EFFECTS OF DIFFERENT SOURCES OF FAT (CALCIUM SOAP) ON CARCASS QUALITY AND FATTY ACID PROFILE OF MUSCLE AND ADIPOSE TISSUES IN LAMBS

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Abstract

The objective of this study was to establish the effect of dietary supplementation of lambs subject to intensive fattening, with different types of protected fat (calcium salts of fatty acids) on the carcass quality and fatty acid profile of intramuscular and fat deposit. Our research was conducted on 32 male lambs belonging to Tsigai (sheep with prime wool) variety and which were submitted to four dietary treatments: C – ration without saponified fat supplement, SO – ration supplemented with saponified sunflower oil, CO - ration supplemented with saponified canola oil, and GR - ration supplemented with saponified lard. Saponified fat was included in the diet at a 4% ratio. Protected fat in the lambs dietary resulted in reduction of the share of meat and protein level, respectively (N x 6.25) in meat in favour of adipose tissue and fat from meat, resulting the following hierarchy: C > GR > SO > RO. Lambs in whose food protected fat was used (SO, RO) recorded higher concentrations of n-3 FA and CLA in carcass by up to 59.3% when compared to control group. In comparison with intramuscular fat, fat deposit (subcutaneous and kidney) is characterized by a higher content of monounsaturated and saturated fatty acids (especially kidney fat) and proportionately lower polyunsaturated fatty acids and in particular Omega 3 PUFA series . It was found that there is a direct relationship between the linoleic acid content in fat from food and intramuscular fat and fat deposit. Analyzed under in terms of impact on human health, the best quality of fat provided by a high content in n-3 FA and CLA and low content in SFA was recorded in the carcases of those lambs in whose dietary there were used calcium soaps of vegetable oils.

Key words: lambs, Ca salts of fatty acids, functional fatty acids, n-3 FA, c9, t11-CLA.

INTRODUCTION

Fats, regardless of the length chain of carbon atoms in the structure of fatty acids, are an effective energy supplement for animals, being very digestible, with a high metabolic use (Ramdane et al., 2010; Zsédelyet al., 2012) and which provides a good use of nitrogen in food, a proper functioning of the rumen during the initiation of fermentation activity (pH, digestive transit speed), and reduces the proportion of fatty acids and

volatile fatty acid precursors responsible for the inconsistency of skeleton fat (Velasco et. al., 2001). In addition, fat supplement in food helps in preventing rumen acidosis, facilitates the absorption of fat soluble nutrients and allow for modelling the share of fat in carcass in relation to consumer' preferences (Manso et. al., 2006).

Triglycerides which are the basis of fats are hydrolyzed in rumen and form free fatty acids and glycerol. Glycerine ferments and turns into propionic acid which is the precursor of glucose and some amino acids, thus enhancing protein synthesis. Meanwhile fats from ruminant feed lead to substantial reduction of gases occurred in rumen and released into the environment (Nigdi et. al., 2000; Mierlita et al., 2010). The negative effects of fat on rumen fermentation processes can be diminished or even eliminated by means of fat hydrogenation, and their conversion in calcium salts or by fat or encapsulation (Naik, 2007). Calcium soaps are obtained in the most simple and economical way and at the same time they can be easily incorporated in animal feed since they are "dry fat" (Nigdi et. al., 2000; Jenkins, 1993; Zsédelyet al., 2012).

Previous research has demonstrated the effectiveness of protected vegetable fat in feeding lambs subject to fattening (Russo et. al., 1999; Bas and Morand-Fehr, 2000; Machmuller et. al., 2000; Kott et. al., 2003; Ivan et. al., 2004). However, there are no studies to compare the effect of different degree of fat saturation from the food of lambs which are subject fattening on the bio performance, and especially on the fatty acid profile of the carcass.

The objective of this study was to establish the effect of dietary supplementation of intensive fattening of lambs, with different types of protected fat (calcium salts of fatty acids) on the carcass quality and fatty acid profile of intramuscular and fat deposit.

MATERIAL AND METHOD

In line with its objectives, our research has been conducted on a number of 32 male lambs of aged 70-75 days (immediately after weaning) belonging to Tsigai (sheep with prime wool) variety and which were distributed randomly into four groups of eight individuals each, corresponding to the 4 dietary treatments, as follows:

C – ration without saponified fat supplement,

SO – ration supplemented with saponified sunflower oil,

CO – ration supplemented with saponified canola oil, and

GR – ration supplemented with saponified lard.

Fat was saponified in the laboratory, using the method described by Akers et al. (1981), and which consisted of hydrolysis of fats in the presence of acetone at high temperature (50-60°C) to which a treatment with calcium

hydroxide powder was applied, and thus obtaining calcium salts of fatty acids. Saponified fat was incorporated in the lambs' dietary at a rate of 4% (% by TMR weight – total mixed ration). TMR consisted of alfalfa hay, corn, triticale, soybean meal and vitamin-mineral premix, providing a forages:concentrates ratio of 40 : 60. Diets were isoprotein kind (16.4% CP) and the caloric energy value calculated were 0.87 UNC/kg in the control group and 0.90 UNC/kg for the groups SO, CO and GR, respectively.

At the end of the experiment there were performed control slaughters using eight individuals from each group to determine the carcass quality thus obtained (tissue structure and chemical composition of meat). The tissue structure of the carcass was performed manually for each anatomical part and the outcomes are averaged for each meat quality grade. Cutting the carcasses by quality grades was made in agreement with recommendations made by Pascal C. (2007). The chemical composition of the *longissimus dorsi* and *bicepfemoris* was determined using established laboratory techniques: DM (ISO, 1999a); N (Kjeldahl technique) and ether extract (AOAC, 1996).

In order to establish fatty acid profile of carcass individual samples were taken from muscle tissue (*longissimus dorsi* - the lumbar and biceps femoris muscle) and adipose tissue (subcutaneous - lower back and perirenal areas). Muscle and adipose tissue samples were stored at -20°C in order to establish the fatty acids profile by means of gas chromatography. Lipid extraction was done through a mixture of chloroform: methanol (2:1, vol./vol.), and the fatty acid methyl esters (FAME) were obtained using Boron trifluoride (BF_3), simetanol (14% w/w) according to the procedure described by Watkins et. al. (1997). For the quantification of conjugated linoleic acid (CLA), lipids extracted from tissue samples were methylated (sodium methoxide) based on the procedure described by Li and Watkins (1998). FAME were identified using HP 5890 gas chromatography apparatus, equipped with a DB23 column of 30 m length. Gas chromatography worked at 140°C for 2 min., the temperature rising by 1.5°C/min., up to 198°C and then maintained at that level for 7 minutes. For each operation with gas chromatography, the injector and flame ionization detector indicated temperatures of 225°C and 250°C respectively. The identification of peaks was made by comparison of retention times with the one obtained for fatty acid methyl esters (FAME) standard mixtures acquired from Un-Check-PrepInc.(Elysian, MN, USA) and from SupelcoInc. (Bellefonte, PA, USA).

All data were statistically processed and interpreted, using the SAS procedure (version 8.0; SAS Inst., Inc, Cary, NC) for repeated measurements and 't' test. Effects included in the statistical model were as follows: control vs. saponified fat; vegetable fat vs. animal fat and

sunflower oil vs. canola oil. The differences caused by lambs dietary supplementation by saponified fat were considered significant for a $p < 0.05$.

RESULTS AND DISSCUSIONS

Protected fats tested in the dietary of lambs subject to fattening were characterized by a high content of polyunsaturated fatty acids and especially linoleic acid (C18:2 n-6), in the case of the group SO (sunflower oil) and CO group (canola oil). Compared to the aforementioned, saponified lard administered in the case of the group GR had a higher content in saturated fatty acids, particularly palmitic acid (C16:0) and stearic acid (C18:0), and monounsaturated acids, mainly oleic acid (Table 1). The particularity of group CO as the high level of linolenic acid (C18:3 n-3), which was roughly six times higher than the sunflower oil and lard.

Tabel 1

The structure of the fatty acids of the saponified fat sources used

Fatty acid (g/100g FAME)	SO	RO	GR
C16:0 palmitic	7,45	5,44	18,12
C16:1 palmitoleic	0,30	NI	1,74
C18:0 stearic	4,29	3,40	15,50
C18:1 oleic	21,35	20,10	34,20
C 18:2 n-6 linoleic	60,45	61,60	16,60
C 18:3 n-6 linolenic	1,21	7,40	1,12
C 20:1 eicosenoic	0,52	1,96	0,72
SFA	11,86	10,05	40,34
MUFA	21,89	22,56	36,66
PUFA	66,25	67,39	23,00

SFA – saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Protected fat in food lamb did not significantly affect the carcasses quality or the proportion of the parts cut from carcass, the results being consistent with those obtained by Manso et. al. (2006), Radunz et al., (2009) and Brooks (2013), who supplemented at their turn the dietary of lambs with various vegetable fat.

Increasing the energy level provided by food by supplementing the latter with different types of protected fat had a negative influence on the meat proportion in carcass. Thus the share of both meat in carcass tissue structure and cut parts dropped in favour of fat in the case of groups SO, CO and GR compared to control group C. A similar effect was observed in the case of bone weight, and thus the meat (meat + fat) / bone ratio was positively influenced by the protected fat supplement in the food, and the following hierarchy was established: C<GR<RO<SO (Table 2). The lowest

musculature:fat ratio was recorded in the case of the group GR and the hierarchy was GR<C<RO<SO, both in the carcass and cut parts divided by quality grades.

Table 2

The effect of feed on protected fat tissular and chemical composition

		Groups				p values of effects		
		C	SO	RO	GR	1	2	3
Tissular composition (%):								
Carcass	Muscle	58,01	56,73	56,48	57,31	*	*	NS
	Fat	22,76	24,91	25,10	24,01	*	*	NS
	Bone	19,23	18,36	18,42	18,63	*	NS	NS
Class Ia	Muscle	60,00	58,81	58,10	58,73	*	NS	NS
	Fat	20,09	22,40	22,66	21,64	*	*	NS
	Bone	19,91	18,79	19,24	19,63	NS	NS	NS
Classb	Muscle	59,15	56,79	57,07	58,42	**	*	NS
	Fat	19,80	23,07	22,83	21,04	**	*	NS
	Bone	21,05	20,14	20,10	20,54	*	NS	NS
Classc	Muscle	55,70	54,61	54,36	54,97	*	NS	NS
	Fat	27,90	30,29	30,52	29,40	**	*	NS
	Bone	16,40	15,10	15,12	15,63	*	NS	NS
Ratio:								
Muscle : bone	Carcass	4,20/1	4,45/1	4,43/1	4,35/1	*	NS	NS
	Class I	4,02/1	4,32/1	4,20/1	4,09/1	*	NS	NS
	Class II	3,75/1	3,96/1	3,97/1	3,87/1	*	NS	NS
	Class III	5,10/1	5,62/1	5,61/1	5,49/1	*	NS	NS
Muscle : fat	Carcass	3,02/1	3,08/1	3,07/1	2,39/1	NS	*	NS
	Class I	2,99/1	3,12/1	3,02/1	2,72/1	NS	*	NS
	Class II	2,99/1	2,82/1	2,84/1	2,78/1	NS	NS	NS
	Class III	2,00/1	3,61/1	3,60/1	1,87/1	*	**	NS
Chemical composition (% of dry matter -DM)								
Longissimus dorsi	DM	24,65	26,21	27,06	26,81	**	NS	NS
	Crude protein	77,08	70,14	70,95	72,16	**	*	NS
	Crude fat	19,96	23,05	22,28	22,17	*	NS	NS
	Ash	4,79	5,72	5,40	4,29	NS	NS	NS
Biceps femoris	DM	25,29	26,91	26,70	26,07	*	NS	NS
	Crude protein	77,41	73,47	73,74	76,87	**	*	NS
	Crude fat	16,10	19,54	19,89	17,53	**	*	NS
	Ash	5,18	5,16	4,76	4,22	NS	NS	NS

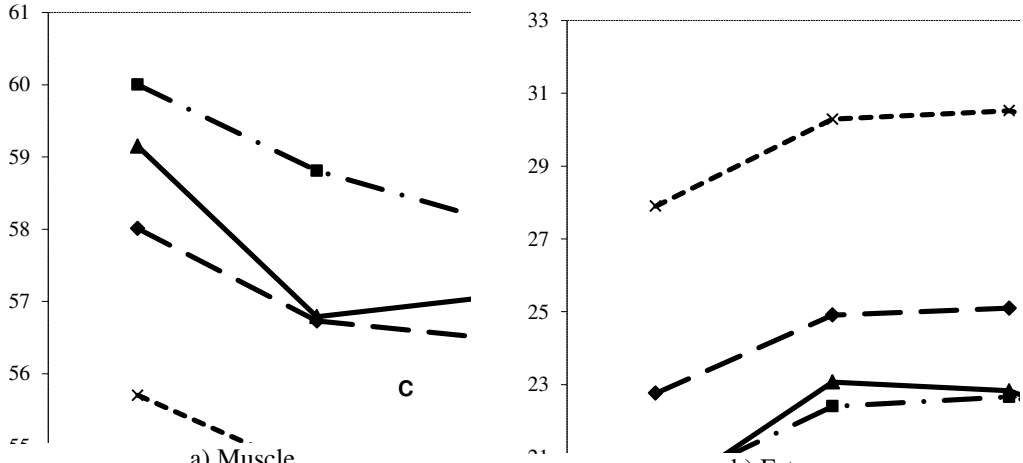
C – ration without saponified fat supplement, SO – ration supplemented with saponified sunflower oil, CO – ration supplemented with saponified canola oil, and GR – ration supplemented with saponified lard.
[a]leg and chop; [b]shoulder, arm, chest and ribs; [c]neck and tail meatloaf.
1 = Control *vs*. Protected fats ; 2 = Protected plant oils (SO and RO) *vs.* Protected animals fat (GR) ; 3 = SO *vs*. RO. NS – p > 0,05, * - p < 0,05

Tissue structure of the carcass is closely linked to the chemical composition of meat determined at *longissimus dorsi* and *bicepfemoris* muscles level. Placement of different types of protected fat in food in the

case of lambs which are subjects to intensive fattening led to decreasing the proportion of meat and protein levels respectively (N x 6.25) in meat in favour of adipose tissues and fat (ether extract) in meat (Fig. 1) which normally attracted increased meat content in DM. Protected vegetable fat has a stronger influence, the following group hierarchy on content of musculature and protein content in carcass being established: C>GR> SO>RO.

Placement of protected fats in the dietary of lambs subject to intensive fattening had a positive influence on fatty acid profile of intramuscular fat and deposit fat, increasing the share of functional fatty acids (n-3 PUFA series and CLA-conjugated linoleic acid) which have positive effects on human health, to the detriment of saturated fatty acids (SFA), held responsible for many pathological conditions in humans.

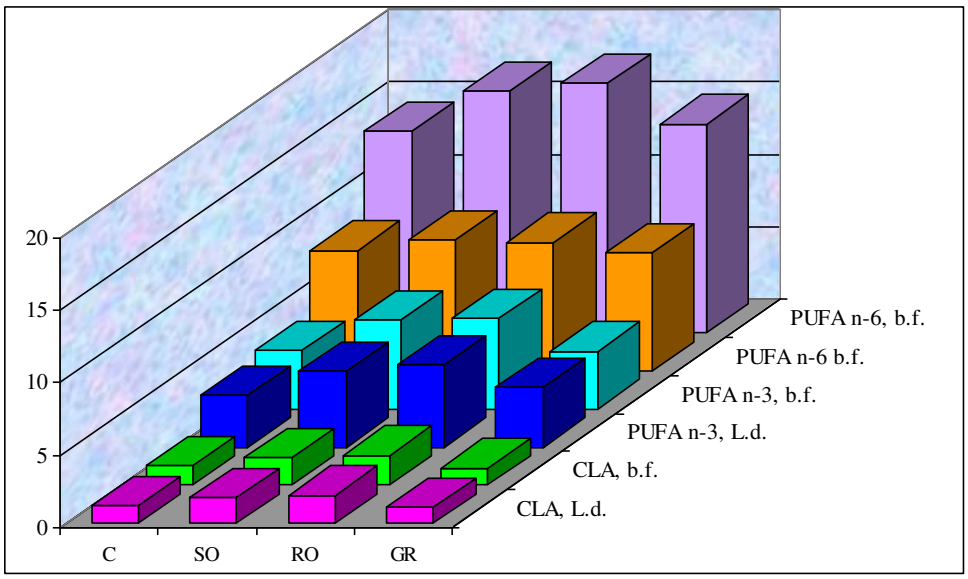
The highest amount of Omega 3 PUFA fatty acids was found in muscle tissue coming from the lambs in whose food there was used saponified vegetable fat (groups SO and RO) and especially rapeseed oil (Fig. 2), being set a direct relationship between linolenic acid intake in food and the n-3 FA level in intramuscular fat (Guler et al., 2011). Regarding the effects of lard (GR group) in fatty acid composition, these are almost negligible in the current experiment when compared to control group. In this sense, previous works have shown that the effects of palmoil supplements on fatty acid composition are more likely to be evident in internal depots such as omental and mesenteric fat (Manso et al., 2006).



a) Muscle b) Fat

Fig. 1. Effect of feed supplementation with different types of protected fat on the proportion meat and fat.

Numerous studies have demonstrated the effectiveness of using vegetable fats (canola, flax, safflower, soybean, sunflower) in the diet of lambs subject to fattening to improve the fatty acid profile in carcass (Wachira et al., 2002; Demirel et al, 2004; Copper et al., 2004; Radunz et al., 2009). Thus fat dietary supplementation with fat rich in n-3 PUFA has increased the ALA content (C18:3 n-3) in meat by 2.2 times compared to the control group. The functional fatty acids share in *biceps femoris* muscle was higher than in the *longissimus dorsi* muscle.



CLA = conjugated linoleic acid (C18:2 c9, t11 + C18:2 t10, c12) ; L.d. Longissimus dorsi, b.f. = biceps femoris.

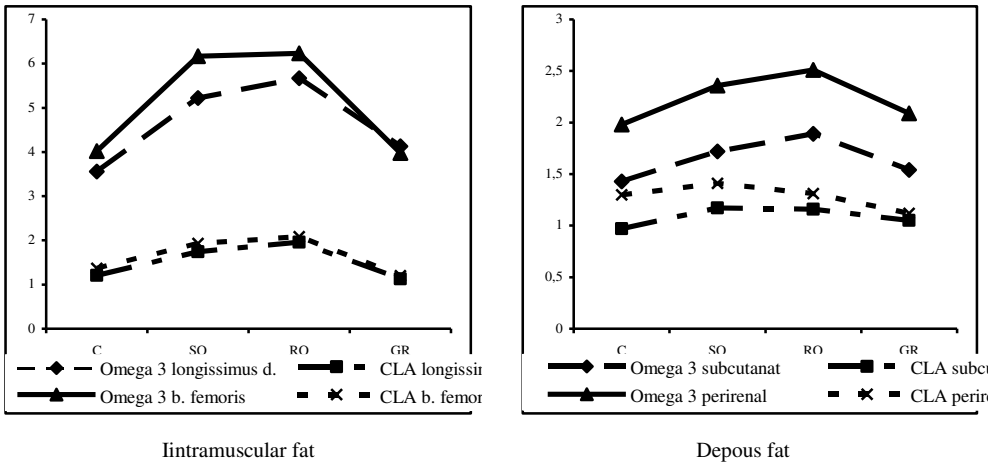
Fig. 2. Fatty acid profile of intramuscular fat

Compared to the muscle tissue, fat deposit (subcutaneous and kidney) is characterized by a higher content of saturated and monounsaturated fatty acids (especially kidney fat) and a proportionately lower content in polyunsaturated fatty acids, in particular OMEGA-3 series PUFAs (Fig. 3). Protected fats from the food had a similar influence on the fatty acid profile of the fat deposit, as in the case of fat in muscle tissue, but with much lower intensity.

Diets high in LA and ALA have been shown to increase the production of CLA (Wachira et al., 2002). This pattern was not observed in adipose tissue.

Lambs in whose food protected vegetable fats (groups SO, RO) were used recorded the highest concentrations of n-3 FA and CLA in carcass by up to 59.3% when compared to control group. Thus it was found a direct

relationship between linoleic acid content of the fat from food and the intramuscular and deposit fat.



Iintramuscular fat

Depous fat

Fig. 3. Content of n-3 FA and CLA in intramuscular and depous fat

Analyzed under the influence on human health, the top quality of fat provided by a high content in functional fatty acids (n-3 FA and CLA) and low content in SFA was recorded in carcasses of lambs in whose food calcium soaps of vegetable oils were used. In the intramuscular fat from legs the lowest content of SFA and the highest concentration of CLA and especially PUFA (C18: 2 cis-9, trans-11) were found. Compared to the subcutaneous fat, fatty acid profile of the kidney region is characterized by a higher content in SFA (+ 9.6 to 14.3%) and the lowest content in MUFA; while the share of PUFA and especially the share of PUFA n-3 series FA and CLA have not changed basically.

CONCLUSIONS

Tissue structure of the carcass is closely linked to the chemical composition of meat determined at the *longissimus dorsi* and *bicepfemoris* muscles. The placement of different types of protected fat in the dietary of lambs subject to intensive fattening led to decreasing the proportion of meat and protein levels respectively (N x 6.25) from meat in favour of adipose tissues and fat (ether extract) in meat, which led normally to an increased DM content in meat. Protected vegetable fat have protected more pronounced influence, being established following hierarchy the content muscled carcass and protein respectively: C > GR > SO > RO.

Lambs in whose food protected fat was used (SO, RO) recorded higher concentrations of n-3 FA and CLA in carcass by up to 59.3% when

compared to control group. In comparison with intramuscular fat, fat deposit (subcutaneous and kidney) is characterized by a higher content of monounsaturated and saturated fatty acids (especially kidney fat) and proportionately lower polyunsaturated fatty acids and in particular Omega 3 PUFA series . It was found that there is a direct relationship between the linoleic acid content in fat from food and intramuscular fat and fat deposit.

Analyzed under in terms of impact on human health, the best quality of fat provided by a high content in n-3 FA and CLA and low content in SFA was recorded in the carcases of those lambs in whose dietary there were used calcium soaps of vegetable oils.

Acknowledgments: This work was supported by CNCSIS – UEFISCSU, project number PN II – IDEI 679/2008.

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