

OPTIMIZATION OF A CRYOHISTOLOGICAL TECHNIQUE TO PERFORM MICRO-MORPHOMETRIC ANALYSES OF INTRAMUSCULAR FAT MARBLING IN THE IBERIAN PIG (S4P16)

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Intramuscular fat is positively associated to meat quality. Besides chemical analysis of intramuscular fat, microscopic image analysis might allow for a detailed assessment of intramuscular fat distribution and changes in adipocyte size and number. These features, which could be different for a given intramuscular fat percentage, could facilitate comparisons between growth stages, feeding systems and swine breeds, e.g., Iberian or other fatty pigs vs. commercial, fast-growing breeds. On the other hand, certain differences in microscopic marbling structure could account for important pork quality traits. The aim of this work is to adapt a cryohistological technique to perform microphotometric studies involving intramuscular fat deposition in pig muscle. For these purposes, a preliminary study involving Iberian pigs (*Retinto* strain, *Valdesequera* line) finished in “*montanera*” system has been carried out. Animals were previously fed with three diets differing in fibre content (control, moderate and high) during the pre-finishing stage (*pre-montanera*) and then were submitted to *montanera* (free-range acorn feeding system). Histological studies were performed in 1 cm samples excised from specific and consistent locations within a central stripe taken from the medial to the lateral surfaces of the *Longissimus dorsi* muscle at the level of the 10th intercostal space. These segments, obtained from frozen loin pieces, were fixed in paraformaldehyde and then cryoprotected in a sucrose and gelatin solution. Next, sections were embedded, and 30- μ m thick cryosections were obtained and stained with Sudan III and Methylene blue. Microphotographs of the sections were analysed by using an image analysis software. Adipose tissue area and number and mean area of adipocytes within the muscle were quantified. The experimental outcome shows that this technique is feasible and prevents the formation of ice crystals. Therefore, it can be used instead of the classic paraffin-inclusion method, thus avoiding tissue dehydration. In addition, muscular and fat tissues were easily discriminated for morphometrical

measurements. Preliminary results suggest that the *premontanera* high-fiber regime resulted, after *montanera*, in smaller adipose tissue area and adipocyte number in comparison with the other two groups, although adipocyte size did not differ. Moreover, these results correlate with intramuscular fat quantified by chemical methods.

Keywords: adipocytes, *Longissimus dorsi* muscle, image analysis, meat histology

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