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## Influence of antioxidants to improving meat quality: histochemical characteristics of lamb muscle

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### TITOLO

Influenza degli antiossidanti per migliorare la qualità della carne: caratteristiche istochimiche del muscolo di agnello

### KEY WORDS

meat quality, fiber type, fatty acids  
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Qualità carne, fibra, acidi grassi  
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### Summary

A strategy to obtain "functional" meat is the change in vivo of carcass composition, which is affected by feeding in primis. Thirty Delle Langhe breed lambs were divided in three groups according to: post weaning rationing with no omega-3 in diet, or diet containing 4% of omega-3, or third group with diet containing 7% of omega-3. A portion of the longissimus dorsi and semimembranosus muscles was sectioned and evaluated histochemically to fiber type. Intramuscular fat cells were stained and their area determined using a particle size analyzer. The enrichment of diet with omega-3 fatty acid significantly affects the morphometric parameters of adipocytes and fiber of the two muscles under study. However, the percentage of fiber types varied between treatment groups with a lower percent of  $\beta$ red fibers being observed. These findings add interest and meaning to our understanding of meat characteristics as food that may contribute to the improvement of human health.

### Riassunto

La ricerca ha la finalità di ottenere il prodotto carne quale alimento "funzionale" ottenuta da soggetti la cui dieta risulta arricchita di antiossidanti. Trenta agnelli di razza Delle Langhe sono stati divisi in tre gruppi in base alla dieta post svezzamento: primo gruppo senza omega-3 nella dieta, il secondo gruppo con una dieta contenente il 4% di omega-3, e il terzo gruppo con una dieta contenente il 7% di omega-3. Un campione dei muscoli longissimus dorsi e semimembranosus è stato prelevato e valutato istochimicamente in relazione al tipo di fibra. Mentre alcune cellule di grasso intramuscolare sono state colorate e la loro superficie valutata con un analizzatore d'immagini. L'arricchimento della dieta con acidi grassi sembra influenzare in modo significativo le analisi morfometriche del grasso intramuscolare e della fibra muscolare nei due muscoli studiati. Tuttavia, la percentuale dei tipi di fibre varia tra i gruppi con una percentuale inferiore delle fibre rosse. Questi risultati aggiungono interesse e significato alla nostra comprensione delle caratteristiche della carne quale alimento che può contribuire al miglioramento della salute umana.

## Introduction

In the last few years the interest in omega-3 fatty acids has greatly increased both in the medical-scientific field and public opinion. Number of studies have shown that such a class of polyunsaturated fatty acids owns favourable effects on the human health and is particularly effective in preventing different kinds of diseases. Omega-3 fatty acids are of vital importance for the human being because, this last like all animal species, is not able to synthesize them autonomously starting from base food components. However, man is able to elongate the molecules of fatty acids of this family. He is able, then, to produce long chain omega-3 fatty acids from an omega-3 fatty acid available in plants, that is the alpha-linoleic acid. Such a transformation mechanism is characterized by a variable effectiveness over different ages of life. Long chain omega-3 acids (particularly the eicosapentaenoic acid, or EPA and docosahexaenoic acid, or DHA, which account for the compounds of this family very important for the human body) must be derived from food. Among other things, these compounds are present in most common food, except for fish and its derivatives. A further contribution of omega-3 fatty acids to man's nutrition, can be obtained

by means of fish oils consumption and by feeding stock farming with feed enriched in omega-3 acids. It is noteworthy the transfer of omega-3 in meats especially the cattle ones, in order to improve meat quality (Nicastro et al. 2001; Nicastro et al. 2002; Nicastro F. 2003). Muscle fiber type composition is highly variable, depending on several factors such as breed, sex, ambient temperature exercise and diet (L. Lefaucheur 2010). There are no reports concerning muscle fiber characteristics and diet added with antioxidants. The aim of this work is to investigate histological characteristics relevant to morphometric study (surface and area) of muscle fibres and intramuscular fat associated with different feed diets.

## Methods

Thirty Delle Langhe breed lambs have been used. They were fed with weaning feed over a 30 day period, and later with growth feed added with omega-3. The most representative fatty acid  $\omega_3$  was DHA with a presence of 15,8%. Lambs were divided in three groups according to the following treatments: First group: Post weaning rationing with no omega-3 in diet; Second group: Post weaning rationing with diet containing 4% of omega-3; Third

group: Post-weaning rationing with diet containing 7% of omega-3. Lambs were slaughtered at 110 days of age and the carcasses were chilled at 3° C for 24 h. Samples of longissimus dorsi (LD) and semimembranosus (SM) muscles were collected from all animals 4h after slaughter for the histochemical characterization of the muscular fibres. Some pieces of said muscles have been taken, and then immersed in liquid nitrogen for about 10 seconds. Cross section were cut and mounted on spindles before sectioning 15 mm thick using a Reichert-Jung freezing microtome. Serial sections mounted on glass microscope slides were stained with NADH-Tr, myofibrillar ATPase reacted at alkaline pH to differentiate muscle fibre type according to their oxidative and glycolytic capability (Nicastro, 1989), and hematoxylin according to the procedure outlined by Lillie (1965) in order to stain fat cells in the intercellular space. Fibers were classified on the basis of stain reactions using the technique of Ashmore and Doerr (1971): Beta-red fibers were dark brown, Alpha-red fibers were clear in the middle and surrounded by a blue ring, Alpha-white fibers were clear. Sections were analysed using an Image Analyzer Vidas by Zeiss to determine fiber diameter and fiber percentage type for each fiber

type. All data collected were subjected to analysis of variance and differences between the estimated average, evaluated with the “t” Student.

**Results and Discussion**

Detailed fibre type and intercellular fat was conducted on the LD and SM muscles of lambs from Delle Langhe breed, reared on three different diets. Least square means for longissimus fiber type area and percentages for each dietary group are shown in Fig. 1 and 3. There is a significant effect ( $P < .05$ ) on the fiber size of red and white muscle fibers after lamb had been on feed control and 7% of omega-3 fatty acids respectively. The alpha-white fibers tended to be larger in area than alpha-red (Intermediate) and beta-red fibers (3725 m<sup>2</sup> compared to 1525 m<sup>2</sup> and 735 m<sup>2</sup> respectively).

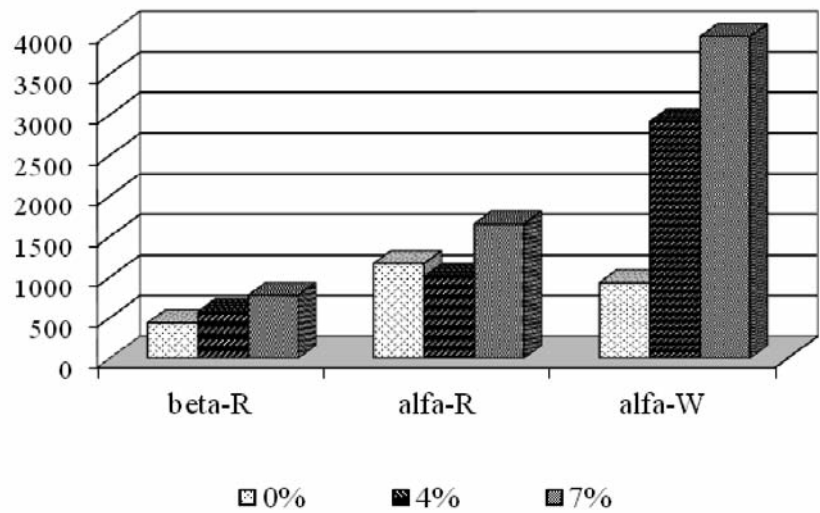
The diet enriched with omega-3 did not influence the distribution of fiber types. However, a significant ( $P < .05$ ) decrease in percentage of alpha-red fibers was noted in the lamb fed with 7% of fish oil.

Muscle fibers characteristics for the SM are presented in Fig. 2 and 4. The least square means of all three fiber types (red, intermediate and white) in this muscle revealed little variation ( $P < .05$ ) in

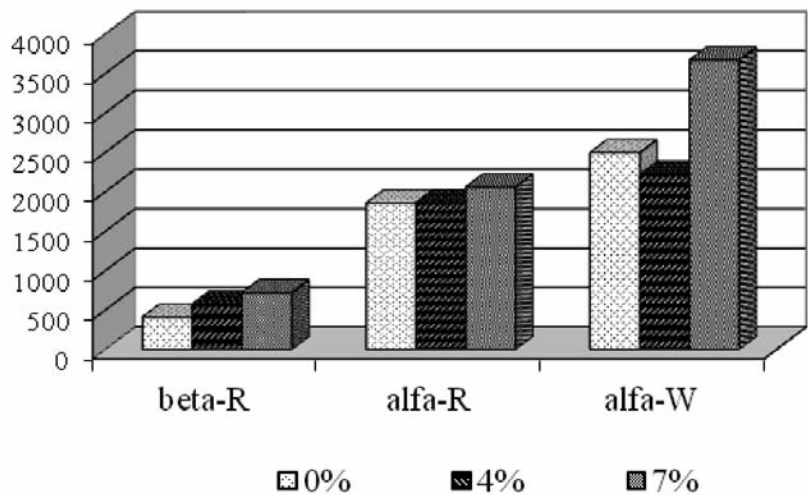
muscle fiber size in lamb that were fed three different levels of feed added with omega-3. However, feed appeared to have had a sig-

nificant effect on red and white muscle fiber size and percentage of lamb in group one (control) and three (7% of fish oil). Mean fiber

**Figure 1** - Least squares means for area ( $\mu\text{m}^2$ ) of fibers in *longissimus dorsi* muscle of lamb Delle Langhe fed different amount of omega-3 fatty acids



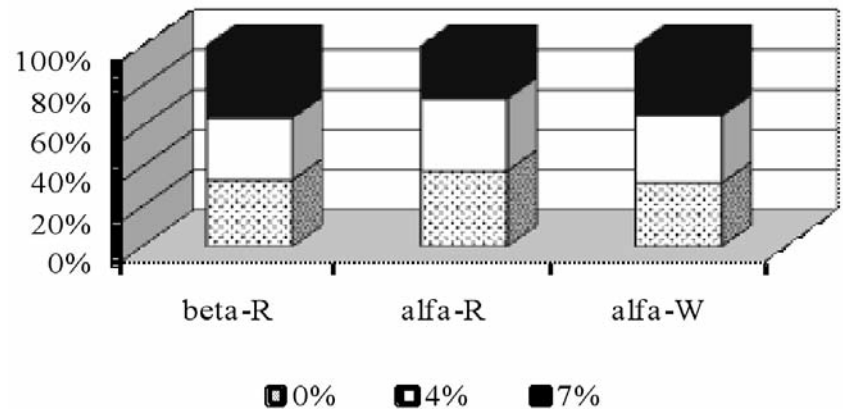
**Figure 2** - Least squares means for area ( $\mu\text{m}^2$ ) of fibers in *semimembranosus* of lamb Delle Langhe fed different amount of omega-3 fatty acids



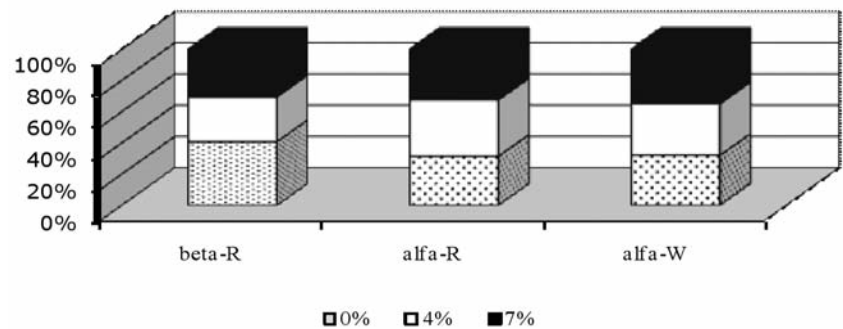
area of the three fiber types showed the same pattern as the LD muscle where alpha-white fibers appeared larger than beta-red or alpha-red (intermediate). Variation of fiber type distribution within muscles is important when studying fiber type composition in relation to meat quality. Muscles involved in posture are more oxidative than those involved in movements (Totland and Kryvi, 1991; Henckel, 1995). Unfortunately, no definite conclusions can be drawn to associate fiber transformation with animal feed age, since the experiment was terminated before long term effects could be observed. More research is needed using a biopsy technique to observe fiber types in animals over a longer age span before giving definite answers can be to this important issue.

Fig. 5 and 6 show the diameter of intracellular fat in LD and SM muscles for each dietary group. In semimembranosus muscle the area of the adipocytes was larger in lamb fed with 7% of fish oil ( $P > .05$ ) while in the longissimus muscle no significant differences are noted. The intracellular fat content is an important factor that affects sensory quality. Lipids are mainly stored in beta-red and some alpha-red fibers (Essen-Gustavsson et al. 1994). However, Henckel et al. (1997) found that the intracellular fat content was

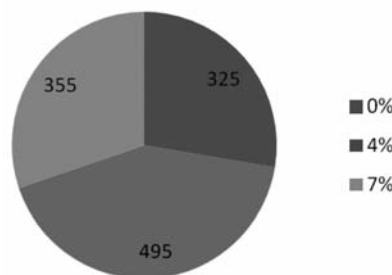
**Figure 3** - Fibers percentage of *longissimus dorsi* of lambs Delle Langhe fed with different amount of omega-3 fatty acids



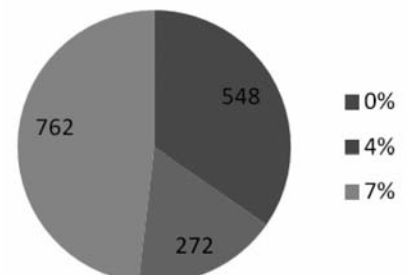
**Figure 4** - Fibers percentage of *semimembranosus* muscle of lambs Delle Langhe fed with different amount of omega-3 fatty acids



**Figure 5** - Area ( $\mu\text{m}^2$ ) of intramuscular fat cells of LD muscle of lamb Delle Langhe fed different amount of omega-3 fatty acids



**Figure 6** - Area ( $\mu\text{m}^2$ ) of intramuscular fat cells of SM muscle of lamb Delle Langhe fed different amount of omega-3 fatty acids



positively correlated with the frequency of alpha-white fibres. Essen-Gustavsson and Fjelkner-Modig (1985) concluded that meat tenderness is related to the metabolic muscle profile.

### Conclusions

Within a carcass there is considerable variation in meat quality characteristics between various muscles. The inter-muscle variation is often directly related to the metabolic and contractile properties as determined by their muscle fiber type distribution. The present study relates to the influence of dietary omega-3 fatty acids on the fiber type characteristics, but no clear pattern was obtained in this experimental design. More research is needed to verify these findings.

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