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Effect of Dietary Rapeseed Meal on Fatty Acid Profile of Lamb Carcass

Research Article

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ABSTRACT

The objectives of this study were to determine the effects of feeding rapeseed (27%, DM basis) and sunflower biodiesel by-products as protein supplements on fatty acid composition (FA), profile (ratios and indices), enzyme activities from the carcass in lambs fed cereal-based diets. Simultaneously, it was examined the relationship between ingested FA and carcass FA. Samples were obtained from male fattened lambs (n=10, Bulgarian synthetic dairy population, 146-d aged, final body weight FBW=36.95±0.91 kg) involved at two iso-caloric, iso-nitrogenous and equal in calcium: phosphorus (Ca:P) ratio dietary treatments: control–with sunflower meal (SFD) and experimental–with rapeseed meal (RSD). Lipids, extracted from carcass were analyzed for FA profile. Animals feeding RSD had significantly higher (P<0.01) performance (FBW=38.80 *vs.* 35.1 kg) and meat yield (5.26 *vs.* 4.67 kg). RSD increased C18:3 (P<0.01) and C18:3 / CLA (P<0.05), but decreased (P<0.01) C18:2 / C18:3 ratio compared with SFD. Deduced relationships between ingested dietary FA and carcass FA shows good parity and high correlation (R>0.55). In conclusion, animals feeding RSD, as a method to modify carcass FAs, decreased the amount of saturated and polyunsaturated FA, and increased monounsaturated, total unsaturated and desirable FAs of carcass.

KEY WORDS carcass, fatty acids, lamb, meal, rapeseed.

INTRODUCTION

The majority physiological roles of fatty acids are building blocks of phospholipids and glycolipids in biological membranes and fuel molecules. In this regard, nutritionists explain the essential role of dietary fat in human health (Lichtenstein *et al.* 1998; Wood *et al.* 2003). World health organization and food and agriculture organization have been recommending reduction in total fat intake (especially saturated fatty acids, FA) and greater ratio of polyunsaturated to saturated FA (PUFA/SFA) as a healthy choice for contemporary consumer. Otherwise, diets have been considered as major risk factor for obesity, cardiovascular diseases (atherosclerosis, coronary heart disease, hypercholesterolemia, etc.), cancer, etc. resulting in human mortality in developed countries (WHO, 2003; WHO/FAO, 2003). A lipid in ruminant products, especially meat, has been criticized for a great SFA and low PUFA concentration, e.g. lower PUFA / SFA ratio. Concern to augment the PUFA concentration of ruminant meat is difficult due to biohydrogenation by rumen microbes (Jenkins, 1993). So, if PUFAs are protected against microbial attack, they can be passed and absorbed in small intestines.

Several studies observed nutritional effect of diet supplement on greater UFA in ruminant muscles and fat depots (Solomon *et al.* 1991; Lough *et al.* 1992; Tripathi and Mishra, 2007). However, ruminant fat is not abundant in conjugated linoleic acid (CLA) (Gomez-Cortes *et al.* 2009). Thus, the present study was an extension of our investigations on rapeseed meal, and reported protein supplement

effects on animal metabolism and performance (Yossifov and Kozelov, 2011; Kozelov and Yossifov, 2013; Yossifov, 2013; Yossifov and Kozelov, 2013; Yossifov and Kozelov, 2014). Rapeseed meal (RSM) is a by-product of biodiesel industry, obtained after oil extraction. It is high in protein with balanced amino acid profile and neutral detergent fibre stipulated that it is the second largest animal feed meat produced after soybean meal (Emanuelson, 1994; USDA, 2010; AOF Crop Report, 2012). Higher protein bioavailability is an effective source of diet protein optimized microbial growth and ruminal fibre digestion, and adequate amounts of essential amino acids increased metabolic demands (Casper *et al*. 1999; Yossifov, 2014a).

Nitrogen characteristics indicated partial rumen protection, but no large-scale sheep feeding studies has been performed to investigate the ruminal protection of fat fraction and FA retention in carcass. Despite this, no studies have evaluated the effect of RSM on the lamb carcass FA content, profile (indices and ratios) and enzyme activities. Based upon this, the objectives of this study were to test the hypothesis that substituting sunflower, as traditional protein supplement in lamb diets, with rapeseed meal will affect FA composition, profile (ratios and indices) and enzyme activities of carcasses from lambs fed cereal-based diets. Also, the relationships between ingested FA and carcass FA were induced.

MATERIALS AND METHODS

Animals and experimental design

The study was carried out in the experimental base at the institute of animal science Kostinbrod. Ten male fattening lambs (Bulgarian synthetic dairy population, 59-d aged, initial body weight IBW=16.71 kg) were allotted to two iso-caloric, iso-nitrogenous and equal in Ca:P ratio dietary treatments (5 lambs per diet): control–with sunflower meal (SFD) and experimental–with rapeseed meal (RSD). Animals were fed twice daily to approximately 5% weigh-back to ensure *ad libitum* consumption. The concentrate (offered at 8.00 and 14.00 h) and forage (offered at 10:00 and 16:00 h) were fed separately throughout the experimental period. Diet composition is shown in Figure 1.

Slaughter and sampling

After 87-d fattening period (Yossifov and Kozelov, 2013) and FBW= 36.95 ± 0.91 kg animals were slaughtered (performance data represents the entire 87 d), dressed carcass was weighted (hot carcass weight, kg) and, after 24 h cold storage (at 4 ˚C), divided into halves. The right carcass half was weighed and dissected into compound tissues (muscle, fat and bone), expressed as absolute and relative values of the right half. Dissected muscle and fat were mixed, ground and sampled for consecutive FA analysis. Diets, forage and supplements were also evaluated for individual FA– samples were randomly collected at the begining, middle and end period of the experiment (0, 44 and 87 d).

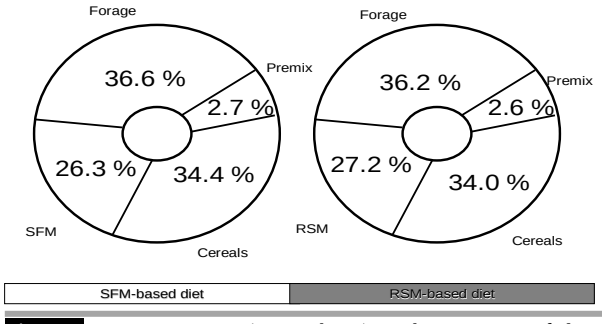

Figure 1 Diet composition (as DM basis). Both groups were fed with equal values forage/concentrate ratio (36:64). Cereal component (34%) was presented by corn and tritikale (1:1). Addopted from Yossifov and Kozelov (2013). SFM-based diet= SFD, RSM-based diet= RSD

Measurements

Total lipids of the samples were extracted in 3.8 mL of 1:2:8 (v:v:v) chloroform: methanol: water (Bligh and Dyer, 1959). Fatty acid methyl esters (FAME) were isolated by preparative TLC, using 0.01% solution of sulphuric acid in dry methanol for 14 h (Christie, 1973). The FA profile of triacylglycerols was determined by GLC analysis with chromatograph CSi 200, equipped with capillary column (TR-FAME: 60 m×0.25 mm i.d.×0.25 µm), and carrier gas–hydrogen. The initial oven temperature was 160 ˚C for 0.2 min then increasing at 5 ˚C / min up to 220 ˚C, where it was maintained for 5 min. Injector and detector temperature set points were stated at 200 ˚C. Individual FAME were identified by comparing their retention times with those of reference compounds and FA were expressed as percentages of the total peak area of the FAME identified on the chromatograms (Christie, 1973).

Statistical analysis

All obtained data are offered as mean values. The results were submitted to calculate standard error of mean (SEM) and analysis of variance (ANOVA) to assess the influence of dietary protein source (RSM *vs.* SFM) on the carcass FA profile.

Means were compared throughout the Student t-test and differences with level of significance below $P < 0.05$ were considered as significant. Pearson's correlation coefficient (R) between variables was also calculated as a measure of the strength and direction of the linear relationship between two variables. It was used MS Office 2007.

Calculations

A number of indices (fatty acid and healthy) and enzyme activities were calculated as follows: SFA or total of saturated FA (C14:0+C15:0+C16:0+C17:0+C18:0+C20:0); OFA or hypercholesterolemic fatty acids= SFA–C18:0;

MUFA or total amount of monounsaturated fatty acids= (C16:1+C18:1); n6 fatty acids= (C18:2+C18:3+C20:4); n3 fatty acids= (C18:3+C20:5+C22:5); PUFA or total of poly-unsaturated fatty acids= (n6+n3); total LC n3 or total of long-chain n3 fatty acids= (C20:5+C22:5 n3); UFA or total of unsaturated fatty acids= (MUFA+PUFA); DFA or sum of desirable fatty acids= (MUFA+PUFA+C18:0); AI or atherogenicity index= (((C14:0×4)+C16:0)/UFA); TI or thrombogenic index= (C14:0+C16:0) / (0.5×MUFA) + (0.5×n6) + (3×n3) + (n3/n6); h / H or hypocholesterolemic / hypercholesterolemic index= (C18:1+C18:2+C18:3) / (C14:0+C16:0); IDSA16:0 or index of D9 desaturase enzyme activity on the conversion of C16:0 to C16:1 n9= (100×(C16:1 n9/(C16:1 n9+C16:0)); IDSA18:0 or index of D9 desaturase enzyme activity on the conversion of C18:0 to C18:1 n9= (100×(C18:1 n9/(C18:1 n9+C18:0))); SCD or stearoyl CoA desaturase= (C16:1+C18:1) / (C16:0+C16:1+C18:0+C18:1); EAI or elongase activity= (C18:0/C16:0).

RESULTS AND DISCUSSION

Animal performance

Animal performance is reported elsewhere (Yossifov and Kozelov, 2013).

Briefly, feed intake was decreased (5%), but growth performance (final body weight, average daily gain, gain efficiency and feed efficiency 12, 19, 28 and 26%, respectively) was significantly (P<0.01) increased. Lambs, fed different protein supplements (RSD *vs.* SFD) had slightly tendency among daily nutrient intake (Table 1). Contrary, dietary protein source had a consistent effect on carcass measurements of lambs fed cereal-based diets–SFD *vs.* RSD (Table 2). Hot carcass weights were higher in lambs fed RSM (17.5 *vs.* 16.3) compared to SFM (P=0.29) Meat yield (5.26 vs. 4.67 kg) was significantly (P=0.04) affected by RSM supplementation in contradistinction (P>0.05) from separable fat yield (1.15 vs. 1.23 kg).

Diet FA composition

Fatty acid composition of diets and supplements are presented in Table 3. SFA were not affected signicantly by diet. The total amounts of C14:0, C16:0 and C18:0 were similar among the forage, concentrates and total rations. C18:1 percentage (Table 3) varied among diets (SFD and RSD) and supplements (SFM and RSM) being lower in forage, SFM and SFD compared with RSD and RSM (10.38, 25.17, 28.67 and 39.04, 49.92%, respectively). These values included different C18:1 trans isomers including vaccenic acid (VA, trans C18:1 n-11) produced by ruminal biohydrogenation of C18:2 n6. However, CLA and VA namely are dependent on the nutritional factor, e.g. source and level of dietary nutrients providing impact yields in meat lipids (Griinari and Bauman, 1999). Some authors rendered this as tissue-related mechanism or response to concentrate-rich diets with a greater stearoyl-CoA desaturase gene expression or with higher substrate (C18:1) availability (Daniel *et al.* 2004). Concentration of n-6 PUFA C18:2 were greater in SFM and SFD than RSD, RSM and forage (57.84, 54.90 and 44.55, 32.01, 29.76%, respectively). The n3 PUFA C18:3 values were higher in forage (34.47%), decreasing in RSM, RSD, SFD and SFM (5.03, 3.76. 2.01 and 0.49% respectively). Increasing dietary levels of forage tended to higher C18:3 with toxic effect of PUFA on ruminal biohydrogenation resulting in greater pool of CLA and its accumulation in tissues. The FA composition of RSM consisted primarily of oleate, linoleate and palmitate (49.92, 31.01 and 11.05%, respectively). Thus, inclusion RSM in the feedlot diets increased the values of these FA in the RSD (Table 3).

Table 1 Mean daily intake of diet DM and nutrients (g)

Item	Forage		Concentrate		Total ration	
	SFD	RSD	SFD	RSD	SFD	RSD
DM (g/kg DM)	378.31	327.31	773.98	768.25	1152.29	1095.56
CP (g/kg DM)	32.01	27.49	165.52	159.46	197.52	186.95
EE (g/kg DM)	4.12	3.57	11.15	18.53	15.27	22.10
CF (g/kg DM)	135.36	117.11	84.52	59.20	219.88	176.31

DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fiber; SFD: sunflower meal-based diet and RSD: rapeseed meal-based diets.

Table 2 Effect of dietary supplement (SFD *vs.* RSD) on carcass characteristics of lambs fed cereal-based diets supplemented with sunflower or rapeseed meal (kg)

Item	Treatment		SEM	P-value
	SFD	RSD		
Final body weight	35.10[a]	38.80[b]	0.91	< 0.01
Hot carcass weight	16.29	17.49	0.53	0.29
Meat yield[1]	4.67[a]	5.26[b]	0.78	< 0.04
Separable fat[1]	1.23	1.15	0.08	0.62

[1] Right side carcass weight.
SFD: sunflower meal- based diet and RSD: rapeseed meal-based diet.
SEM: standard error of the means.

Diet effects on meat FA profile

The effect of protein source (RSM *vs.* SFM) on FA composition of lamb carcass is shown in Table 4. The oleic acid percentage showed the highest values (42.80 *vs.* 43.47%), followed by palmitic (24.59 *vs.* 23.44%), stearic (15.94 *vs.* 16.80%) and linoleic (5.44 *vs.* 5.03%) acids in SFD and RSD, respectively. All differences were not significant. Diet supplementation with RSM (RSD) significantly increased the values of C18:3 (P<0.01). FA profile (ratio and indices) are presented in Table 5. SFA, OFA, n3, n6 and PUFA % were higher in SFD compared with RSD (47.50, 31.55, 0.26, 6.85 and 7.11 *vs.* 46.89, 30.09, 0.20, 6.50 and 6.70%, respectively). Contrary, MUFA, UFA and DFA were lower in SFD than in RSD (44.73, 51.84, and 67.78 *vs.* 45.59, 52.29 and 69.09%, respectively). The C18:2 n6 / C18:3 n3 and C18:3 / CLA ratios were linearly and significantly (P<0.01) affected by the protein supplement in the diet (Table 5). The values of n6/n3, DFA/OFA, (C18:0+C18:1) / C16:0, MUFA / SFA and UFA / SFA ratios were higher in RSD than in SFD, contrary to lower values obtained in RSD than in SFD for PUFA / SFA, C20:4 / C20:5 and C18:2 / CLA ratio.

Table 3 Fatty acid composition of diets and supplements (% by weight of identified FA)

Item	Forage	Diet		Supplement	
		SFD	RSD	SFM	RSM
EE, %	1.43	1.57	2.06	1.28	2.45
C14:0	1.16	ND	ND	ND	ND
C16:0	22.05	12.12	10.98	12.34	11.05
C18:0	2.18	2.30	1.67	4.16	2.00
C18:1	10.38	28.67	39.04	25.17	49.92
C18:2	29.76	54.90	44.55	57.84	32.01
C18:3	34.47	2.01	3.76	0.49	5.03

ND: not detected; EE: ether extract; SFD: sunflower meal-based diet; RSD: rapeseed meal-based diet; SFM: sunflower meal and RSM: rapeseed meal.

Table 4 Effect of protein source on fatty acid composition of meat in lambs fed cereal-based feedlot diets (SFD *vs.* RSD)

Item	Treatment		SEM	P-value
	SFD	RSD		
C14:0	4.30	4.44	0.27	0.80
C15:0	0.77	0.72	0.06	0.69
C16:0	24.59	23.44	0.91	0.56
C16:1	1.93	2.12	0.20	0.65
C17:0	1.90	1.49	0.11	0.07
C18:0	15.94	16.80	0.54	0.46
C18:1	42.80	43.47	0.81	0.71
C18:2	5.44	5.03	0.30	0.52
C18:3	0.43^{b}	0.60^{b}	0.04	<0.01
Total CLA	0.71	0.82	0.04	0.13
C20:4	0.98	0.87	0.12	0.67
C20:5	0.11	0.08	0.01	0.17
C22:5	0.15	0.12	0.02	0.56

SFD: sunflower meal-based diet and RSD: rapeseed meal-based diet

SEM: standard error of the means.

Means within a row with different superscript are significantly different ^{aa}P<0.05, ^{bb}P<0.01.

The abundance of expressed ratios, indices and enzyme activities, involved in various metabolic aspects, induces physiological effects and could be used as indicators of the rumen environment and its conditions following appointed patterns and pathways. More of them are important nutritional ratios and indices for healthiness of meat products. In this regard, they could be profitable to be examined and responded to consumer nutritional recommendations (Figure 2).

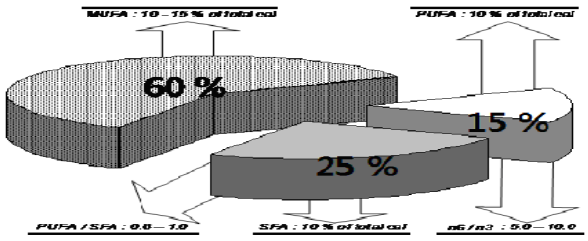

Figure 2 Consumer dietary fat recommendations (Legrand, 2001; WHO/FAO, 2003). Different FA classes depicted healthiness of meat products. Their profitability recommended saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) FA values not exceeded 10, 15 and 20% of total caloric intake per day, respectively. SFA, MUFA and PUFA must be separated as follow: 25, 60 and 15% of total FA input. PUFA / SFA and n3 / n6 ratios must be ranged between 0.8-1.0 and 5.0-10.0

The index of D9 desaturase enzyme activity on the conversion of C16:0 to C16:1 was greater in ED than CD, but with lower activity on the conversion of C18:0 to C18:1. The lower desaturase activity and higher CLA is associated with higher substrate (C18:1) availability (Table 4 and 5).

Pearson's correlation coefficients

The results of the regression analysis of the experimental data are presented graphically (Figures 3, 4, 5 and 6). Examined relationships between ingested FA and carcass FA in slaughtered lambs shows good parity and as could be seen below, are characterized with significant Pearson's correlation coefficients (R>0.55).

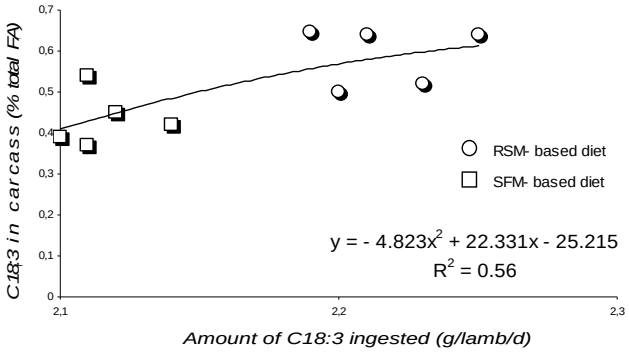

Figure 3 Relationship between the level of carcass C18:3 (% total FA) and amount of C18:3 ingested (g/lamb/d) in fattening lambs assigned to SFD (SFM-based diet) *vs.* RSD (RSM-based diet) for 87 days

Table 5 Fatty acid profile (ratios and indices) and enzyme activities of carcass lipids of lambs fed SFD *vs.* RSD

Item	Treatment		SEM	P-value
	SFD	RSD		
SFA	47.50	46.89	0.96	0.77
OFA	31.55	30.09	1.24	0.59
MUFA	44.73	45.59	0.78	0.61
n6	6.85	6.50	0.39	0.67
n3	0.26	0.20	0.03	0.39
n6 / n3	27.62	37.88	3.82	0.19
PUFA	7.11	6.70	0.41	0.64
DFA/OFA	2.18	2.35	0.12	0.49
(C18:0+C18:1) / C16:0	2.41	2.63	0.13	0.44
Total LC n3	0.26	0.20	0.03	0.39
UFA	51.84	52.29	0.93	0.83
MUFA / SFA	0.95	0.98	0.03	0.63
PUFA / SFA	0.15	0.14	0.01	0.79
UFA / SFA	1.10	1.13	0.04	0.74
DFA	67.78	69.09	1.21	0.62
C18:2 / C18:3	12.58^{b}	8.47^{b}	0.87	< 0.01
C20:4 / C20:5	11.27	10.43	0.73	0.65
C18:2 / CLA	7.70	6.19	0.43	0.07
C18:3 / CLA	0.62^{a}	0.73^{a}	0.03	< 0.02
AI	0.81	0.80	0.05	0.94
TI	1.09	1.06	0.07	0.84
h/H	1.70	1.81	0.09	0.60
SCD	0.52	0.53	0.01	0.74
EAI	0.66	0.73	0.04	0.38
$IDSA_{16:0}$	7.13	8.38	0.72	0.42
$IDSA_{18:0}$	72.93	72.09	0.67	0.56

SFD: sunflower meal-based diet; RSD: rapeseed meal-based diet; FA: fatty acids; SFA: saturated FA; OF: undesirable FA; DFA: desirable FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; UFA: unsaturated FA; LC: long-chain; $IDSA_{16:0}$: index of D9 desaturase enzyme activity on the conversion of C16:0 to C16:1 n9; $IDSA_{18:0}$: index of D9 desaturase enzyme activity on the conversion of C18:0 to C18:1 n9; CLA: conjugated linoleic acid; AI: Atherogenicity index; TI: thrombogenic index; h / H: hypocholesterolemic / hypercholesterolemic index; SCD: stearoyl CoA desaturase and EAI: elongase activity. SEM: standard error of the means.

Means within a row with different superscript are significantly different $^{aa}P<0.05$, $^{bb}P<0.01$.

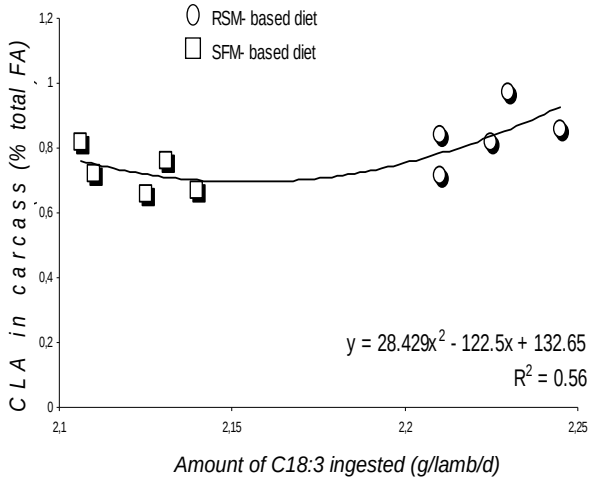

Figure 4 Relationship between the level of CLA in lipids of carcass (% total FA) and amount of C18:3 ingested (g/lamb/d) in fattening lambs assigned to SFD (SFM-based diet) *vs.* RSD (RSM-based diet) for 87 davs

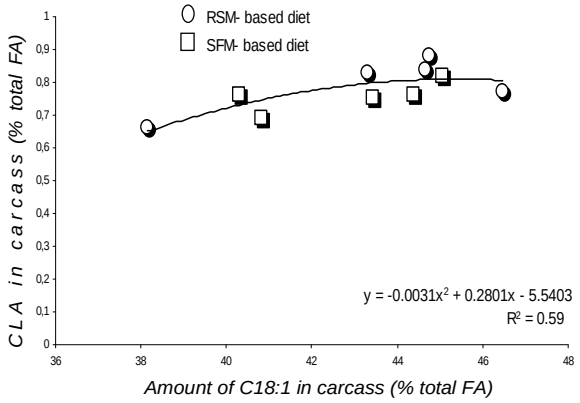

Figure 5 Relationship between the level of CLA in lipids of carcass (% total FA) and C18:1 (% total FA) in fattened lambs assigned to SFD (SFM-based diet) *vs.* RSD (RSM-based diet) for 87 days

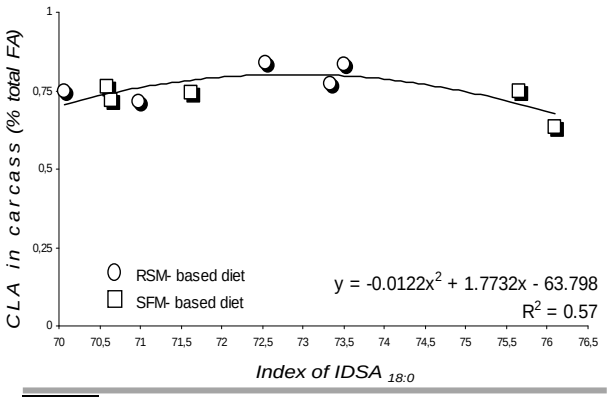

Figure 6 Relationship between the level of CLA in lipids of carcass (% total FA) and IDSA 18:0 in fattened lambs assigned to SFD (SFM-based diet) *vs.* RSD (RSM-based diet) for 87 days

In the first graph (Figure 3) the amount of C18:3 as percentage of total carcass FA displayed high coefficient of determination (R=0.56) with concentrations of ingested C18:3 (g.lamb-1.d-1). Similar trends were observed between total amount of CLA in carcass (% total FA) and level of ingested C18:1 (R=0.59) or C18:3 (R=0.56) (Figures 4 and 5). The relationship between the values of CLA in lamb carcass and index of D9 desaturase enzyme activity on the conversion of C18:0 to C18:1 n9 is revealed with high coefficient of determination (R=0.57) (Figure 6).

CONCLUSION

Obtained results revealed that in general diet supplementation with RSM (RSD) have an impact on carcass FA composition and profile (ratio and indices) due to modified rumen and tissue metabolism. RSM feeding decreased the amount of SFA and PUFA while increased MUFA, UFA and DFA affecting the nutritional value of lamb carcass. Examined Pearson's correlation coefficients show good parity and high correlation (R>0.55) among ingested FA and carcass FA in slaughtered lambs.

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