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Dietary Supplementation with Oregano and Linseed in Garganica Suckling Kids: Effects on Growth Performances and Meat Quality

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GM conceived and designed the study. PR performed experimental work and laboratory analysis. MAC carried out meat analysis. FG and MR helped in preparation of the manuscript. AMF analysed the data and wrote the article.

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ABSTRACT

Meat from Garganica kids fed diets containing oregano and linseed was analysed for physical and sensory properties, chemical and fatty acid composition of intramuscular lipids. Twenty-one three-week-old kids were divided into three homogeneous groups (n = 7), according to age and body weight, and assigned to one of the following feeding treatments: C) control: commercial pelleted feed; L) pelleted feed containing 3% extruded linseed; LO) pelleted feed containing 3% extruded linseed and 0.6% dried oregano inflorescences. Kids were slaughtered at 60 days of age. Diet did not affect *in vivo* performances, dressing percentage, pH and meat colour, but it influenced meat tenderness, that was lower (P<0.01) in meat from kids receiving oregano, probably due to the lesser (P<0.05) fat content of their meat. The use of oregano also resulted in a lower muscle fat oxidation and in a better meat flavour. As for human health, the dietary supplementation with linseed improved the muscle fatty acid composition, resulting in higher levels of α-linolenic acid (P<0.05) and in a lower n-6/n-3 ratio.

INTRODUCTION

The crisis affecting animal production may be overcome by rediscovering "biodiverse" animal breeds. The recovery of autochthonous breeds is an important factor in maintaining biodiversity. The Garganica breed is a native Apulian goat breed well adapted to the harsh environmental conditions of Southern Italy marginal areas. The exploitation of this goat breed passes through the improvement of meat production and quality, which has a relationship with human health.

In the last ten years, research on human nutrition has shown that a diet containing high levels of saturated fatty acids (SFA) increases the risk of cancer, atherosclerosis and cardiovascular diseases (Hooper *et al*., 2006; Erkkilä *et al*., 2008; Webb and O'Neill, 2008; Leon *et al*., 2009).

Consequently, consumers of the technologically advanced countries have begun paying attention to the link between health and nutrition, directing their food choices towards good quality, healthy and wholesome products. People prefer meats with a low fat and cholesterol content along with high levels of n-3 polyunsaturated fatty acids (n-3 PUFA).

Ruminant meat can be considered a good dietary source of several nutrients (Wahle *et al*., 2004), however, it has also been censured for its high level of intramuscular fat, which is particularly rich in SFA due to rumen biohydrogenation of dietary fats, especially PUFAs (Doreau and Ferlay, 1993; Glasser *et al*., 2008).

Therefore, the improvement of ruminant meat requires feeding strategies (Wood *et al*., 1999; Wachira *et al*., 2002; Demirel *et al*., 2004) able to optimize its chemical and nutritional composition and fatty acid profile, in order to obtain a product with a low SFA content, a high PUFA concentration (especially n-3; Cooper *et al*., 2004), and n-6/n-3 and PUFA/SFA (Chilliard *et al*., 2000; Vatansever *et al*., 2000; Caputi Jambrenghi *et al*., 2004) ratios close to the levels recommended by the Department of Health (1994).

Several studies have shown that the supplementation of the animal diet with linseed resulted in a higher content of α-linolenic acid in the muscle and increased the amount of long-chain n-3 PUFAs (Wachira *et al*., 2002; Demirel *et al*., 2004; Bas *et al*., 2007; Berthelot *et al*., 2010); moreover, changes in the feed level in the diet affect the ruminal ecosystem reducing PUFA biohydrogenation

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(Glasser *et al*., 2008).

Although dietary supplementation with PUFAs improves its nutritional characteristics, meat becomes more susceptible to lipid oxidation and peroxidation, resulting in consistent worsening of its colour (browning), flavour and shelf life (Wood *et al*., 2004).

Animal feeding supplementation with natural antioxidant essences such as oregano, sage, thyme and rosemary (Rice-Evans *et al*., 1997) has proved to delay or limit the oxidative process (Elmore *et al*., 1999) during meat storage and exposure at the meat counter, thus prolonging its shelf life.

Oregano (*Origanum vulgare* L.) is a common aromatic plant in the Mediterranean area (Kokkini *et al*., 2004). Its essential oil is known to have several properties: antimicrobial (Lambert *et al*., 2001; Marino *et al*., 2001; Friedman *et al*., 2002), antifungal (Paster *et al*., 1995; Adam *et al*., 1998) and antioxidant (Bendini *et al*., 2002; Botsoglou *et al*., 2002; Papageorgiou *et al*., 2003). Oregano's antioxidant activity is attributed to carvacrol and thymol; these molecules make the bacterial cell membrane permeable (Lambert *et al*., 2001) and convert lipids and hydroxyl radicals into stable products (Yanishlieva-Maslarova, 2001).

Studies on animal feeding supplementation with oregano have given controversial growth performance results for pigs (Namkung *et al*., 2004) and broilers (Botsoglou *et al*., 2004a; Giannenas *et al*., 2005). However, oregano improves the oxidative stability of chicken (Botsoglou *et al*., 2003) and rabbit meat (Botsoglou *et al*., 2004b), protecting it from the negative effects that slaughtering stress may have on quality (Young *et al*., 2003). Studies on oregano used in lamb feeding have confirmed its effectiveness in giving greater oxidative stability and in improving the sensory qualities of meat (Bampidis *et al*., 2005; Simitzis *et al*., 2008), although little information is available regarding the effects of oregano supplementation to the kid ration.

This study aimed to evaluate the effect of dietary supplementation with oregano and extruded linseed on the chemical, physical and sensory characteristics, intramuscular fat oxidation and fatty acid profile of meat from suckling Garganica kids.

MATERIALS AND METHODS

Animal management and diet

The study involved twenty-one male Garganica kids, born as twins, carried out in the Research Unit for Extensive Animal Husbandry (Zootecnia Estensiva - ZOE) in Muro Lucano (PZ, Italy, latitude: 40°45'13"64 N, longitude: 15°29'17.1" E, 650 m a.s.l.) between March and May 2015 for a total of 40 days. Kids were reared according to the traditional farming system for the Garganica breed: they were exclusively milk-fed, suckling from the dams until they reached the age of about 21 days. Then they were assigned to one the following groups (n=7), homogeneous for body weight and age: C) control group, that received a commercial pelleted feed; L) group that received a pelleted feed containing 3% extruded linseed; LO) group fed a pelleted feed containing 3% extruded linseed and 0.6% dried oregano inflorescences. The three pelleted total mixed rations (PTMR) (Tables I, II) were formulated to be isocaloric and isonitrogenous, in order to meet the nutritional requirements of kids (INRA, 1988) and were provided *ad libitum*.

Feed was offered daily at 08:00 h at a rate of 110% of *ad libitum* intake calculated by weighing-back refusal weekly. Feed samples were taken weekly and stored at -20°C until analysis. In addition, kids had access to maternal milk throughout the trial period. Suckling occurred twice daily, in the morning at about 07:00, before taking the dams out to pasture, and in the evening at about 19:00, when the dams came back from pasture. The amount of milk suckled by the kids was calculated by weighing them before and after suckling.

Table I.- Composition of experimental diets.

	Diets[1]		
	C	**L**	**LO**
Ingredient % (as-fed basis)			
Dehulled soybeans	6.00	6.00	6.00
Corn	31.00	31.00	30.4
Barley	9.00	9.00	9.00
Wheat flour shorts	9.00	9.00	9.00
Faba bean	10.00	8.50	8.50
Bran	10.00	10.00	10.00
Sugar beet pulp dehydrated	6.00	6.00	6.00
Extruded linseed	-	3.00	3.00
Soybean oil	1.00	-	-
Sunflower meal	8.00	7.50	7.50
Molasses	3.00	3.00	3.00
Soybean hulls	4.00	4.00	4.00
Oregano (dried inflorescences)	-	-	0.60
Vitamin mineral premix	3.00	3.00	3.00
Total	100.00	100.00	100.00

[1]Diets: C, control feed; L, control feed + 3% extruded linseed; LO, control feed + 3% extruded linseed + 0.6% Oregano.

The dams grazed during the day, receiving hay *ad libitum* and commercial feed (500 g/head/day) at housing, in the evening.

Samples of maternal milk were taken every two weeks for analysis of chemical and fatty acid composition

(Table II).

The kids were housed in individual pens (0.8 m^2/head) with free access to water, and the temperature in the pens ranged from 7 °C to 15 °C.

Feed refusals were recorded weekly to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR).

Table II.- Chemical and fatty acid composition of the diets and maternal milk.

	Diets[1]			Maternal milk
	C	L	LO	
Chemical composition % (dry matter basis)				
Moisture	88	88.9	88	87
Crude protein	16.795	16.682	17.227	3.456
Ether extract	4.598	5.589	6.156	4.729
Ash	9.098	9.065	9.008	0.861
Lactose	-	-	-	4.351
Crude fiber	15.183	13.432	14.970	-
Indeterminate	54.325	55.232	52.639	-
NDF[2]	33.850	36.001	36.553	-
ADF[2]	10.936	11.685	11.953	-
ADL[2]	2.641	2.919	3.073	-
AIA[2]	0.405	0.486	0.453	-
Fatty acid composition (% fatty acid methyl esters)				
C6:0	-	-	-	1.18
C8:0	-	-	-	2.17
C10:0	-	-	-	9.15
C12:0 (lauric)	0.950	-	-	4.42
C14:0 (myristic)	0.949	-	-	9.47
C16:0 (palmitic)	9.171	7.642	7.632	24.70
C17:0	0.667	-	-	0.63
C18:0 (stearic)	1.155	3.695	4.033	10.94
C18:1 n-9, cis 9 (oleic)	17.913	18.837	17.953	22.32
C18:2 n-6 (linoleic)	39.166	22.037	20.589	3.04
C18:3 n-3 (α-linolenic)	4.546	31.067	30.707	0.88
C20:4 n-6 (arachidonic)	0.213	-	-	0.32
C22:5 n-3 (DPA)	0.545	0.176	0.272	0.54
C22:6 n-3 (DHA)	0.297	0.280	0.283	0.30

[1]Diets: C, control feed; L, control feed + 3% extruded linseed; LO, control feed + 3% extruded linseed + 0.6% Oregano. [2]NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; AIA, acid insoluble ash.

The kids were slaughtered at 60 days of age, by exsanguination, according to the veterinary police rules (D.P.R. 320/54) after fasting for 12 h, with free water access, and recording of body weights. The hot carcass, skin, fleece, pluck, and full and empty gastro-intestinal tract (GIT) were weighed. Carcasses were hung by the Achilles tendon, chilled at 4 °C (80-82% relative humidity) for 24 h and then reweighed. The weight of the digestive content (full-empty GIT) was used to calculate the net dressing percentage after dressing and chilling (hot carcass weight/empty body weight; cold carcass weight/empty body weight).

Chemical composition of feed

Representative samples of the pelleted feeds were taken at fifteen-day intervals, and mixed to obtain a single final pool for each diet, which was then analysed to determine its chemical composition and fatty acid profile. Samples of each PTMR were ground in a hammer mill with a 1 mm screen and analysed using the following AOAC (2004) procedures: DM (Method 934.01), EE (Method 920.39), ash (Method 942.05), CP (Method 954.01), CF (Method 945.18), ADF and ADL (Method 973.18), and amylase-treated NDF (Method 2002.04).

The chemical composition of the dams' milk was analysed as follows: total protein (N×6.38), fat and lactose content, using an infrared spectrophotometer (Milko Scan 133B; Foss Electric, DK-3400, Denmark).

Physical and chemical parameters of meat

The pH values were measured on the *Longissimus lumborum* (*Ll*) muscle at the time of slaughter (pH_0) and after that the carcasses were refrigerated at 4 °C for 24 h (pH_{24}), using a portable instrument (Model HI 9025; Hanna Instruments, Woonsocket, RI) with an electrode (FC 230C; Hanna Instruments) and performing a two-point calibration (pH 7.01 and 4.01). The refrigerated carcasses were divided into left and right halves, and samples of the *Ll* muscle were taken from the right side in order to measure meat colour and tenderness.

Meat colour (L* = lightness, a* = redness, b* = yellowness) were determined using a Hunter Lab Miniscan™ XE Spectrophotometer (Model 4500/L, 45/0 LAV, 3.20 cm diameter aperture, 10° standard observer, focusing at 25 mm, illuminant D65/10; Hunter Associates Laboratory Inc., Reston, Virginia, USA). Three readings were taken for each sample by placing the instrument on different meat areas. The instrument was normalized to a standard white tile before performing analysis (Y = 92.8, x = 0.3162, and y = 0.3322). The reflectance measurements were performed after the samples were allowed to oxygenate in air for at least 30 min, in order to take stable measurements (Šicklep and Čandek-Potokar, 2007).

Three *Ll* samples (1.25 cm diameter and thick) were tested for tenderness by the Warner-Bratzler Shear Force (WBSF) system using an Instron 5544 testing machine. Shear forces were determined perpendicular to fiber direction.

Chemical composition and fatty acid analyses

In order to analyse the chemical composition of meat (moisture, ether extract, raw protein, ash) (AOAC, 1995), representative sub-samples of *Ll* were homogenised and fat was extracted according to the method suggested by Folch *et al*. (1957), using a 2:1 chloroform/methanol (v/v) solution in order to determine the fatty acid profile. The fatty acids were then methylated using a KOH/methanol 2N solution (Christie, 1982) and analysed by gas chromatography (Shimadzu GC-17A) using a silicone-glass capillary column (70% Cyanopropyl Polysilphenylene-siloxane BPX 70 by Thermo Scientific, length = 60 m, internal diameter = 0.25 mm, film thickness = 0.25 µm). The starting temperature was 135 °C for 7 min, then increased by 4 °C/min up to 210 °C.

The analysis of milk fatty acids was performed by applying the same extraction procedure described for meat. The methylated fatty acids were prepared following AOAC (2000) procedure and analysed by gas chromatography, the only differences being that the starting temperature was 140 °C, and increased by 5 °C/min up to 230 °C. The single fatty acids were expressed as a percentage of the total methylated fatty acids.

Samples of each concentrate mixture were used for fatty acid analysis according to the method described above for the meat fatty acid profile.

Morover, Δ^9 desaturase and elongase enzymatic activities were determined as described by Malau-Aduli *et al*. (1997), using mathematical indices. The calculations were performed as follows: Δ^9 desaturase 16 index = 100 [(C16:1cis9)/(C16:1cis9 + C16:0)]; Δ^9 desaturase 18 index = 100 [(C18:1cis9)/(C18:1cis9 + C18:0)]; elongase index = 100 [(C18:0 + C18:1cis9)/(C16:0 + C16:1cis9 + C18:0 + C18:1cis9)].

The food risk factor of meat was evaluated by calculating the n-6/n-3 and PUFA/SFA ratios and the nutritive value of the fat (Bonanome and Grundy, 1988); the latter was calculated as follows: (C18:0 + C18:1cis9)/C16:0.

Lipid oxidation

Lipid oxidation on meat samples from *Ll* muscle was evaluated by measuring 2-thiobarbituric acid reactive substances (TBARS), according to the method of Salih *et al*. (1987), and was expressed as mg MDA/kg meat.

Sensory properties of meat

Consumer tests were carried out in a single session to record consumer responses to the sensory properties of meat: tenderness, juiciness, flavour and overall acceptability (Meilgaard *et al*., 1991). Each consumer was asked to score each attribute on an intensity scale ranging from 1 (very bad) to 9 (excellent).

The lumbar region from the kids' half carcasses was refrigerated at 4 °C for 24 h and cut into ribs. The meat was grilled at 180 °C until it reached an internal temperature of 75 °C, monitored using an internal thermocouple. It was then served to the members of the consumer panel, consisting of 41 adult men and women aged between 18 and 60. The educational level was medium-high and panel members belonged to three job categories of approximately equal numbers: (1) students, (2) office workers and (3) technicians. Most were non-smokers and habitual meat eaters (eating meat more than once a week).

Statistical analysis

Statistical analysis was performed using the GLM procedure of the SAS application package (SAS, 2000). The statistical model included the effect of diet treatment and experimental error. When the diet effect was significant ($P<0.05$), means were compared by the Student's t-test.

Table III.- Effect of diet on *in vivo* performances and slaughtering data of kids.

	Diets[1]			SEM[2]	P value
	C	L	LO		
Initial BW (Kg)	6.21	5.33	6.34	1.831	0.573
Final BW (Kg)	11.50	11.42	11.66	2.091	0.978
Average daily feed intake (Kg/d)	0.118	0.113	0.107	0.011	0.228
Average daily milk intake (Kg/d)	0.910	0.840	0.900	0.233	0.844
Average daily gain (Kg/d)	0.123	0.127	0.118	0.028	0.852
Feed conversion ratio (F+M)[3] (Kg/Kg)	8.47	7.85	9.40	3.127	0.671
Net hot dressing percentage[4]	68.23	68.98	69.17	1.837	0.381
Net cold dressing percentage[4]	64.51	65.63	65.99	2.345	0.430

[1]Diets: C, control feed; L, control feed + 3% extruded linseed; LO, control feed + 3% extruded linseed + 0.6% Oregano. [2]Standard error of means. [3]Feed + milk. [4]% of empty body weight.

RESULTS AND DISCUSSION

Growth performances in vivo *and slaughtering data*

Performances *in vivo* and slaughtering data were not affected by the diet (Table III). Feed intake was very low for all the groups (107-118 g head/day), therefore growth performances were more affected by milk consumption (840-910 g head /day) than by the experimental diets.

Other studies in lambs showed that dietary supplementation with linseed oil or seeds did not influence slaughtering weight or daily weight gain (Bas *et al*., 2007; Radunz *et al*., 2009; Berthelot *et al*., 2010, 2012). In agreement with previous observations involving lambs (Simitzis *et al*., 2008), broilers (Botsoglou *et al*., 2004a) and rabbits (Botsoglou *et al*., 2004b), the oregano inclusion in the diet did not influence growth performances, although the components of oregano could have affected these by limiting the growth and colonisation of many pathogenic and non-pathogenic bacteria species in the gut (Bampidis *et al*., 2005).

The dressing percentage is similar to that reported by Todaro *et al*. (2006) in Girgentana kids slaughtered at the same age, but higher in comparison with other studies (Santos *et al*., 2007, 2008; Peña *et al*., 2011). The discrepancies can probably be attributed to differences in slaughtering weights (Marichal *et al*., 2003) and genotype (Peña *et al*., 2011).

Table IV.- Effect of diet on physical characteristics and lipid oxidation of meat from the *Longissimus lumborum* muscle.

		Diets[1]			SEM	P value
		C	L	LO		
pH_0		6.67	6.75	6.70	0.065	0.134
pH_{24}		5.49	5.60	5.45	0.179	0.772
MDA (mg/Kg meat)		0.38^{A}	0.42^{A}	0.23^{B}	0.088	0.001
1 d	L*	46.50	45.98	46.85	3.599	0.889
	a*	6.24	6.36	6.82	1.033	0.501
	b*	11.63	11.43	12.08	0.884	0.343
	WBS (kg/cm^2)	5.23^{b}	4.35^{B}	6.27^{Aa}	0.892	0.001
5 d	L*	44.09	44.21	44.86	3.625	0.902
	a*	6.58	7.02	6.72	1.283	0.788
	b*	11.52	11.52	11.77	0.940	0.829
	WBS (kg/cm^2)	5.06	4.57	5.31	1.314	0.531

a, b, P<0.05; A, B, P<0.01. [1]Diets: C, control feed; L, control feed + 3% extruded linseed; LO, control feed + 3% extruded linseed + 0.6% Oregano.

Physical and chemical parameters of meat

The data regarding pH, colour and tenderness of the *Longissimus lumborum* muscle are reported in Table IV.

The pH values were not significantly affected by the diet. The final pH that we recorded was between 5.45 and 5.60, with values similar to those reported by Stanisz *et al*. (2009) and lower than those of other genotypes (Dhanda *et al*., 2003; Santos *et al*., 2007; Bonvillani *et al*., 2010). In agreement with observations recorded in Manchego lambs (de la Fuente-Vázquez *et al*., 2014), the final pH values were not influenced neither by linseed nor by oregano supplementation, while Simitzis *et al*. (2008) reported significant increases in final pH values only for female Chios lambs, but not for males, when diet was supplemented with oregano essential oil.

Considering the effects on shelf life, colour and quality of meat, the final pH values are essential for refrigerated meat; levels above 5.8 generally indicate poor quality caused by pre-slaughtering stress (Lawrie, 1998; Dhanda *et al*., 2003). However, the levels we found are within the acceptable range (5.5-5.8) considered as optimal for high quality goat meat (Herold *et al*., 2007; Solaiman *et al*., 2011).

The colorimetric characteristics (L*, a*, b*) measured at 1 and 5 days after slaughter were not influenced by the diet. de la Fuente-Vázquez *et al*. (2014) found that dietary linseed had no effect on the colorimetric features of lamb meat. Dietary supplementation with oregano, instead, determined slightly higher levels of the parameters studied, in partial agreement with observations on lambs (Simitzis *et al*., 2008) fed with a diet containing oregano essential oil, that showed slightly increased meat lightness and significantly increased a* and b* values.

The lightness values (L*) were intermediate results compared to those observed by Peña *et al*. (2009) in Criollo Cordobes (L* = 42.54) and Anglonubian (L* = 48.82) kids slaughtered at the same weight of the kids of our study. The same suthors, moreover, registered a significant effect due to genotype and reported, like Santos *et al*. (2007), higher a* and b* values, in contrast with our observations.

Meat tenderness recorded the day after slaughtering was significantly affected by the diet; WBS values (kg/cm^2) were higher in group LO compared with group C (P<0.05) and especially with group L (P<0.01). However, these differences disappeared at the 5th day (Table IV). Linseed used alone, however, lowered tenderness in comparison with group C (Table IV), although not significantly.

This result is consistent with previous studies on kids (Abuelfatah *et al*., 2016), lambs (Nute *et al*, 2007; Díaz *et al*., 2011) and beef cattle (Juárez *et al*., 2012) fed using different PUFA sources.

The higher WBS values in kids receiving oregano can probably be ascribed to the lower fat content of their meat (Table V); Kemp *et al*. (1981) found that fatter lambs actually had more tender meat.

Table V.- Effect of diet on chemical composition of the meat from *Longissimus lumborum* muscle.

	Diets[1]			SEM	P value
	C	L	LO		
Moisture (%)	76.65	76.28[a]	77.23[b]	0.595	0.043
Crude protein (%)	19.97	20.27	19.51	0.503	0.055
Ether extract (%)	1.63[a]	1.74[a]	1.32[b]	0.232	0.018
Ash (%)	1.33	1.46	1.52	0.199	0.297

a, b, P<0.05. [1]Diets: C, control feed; L, control feed + 3% extruded linseed; LO, control feed + 3% Extruded linseed + 0.6% Oregano.

The WBS values are also similar to those reported by other researchers (Dhanda *et al*., 2003; Todaro *et al*., 2004; Bonvillani *et al*., 2010) and lower than those recorded by Santos *et al*. (2007), who observed significant differences due to genotype, sex and to the different muscle samples. The shear force values reported for kid meat vary widely in relation to factors such as pre-slaughter treatment, *post mortem* carcass treatment, the type of muscle and the sample preparation method (Webb *et al*., 2005).

TBARS values (mg malondialdehyde MDA/kg^{-1} meat) were similar for groups C and L, and were significantly (P<0.01) reduced by using oregano. The higher level of meat oxidation due to the linseed diet agrees with observations reported in Manchego lambs (de la Fuente-Vázquez *et al*., 2014); the antioxidant activity of oregano is confirmed in trials on lambs (Simitzis *et al*., 2008), calves (Zinoviadou *et al*., 2009), pigs (Simitzis *et al*., 2010) and rabbits (Cardinali *et al*., 2015). Oregano is thought to delay oxidation because lipids and hydroxyl radicals are rendered more stable by reaction with its phenolic components (Jadhav *et al*., 1996; Yanishlieva-Maslarova, 2001).

In agreement with Simitzis *et al*. (2008), it is therefore possible to state, although indirectly, that after oregano was ingested with feed, its phenolic compounds acted at tissue level after being absorbed.

Chemical composition of meat

The chemical composition data of the *Longissimus dorsi* are shown in Table V. The moisture content was higher (P<0.05) in group LO than in group L. However, the fat proportion was lower (P<0.05) in group LO than in the other two groups, while the protein and ash proportion was not influenced by diet. No information is available about the influence of oregano on the chemical composition of kid meat, while a study on rabbit meat did not report any significant differences in chemical composition that could be ascribed to oregano included in the diet (Cardinali *et al*., 2015).

Table VI.- Effect of diet on fatty acid profile (% of total fatty acid methyl esters) and indices related to human health of meat from the *Longissimus lumborum* muscle.

Fatty acids	Diet[1]			SEM	P value
	C	L	LO		
C12:0 (lauric)	0.38	0.35	0.49	0.201	0.479
C14:0 (myristic)	4.89	4.66	5.26	1.170	0.677
C16:0 (palmitic)	24.14	22.78	24.94	2.608	0.372
C17:0	0.99	0.88	1.00	0.102	0.095
C18:0 (stearic)	15.03	13.63	15.30	1.563	0.173
Total SFA[2]	53.23	48.49	53.07	3.853	0.084
C16:1 n-7 (palmitoleic)	0.94	0.86	0.87	0.318	0.883
C18:1 n-9 t9	1.11	1.08	1.01	0.423	0.919
C18:1 n-9 c9 (oleic)	23.17	25.12	24.66	2.784	0.468
Total MUFA[3]	26.72	28.74	28.03	3.124	0.536
C18:2 n-6, c9, c12 (linoleic)	14.21	13.09	11.89	2.311	0.251
C18:3 n-3 (α-linolenic)	0.73[b]	0.99[a]	0.92[a]	0.184	0.049
Total n-6[4]	15.84	14.77	13.25	2.528	0.237
Total n-3[5]	1.15	1.45	1.34	0.324	0.299
Total PUFA[6]	16.99	16.22	14.60	2.744	0.331
Nutritive value[7]	1.59	1.73	1.63	0.269	0.681
n-6/n-3	14.95[a]	10.39[b]	9.85[b]	3.288	0.032
PUFA/SFA	0.32	0.33	0.28	0.068	0.389
Δ^9 desaturase 16 index[8]	4.05	4.30	3.97	0.436	0.421
Δ^9 desaturase 18 index[9]	60.54	64.79	61.61	3.744	0.158
Elongase index[10]	60.39	61.93	60.88	4.296	0.819

a, b, P<0.05. [1]Diets: C, control feed; L, control feed + 3% extruded linseed; LO, control feed + 3% extruded linseed + 0.6% Oregano. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [2]Sum of C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C22:0 + C24:0. [3]Sum of C16:1n-7 + C18:1trans-9 + C18:1cis-9. [4]Sum of C18:2cis-9,cis-12 + C18:2cis-9,trans-11+ C18:3 + C20:4. [5]Sum of C18:3 + C20:3 + C20:5 + C22:6. [6]Sum of n-6 + n-3. [7]Nutritive value: (C18:0 + C18:1cis9)/C16:0. [8]Δ^9 desaturase 16 index = index of desaturase enzyme activity in C16 fatty acids = 100 [(C16:1cis9) / (C16:1cis9 + C16:0)]. [9]Δ^9 desaturase 18 index = index of desaturase enzyme activity in C18 fatty acids = 100 [(C18:1cis9) / (C18:1cis9 + C18:0)]. [10]elongase index = 100 [(C18:0 + C18:1cis9) / (C16:0 + C16:1cis9 + C18:0 + C18:1cis9)].

Fatty acid profile

The fatty acid profile of the *Longissimus lumborum* intramuscular fat is shown in Table VI.

Dietary treatment did not significantly influence the percentage of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The most represented (23-25 %) fatty acids were oleic (C18:1 n-9 cis9) and palmitic acids (C16:0), followed (12-15%) by stearic (C18:0) and linoleic acids (C18:2 n-6 cis9 cis12). This trend, except for linoleic acid, is in agreement with that observed in other studies

(Banskalieva *et al*., 2000; Todaro *et al*., 2006; Ebrahimi *et al*., 2014), which reported oleic, palmitic and stearic acids (in decreasing order) as the fatty acids found in the largest amounts; however, the percentage of stearic and oleic acids was lower than that found in the literature for lambs and kids, while the levels of linoleic acid were higher (Bas *et al*., 2005; Werdi Pratiwi *et al*., 2006; Bonvillani *et al*., 2010). In agreement with Kim *et al*. (2007), the lower levels of stearic acid we found in the muscle could have been caused by a greater amount of linoleic acid escaping ruminal biohydrogenation, and given that stearic acid acts as a Δ^9 desaturase substrate (Cook, 1996) in the synthesis of oleic acid, lower levels of C18:0 in muscle tissue can also determine lower concentrations of C18:1 n-9 cis 9.

In cattle, however, feeding high levels of concentrates (Latham *et al*., 1972) has shown to determine a reduction of the bacteria responsible for the rumen biohydrogenation, probably due to higher acidity (Harfoort and Hazelwood, 1997). The different proportions of acids we found might also be due to the heterogeneity of the trial conditions reported in literature, dissimilar for breeds (de Smet *et al*., 2004), diets (Bas *et al*., 2005; Lee *et al*., 2008) or slaughtering weights (Sañudo *et al*., 1998); it has been observed, in fact, that the fatty acid profile can be significantly affected by diet changes after weaning and by weight increases at slaughtering (Dhanda *et al*., 2003; Beserra *et al*., 2004).

The use of linseed in the diet did not affect the proportion of oleic acid in meat intramuscular fat, as also observed in calves (Aharoni *et al*., 2004; Bartoň *et al*., 2007).

In agreement with previous studies in lambs (de la Fuente-Vázquez *et al*., 2014), linoleic acid levels (11.89-14.21%) were not influenced by linseed supplementation. In Black Goat kids fed with sesame, sunflower or linseed oil, and slaughtered at a higher age than that of our kids, the linoleic acid content in the muscle was approximately a third (3.48-3.89%) of the level we found (Saqhir *et al*., 2012). It is likely that diet, genotype and the age difference were conditioning factors also in this case. Beserra *et al*. (2004) showed, indeed, that the linoleic acid content is influenced significantly by genotype and by slaughtering age of kids.

However, in a study involving Girgentana kids fed with maternal milk, or with maternal milk and feed, and slaughtered at the same age as our kids, the meat linoleic acid level was lower than the level we found (Todaro *et al*., 2006).

Regarding PUFAs, the C18:3 n-3 proportion was significantly lower ($P<0.05$) in the kids of group C than in those fed with linseed and linseed + oregano, in agreement with a previous study carried out in lambs fed with extruded linseed and linseed oil that showed an intramuscular content increase of the α-linolenic acid as related to the diet (Colonna *et al*., 2011).

The total PUFA n-6 and the total PUFA n-3 percentages were not influenced by dietary treatment, whereas the n-6/n-3 ratio was significantly higher ($P<0.05$) in group C than in the other two groups. This ratio reduction due to linseed supplementation agrees with the findings reported in Boer kids (Abuelfatah *et al*., 2016) and Manchego lambs (de la Fuente-Vázquez *et al*., 2014) also fed with linseed.

However, regardless of the diet, the ratio value was higher than that reported in other studies carried out in different genotypes (Todaro *et al*., 2006; Peña *et al*., 2009), and resulted unsatisfactory in terms of meat healthful properties. In relation to human health, this ratio should not exceed 4. The differences with the findings of other Authors may be due to the genotype (Peña *et al*., 2009), or to the amount and composition of the maternal milk received by the kids in our study, which suckled mostly milk, as showed by the feed intake values (Table III). The fatty acid composition of suckling kid meat, in fact, is mainly related to maternal milk composition (Zygoyiannis *et al*., 1992; Dhanda *et al*., 1999).

With regards to the nutritional value, in terms of the fatty acid ratio (C18:0 + C18:1)/C16:0) of meat, Banskalieva *et al*. (2000) suggested that its assessment could be a useful way to describe the potential effects on human health of the different fatty acids ingested with food. It has been highlighted that palmitic acid (C16:0) increases blood cholesterol, while stearic acid (C18:0) has no effect and oleic acid (C18:1) reduces it. In the present study, the ratio ranged from 1.59-1.73 and did not present any differences related to diet. However, the ratio was lower than that recorded in other genotypes (Todaro *et al*., 2002; Santos *et al*., 2007; Peña *et al*., 2009; Bonvillani *et al*., 2010), and consequently less satisfactory for human health.

The PUFA/SFA ratio was unaffected by the diet, and the values ranging between 0.28 and 0.33 are comparable to those obtained in finishing lambs receiving forage (Enser *et al*., 1998a), linseed supplementation (de la Fuente-Vázquez *et al*., 2014), fish, rapeseed or soybean meal (Ponnampalam *et al*., 2001), or fish oil (Wachira *et al*., 2002; Cooper *et al*., 2004). However, the ratio values we obtained were below the level of 0.45, recommended for human health (Enser *et al*., 1998b).

Sensory characteristics of meat

The sensory characteristics of meat are shown in Table VII. Overall acceptability, tenderness and juiciness were not significantly influenced by the feeding treatment, although the tasters gave a higher flavour score to the

meat of kids fed with oregano than to group L subjects and especially to group C (P<0.01). The lack of the diet influence on meat tenderness and juiciness is in agreement with other research carried out in lambs whose ration was supplemented with different lipid sources including linseed oil (Nute *et al*., 2007; Diaz *et al*., 2011) and with the findings reported in Kivircik lambs (Demirel *et al*., 2013) receiving oregano supplementation. About the flavor, instead, the best values were recorded following the administration of oregano in contrast with the observations of Demirel *et al*. (2013), which did not show the influence of oregano oil supplementation on meat flavor in lambs.

Table VII.- Effect of diet on sensory characteristics of meat.

	Diet[1]			SEM	P value
	C	L	LO		
Tasters (n.)	41	41	41		
Overall acceptability	6.29	6.92	6.66	1.224	0.070
Flavour	5.31^{B}	5.50^{b}	6.17^{Aa}	1.474	0.025
Tenderness	6.21	6.60	6.22	1.649	0.491
Juiciness	5.88	6.02	5.97	2.054	0.948

[1]Diets: C, control feed; L, control feed + 3% extruded linseed; LO, control feed + 3% extruded linseed + 0.6% Oregano.

CONCLUSIONS

The results lead us to conclude that, under our experimental conditions, the ration supplementation with extruded linseed does not affect the productive performance, the chemical and physical characteristics of meat and fatty acid profile of muscle lipids. The fatty acid composition, however, shows higher linolenic acid content and a better n-6/n-3 ratio. This poor response may be probably attributed to the low kid feed intake and, therefore, to the greater influence of maternal milk on kid growth and body composition.

Oregano supplementation enhances meat lipids oxidative stability and its flavor, confirming results that have been already observed in other species.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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