(CV diet) g/100 g fatty acid methylesters. On the basis of the per capita sheep meat consumption of 7.6 kg per annum in Iran, the daily sheep meat intake is 37 g [41]. The CLA intake from lamb (based on results in Table 2) would range from 1.8 to 7.0 mg/d, depending on the marbling fat content of the lamb meat and whether the animals were fed diets with oil seeds supplements to deposit CLA in their tissues.

CONCLUSION

Higher CLA values in the muscle tissue of intensively finished lambs are not easily explained. To increase the CLA yield in lamb meat it is essential to provide lamb an appropriate substrate for formation of CLA. The provision of a

source of dietary linoleic acid appears to increase the CLA concentration to the greatest extent. Dietary forage such as grass or legume hay appears to facilitate the establishment of the microflora that enhance the formation and deposition of CLA in the tissues also the provision of modest amounts of grain is more conducive to CLA synthesis rather than high levels of grain. With regarding to the recommended daily intake for appreciation of health benefits in humans (3500 mg/d), this amount of CLA supplied of meat lamb will partially provide the CLA requirement for everyone under conditions this study.

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