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*Full Length Research Paper*

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

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**In recent years, an important objective of world farmers has been to enhance the use of alternative protein sources, preferably from local feedstuffs. In order to an experiment was carried out to study on nutritional manipulation of the saturated fatty acid (SFA) composition of lamb meat to reduce the concentration of SFA in meat from lambs fed legume grains and to raise the concentration of the hypocholesterolemic FA. Forty-two female hybrid lambs were divided into seven groups after weaning at about 60 days of age. The 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). Lambs were fed ad libitum and slaughtered at about 145 days of age. There were no differences in growth or carcass characteristics among the dietaries. The CV and CLV diets had higher contents of the two essential fatty acids, linoleic and linolenic compared to the BV and SBM diets. However, the total percentage of sum of these two fatty acids was ranged for the seven diets from 56.6 g to 57.05g /100 g of FA methylesters. The FA most abundant in the meat from all legume groups was oleic acid, the amounts of which were significantly (P<0.01) different between treatments. The linolenic acid was higher (P<0.01) in meat from CV lambs than in meat from SBM, BV and CLV animals. The provision of modest amounts of grain is more conducive to the oleic acid synthesis rather than high levels of grain. In conclusion the use of legume seeds such as BV, CV and CLV in lamb diets positively affected intramuscular fatty acid composition.**

**Keywords:** Autoclaving; Intramuscular fatty acid composition; Legume seeds; Meat Lamb

## INTRODUCTION

Recently, much focus has been done to increase meat with physiological functions to improve health status and prevent the hazard of diseases. The effect of the use of local legume seeds in lamb nutrition has been considered. Several studies showed that their use did not negatively affect growth, slaughter performances or meat quality (Hadjipanayiotou, 2002; Lanza et al., 2003b; Lanza, 1999; Purroy et al., 1992; Surra et al., 1992). Of the protein feeds commonly used in worldwide, peas, field beans, types of vetch and rapeseed are mainly locally produced. Types of vetch, shch as, bitter vetch

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**Table1.** Ingredient, chemical composition (g/kg dry matter) and fatty acid composition (g/100 g fatty acid methylesters) of the experimental diets.

| 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.

(*Vicia ervilia.*L), common vetch (*Vicia sativa.*L) and chickling vetch (*Lathyrus sativus.*L) seeds are the legume seeds available in the west north area of Iran and are comparatively cheap despite its relatively high nutritional value. Their crude protein content ranges from about 25 to 28 % of dry matter (Abreu and Bruno-Soares,1998; Gonzalez and Andres, 2003; Sadeghi et al., 2004, 2009; Haddad et al.,2006; Ramos Morales et al., 2008; Rezayazdi et al., 2008; Abdullah et al., 2010 and Razmazar et al., 2009).The use of diets based largely on types of vetch for lamb fattening gave similar growth performance and meat characteristics as traditional diets based on soybean meal as the main protein source (Caballero et al., 1992; Lanza et al., 1999).

New feeding strategies in animal nutrition in both ruminants and non-ruminants have as principal objectives to increase polyunsaturated fatty acids(PUFA), especially the ω-3 series, and reduce SFA in animal products (Scollan et al., 2006; Wood et al., 2008). There is little data available on the effects of feeding types of vetch on lamb intramuscular fatty acid composition. The objective of the present study was to evaluate the effect of totally replacing dietary soybean meal by BV, VC and CLV seeds 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 the University of Tabriz (Iran). The experiment was

**Table2.** Fatty acid composition (g/100 g fatty acid methylesters) of meat (longissimus dorsi muscle).

| Fatty acid | Experimental Diets [A] | | | | | | | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | SBM | Raw legume grains | | | Autoclaved legume grains | | | | |
| | | CLV | CV | BV | CLV | CV | BV | | |
| C14:0 | 2.46 | 3.17 | 3.40 | 3.54 | 3.65 | 4.2 | 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 |

[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).

conducted on Forty-two female gizel×merino and moghani×merino lambs, born on the same farm. After weaning, at 60 days of age, the lambs were divided into 7 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, were fed for a further 75 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 female lambs (AFRC, 1993).

The animals were slaughtered at about 145days of age. Twenty-four hours after slaughter carcasses were split in left and right sides and from the right side samples of longissimus dorsi muscle were taken between the 6th thoracic rib and 4th lumbar rib to measure moisture, crude fat, protein and ash according to AOAC procedures

**Table3.** Effect of the diets on lamb growth rate, carcass characteristics and meat chemical composition

| carcass characteristics | Experimental Diets [A] | | | | | | | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | SBM | Raw legume grains | | | Autoclaved legume grains | | | | |
| | | CLV | CV | BV | CLV | CV | BV | | |
| Body weight at 70 d (kg) | 20.34 | 19.99 | 19.99 | 22.05 | 19.95 | 19.46 | 23.44 | 3.89 | 0.76 |
| Body weight at slaughter (kg) | 29.87 | 28.53 | 27.90 | 29.19 | 29.31 | 30.24 | 29.55 | 1.76 | 0.83 |
| Average daily gain(70-145d) (kg) | 0.121 | 0.104 | 0.098 | 0.113 | 0.111 | 0.126 | 0.119 | 0.03 | 0.83 |
| Dry matter intake (g/d) | 687 | 723 | 711 | 756 | 719 | 798 | 764 | 115 | 0.08 |
| Feed/gain (kg) | 6.58 | 6.74 | 7.85 | 7.31 | 6.82 | 6.63 | 7.04 | 1.12 | 0.45 |
| Dressing percentage | 47.35 | 46.86 | 47.7 | 46.29 | 49.96 | 47.72 | 46.53 | 2.26 | 0.52 |
| Cold carcass weight (kg) | 13.85 | 13.04 | 13.04 | 13.33 | 14.16 | 14.13 | 13.57 | 0.82 | 0.96 |
| Eye Muscle area, $cm^2$ | 13.2 | 11.73 | 11.11 | 10.57 | 10.18 | 11.97 | 11.79 | 0.04 | 0.13 |
| GR fat depth (mm) | 0.99 | 0.85 | 0.53 | 0.65 | 0.99 | 0.88 | 0.89 | 0.29 | 0.62 |
| **Longissimus dorsi characteristics** | | | | | | | | | |
| 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 ($P< 0.05$).

(AOAC, 2000). Subsamples of each concentrate mixture were collected weekly and mixed to give a final sample for NDF and ADF analysis, without the use of sodium sulphite as described by Van Soest et al. (1991) using the ANKOM NDF/ADF fiber system (2008), crude protein (method 984.13), ether extract (method 920.39) and ash (method 942.05) according to AOAC (2000).

### *Fatty acid determination*

Samples of longissimus dorsi muscle were taken at the level of the 13th thoracic rib, minced and vacuum-packed (50 g for each animal) and stored at −25 °C until needed. FA composition of meat was determined after chloroform-methanol extraction of total lipids (Folch et al., 1957). Briefly, a 5 g homogenised longissimus dorsi sample was 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 (I.U.P.A.C, 1987). Separation of FAME were also performed on a gas chromatograph (Agilent, Palo Alto, USA) model HP 6890 coupled with a 5973 mass spectrometer detector (Agilent) and HP 7683 Series Injector (Agilent,2001, Palo Alto, CA, USA). 1 µl of the final extract was injected using pulsed splitless mode (140 kPa, 0.4 min, 250 °C).HP-5MS column (30 m × 0.25 mm × 0.25 µm) 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 (HP Mass Spectral Libraries, Agilent, 2001, Palo Alto, USA). 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 the group treatment. The model was:

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

Where principal 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 (2001) after adjusting on covariate effect (initial body weight of lambs) with covariance analysis.

## RESULTS

### *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.

### *Lamb growth performances and meat proximate analyses*

The growth performances data are shown in Table 3. No significant differences were found between treatments. Average daily gain was over 98 g/day in all groups resulting in a final weight of around 28–30 kg with small differences among groups. The Animal growth performances and chemical composition of the meat samples are reported in Table 3. No statistical differences between groups were found for any parameter measured of growth performances and carcass characteristics; thus their growth rate, the DM intakes, Feed/gain and final body weight were not significantly affected by the protein source in the concentrate among the groups (Table 3). Although feed intake (P = 0.08) tended to be lower for the SBM group compared to the other treatments. There was no difference in the dry matter and crude ash contents between the experimental groups. However, chemical composition of the meat samples of lambs fed different diets showed a significant difference in crude fat and crude protein contents (P<0.01). Unlike the crude fat, the crude protein level of the meat in the SBM and CLV diets was slightly higher than in the other treatments.

### *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. (2003a), 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. 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.

## DISCUSSION

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, because rumen microorganisms hydrolyse the glycerides and then hydrogenate the dietary unsaturated FA (Harfoot and Hazlewood, 1988 and McNiven et al., 2004). Thus, ruminant milk or meat has higher SFA/ PUFA than non-ruminant animals. 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 (McNiven et al., 2004 and Scollan et al., 2006). 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 (Sinclair,2007). Sheep meat is characterized as being high in SFA and low in PUFA, attributes that are regarded as being disadvantageous within the human diet (Sinclair, 2007).Despite early hypothesis (Keys et al., 1965) on the effects of dietary fats on human health, there was 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 fat

(MUFA), PUFA or carbohydrates and provided the evidence to deal with this question (Hunter et al., 2010). One goal of animal nutrition research is feasibility study nutritional manipulation of the fatty acid composition of sheep meat (McNiven et al.,2004; Sinclair, 2007) 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 C18:1 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 (Pereira et al., 2003). 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 (Rizzi et al., 2002). 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 (Zhou and Nilsson, 2001). 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 (Kromhout, 1989; Bonanome and Grundy,1998; McAfee et al., 2010). 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 (Rizzi et al., 2002). 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. Also, several studies (Marmer et al., 1984; Chilliard, 1993; Murphy and McNiven, 1994) have shown that dietary FA composition differences can result in differences in tissue FA composition.

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 (Mir et al., 2004; Tilak et al., 2005). Also in current study, no significantly the method of grain processing in the (C18:1, C18:2) 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 (McNiven et al., 2004). They indicated in their report that meat from the extruded soybean-fed group had higher levels of oleic and 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 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.

## CONCLUSIONS

To increase the UFA yield in lamb meat it is essential to provide lamb an appropriate substrate for formation of essential FA. Dietary forage such as grass or legume hay appears to facilitate the establishment of the microflora that enhance the formation and deposition of UFA in the tissues also the provision of modest amounts of grain is more conducive to UFA synthesis rather than high levels of grain.

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