INFLUENCE OF DIET ENRICHED WITH POLYUNSATURATED FATTY ACIDS ON HISTOCHEMICAL CHARACTERISTICS OF LAMB MUSCLE

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Introduction

In industrialized countries meat plays a very important role in human diet. Ovine meat consumption is mostly spread in Mediterranean countries, then the production re-qualification may account for a spring to re-launch home consumption. In the last few years meat demand has greatly decreased also because of their saturated fatty acids likely to affect cardio-vascular diseases and some cancerous forms. The last studies tend to reduce saturated fatty acids in favour of the increase in polyunsaturated ones (PUFA) determining an optimal PUFA/SFA ratio. In diet the assumption of omega-6 and omega-3 fatty acids affects positively various cellular function, in the brain, retina, and prevents various pathology (Nordoy,2001).

Omega-3 polyunsaturated fatty acids are also likely to alter man's plasma lipid composition by lowering triglycerides levels thus reducing the risk of lethal arhythmic in patients with myocardial infraction. Of the feeding strategies in ruminants to increase PUFA muscular concentration it is to be included fish oil or vegetal oil intake (coco, olive, sunflower). It is to be noted that in monogastric the adipose tissue composition reflect the one of ingested fats by the diet (Raes et al., 2004). On the contrary in ruminants this does not happens because many substances in the reaction, including nutraceutic ones and fatty acids, undergo a bio-hydrogenation process by ruminal micro organism (Wood and Enser,1997; Nicastro et al., 2003; Raes et al., 2004).The aim of this work was to investigate histological characteristics relevant to morphometric study (surface and diameter) of muscle fibres associated with different feed diets enriched with Omega-3 polynsatured fatty acids.

Materials and Methods

Thirty six 30 days Val di Belice bred wether lambs (mean live weight 12.1 kg) were randomly divided into three groups of 12. Lambs were randomly assigned to individual pens and housed indoors. After 7 days adaptation, the three groups received hay *ad libitum* and were assigned to one of the following treatments:
First group was control; Second group received concentrated enriched with 1% of omega-3 fish oil; Third group received concentrated enriched with 3% of omega-3 fish oil. At the end of feeding the lambs were fasted overnight and slaughtered at a commercial abattoir at 60 days of age. Samples of longissimus thoracis (LT) and semimembranosus (SM) muscles were collected from all animals 4h after slaughter (from the 10th rib for the first, from the middle point for the second muscle) for the histochemical characterization of the muscular fibres, whereas a part of LT muscle was preserved at -80 °C up to the moment of different analysis.

Duplicate muscle samples were immersed into liquid nitrogen and mounted on spindles before sectioning 12 mm thick using a Reichert-Jung freezing microtome. Serial sections mounted on glass microscope slides were stained with NADH-Tr and myofibrillar ATPase reacted at alkaline pH to differentiate muscle fibre type according to their oxidative and glycolitic capability (Nicastro 1989). Fibres were classified into bRed, aRed and aWhite according to Ashmore and Doerr (1971). Sections were analyzed using an Image Analyzer Vidas by Zeiss to determine fiber diameter and fiber percentage type for each fiber type. The data were subjected to the analysis of variance according to GLM procedure by SAS (SAS Inst. Inc., Cary. NC).

Results and Discussion

Diameter of muscle traits fibers in longissimus thoracis and semimembranosus muscles

The morphometric values of the fibers in LT and SM muscles are reported in table 1. In both muscles it is immediately clear that the animals received concentrated enriched with 3% of omega-3 fish oil present fibers with a larger diameter with statistical significance ($P < 0.05$) is shown only for the beta-Red and alpha-white fibers. In particular for the SM muscle the alpha-white fibers reach the greater size (28.12 mm)) in lambs fed in the third group. The histo-enzymatic typization of muscular fibers is basic for the increase of the muscular tissue and subsequent biochemical evolution of the same in meat after slaughter (Nicastro, 1992). In previous study, we observed that muscles involved in posture are more oxidative than those involved in movements with larger alpha-white glycolytic fiber in semimembranosus muscle after that lambs were fed with concentrated enriched with omega-3 fish oil (Nicastro et al., 2003; Nicastro, 2004). Muscle fiber types reflect a complex interaction of multiple sources of control of protein expression, and the net effect of the control ultimately defines its functional properties. Differences in muscle fiber type between muscles are reflected in differences in fatty acid composition. "Red" muscles have a higher proportion of phospholipids than "white" muscles and therefore a higher percentage of PUFA. The results from this research confirmed what reported in literature.

Table 1. Least-square means for size and population of muscle traits fibers in longissimus thoracis and semimembranosus muscles as influenced by feeding system

Muscle	Fiber	Diameter (mm)			Population (%)		
		Control diet	Omega-3 1%	Omega-3 3%	Control diet	Omega-3 1%	Omega-3 3%
LT	bRed	22.92[a]	26.78[b]	27.16[b]	13.61[a]	14.06[a]	14.77[a]
	aRed	19.66[a]	21.24[a]	21.32[a]	42.21[a]	39.00[b]	41.20[a]
	aWhite	23.39[a]	25.65[b]	27.21[b]	44.18[a]	46.94[b]	44.03[a]
SM	bRed	23.76[a]	24.19[a]	27.24[b]	12.61[a]	13.69[a]	14.20[a]
	aRed	21.01[a]	21.51[a]	22.28[a]	39.63[a]	36.79[b]	36.48[b]
	aWhite	23.48[a]	26.64[b]	28.12[b]	47.76[a]	49.52[b]	49.32[b]

[a–b] Means in the same row without a common superscript letter differ significantly ($P < 0.05$).

Population of muscle traits fibers in longissimus thoracis and semimembranosus muscles

Lambs that were fed supplemented with omega-3 had a greater presence ($P < 0.05$) of aWhite fibers whereas the aRed fibers population is lower in the SM muscle.

The results here discussed may are be indicative of a particular Italian productive technique, that is the milk lamb. The percent distribution of the fibres evidences a lower presence of the red ones in SM muscle of controls (12.61%). This result is found even though not in a significative manner in LD muscle. These differences confirm what Nicastro and Moody (1992) found out, and this depends on the different functionality and physiology of the considered muscles. From the morphometric and percent data of the fibres of the animals fed whit omega3 a trophic potential can be found, that is to be interpreted as a phase in full evolution in the theory of fibre "mutations" (Nicastro 1992).

Conclusions

The present results indicate the beneficial effect of the diet enriched with omega-3 fish oil. This difference appears to be a direct consequence of changes in fatty acid metabolism dependent on the modification of phospholipids components in the skeletal muscle membrane. Further more studies are needed to understand the mechanisms associated with these findings.

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