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Regional differences in micro-structural and biochemical characteristics of growth and metabolism in *semitendinosus* muscle of 28-day old piglets

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ABSTRACT

Micro-structural and biochemical characteristics of myofibre growth and metabolism were compared among three regions (dark [near bone], central, and superficial bright) of the *semitendinosus* muscle mid-belly of 28-day old piglets. The total fibre number as estimated from the dark, central, and bright region, as well as mean fibre area did not differ among regions. Compared with the bright region, the dark region exhibited a larger proportion of red oxidative fibres, a greater capillary density, smaller protein concentration, greater DNA concentration, a lower lactate dehydrogenase (LDH) activity, and a higher isocitrate dehydrogenase (ICDH) activity. High concordance correlation coefficients were found between the central region and the mean of the three regions in terms of micro-structural properties (except fibre type distribution), LDH and ICDH activities per g tissue, which would allow restricting the analyses to the central region for these traits.

1. Introduction

Besides the *longissimus* muscle, the *semitendinosus* (ST) muscle is the most frequently examined muscle in studies on the impact of genetic and environmental factors on muscle development and meat quality in the pig. The round shape of the muscle cross-section, the fibre axis running parallel with the muscle axis, and the fact that it can be easily excised as a whole from the hind limb made ST muscle the standard muscle for the estimation of total fibre number. Muscles contain a mixture of myofibre types, but can be classified into fast and slow or glycolytic and oxidative muscles based on the predominating contractile or metabolic fibre type (Karlsson, Klont, & Fernandez, 1999). In so-called mixed muscles like the ST, at least two regions can be distinguished within the muscle cross-section: the dark, near-bone region with a high proportion of red oxidative/type I fibres and the bright, superficial region with predominantly white glycolytic/type IIb fibres (Lefaucheur, Edom, Ecolan, & Butler-Browne, 1995). Researchers use different approaches to obtain results from ST muscle. One method is to analyze only a single sample from a standardized location (Gondret, Lefaucheur, Juin, Louveau, & Lebret, 2006; Gondret et al., 2005). Alternatively, results can be presented separately for the dark and the bright regions (e. g. Bee, 2004). Thirdly, average values of two or more sampling sites can be presented, e.g. average of the dark and the bright regions (Rehfeldt, Adamovic, & Kuhn, 2007; Rehfeldt, Henning, & Fiedler, 2008), average of three samples (one each from the dark, central, and bright region: Lösel, Kalbe, & Rehfeldt, 2009), average of five samples (one from the dark, two from the central, two from the bright; Nissen, Jorgensen, & Oksbjerg, 2004) or three to six samples, comprising the entire area of the cross-section (Gatford et al., 2003). Whereas some differences in fibre type composition between the dark and bright region within the cross-section have been described previously (Bee, 2004; Bee et al., 2007; Beerman et al., 1990; Gunawan et al., 2007; Hausman, 1989a,b; Markham et al., 2009), less is known about differences in biochemical characteristics like protein and DNA concentrations and enzyme activities, particularly in piglets. Thereby, the early postnatal period is of increasing scientific interest for pig muscle development because of prolonged myofibre formation (Brameld & Daniel, 2008; Rehfeldt, Stickland, Fiedler, & Wegner, 1999; Rehfeldt et al., 2008). Furthermore, differences in histological and biochemical traits among the dark, bright, and central region lying in between have not been described so far. Therefore, it was the aim of this survey to characterize and compare the dark, central, and bright regions taken from the cross-section of the mid-belly of ST muscle of 28-day old piglets in terms of structural and biochemical characteristics of myofibre growth and metabolism. In addition, we tested whether the central region would represent the mean of the three regions in the muscle cross-section, which would allow restricting analyses to the central region.

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2. Materials and methods

2.1. Animals

All procedures were in accordance with the guidelines set by the Animal Care Committee of the state Mecklenburg-Vorpommern, Germany, based on the German Law of Animal Protection.

Offspring of 15 German Landrace gilts from 10 temporally successive repeats were used in this study. A total of 93 female and castrated male piglets with an average weight of 7.11 ± 1.27 kg (mean ± SD) were harvested at weaning on day 28 of age. The right ST muscle was removed as a whole and the circumference was measured at the muscle mid-belly. From the dark (deep), bright (superficial) and central portions of the mid-belly samples were collected for histochemical ($n = 84$) and biochemical ($n = 69$) analyses. In contrast to finishing pigs, the regions hardly differ in colour in 28-day old piglets. Therefore, the dark region was identified by the tendinous insertion. The sampling sites are shown in Fig. 1. Samples were collected within 10 min *postmortem*, snap-frozen in liquid nitrogen and stored at −80 °C.

2.2. Biochemical analyses

Muscle homogenates of each region were analyzed for DNA, protein, activities of creatine kinase (**CK**), lactate dehydrogenase (**LDH**) and isocitrate dehydrogenase (**ICDH**). DNA was measured fluorometrically by a Fluorescence Reader FLx800 (Bio-Tek Instruments GmbH, Bad Friedrichshall, Germany; Ex 360 nm/Em 460 nm) against a standard of calf thymus DNA after using Hoechst 33258 (both from Sigma–Aldrich Chemie GmbH, Steinheim, Germany) according to Rehfeldt and Walther (1997). Protein concentration was determined according to Peterson (1977). Creatine kinase activity was measured using a commercial kit (Biomed, Oberschleisheim, Germany). The activities of LDH and ICDH were measured using modified assay protocols according to SIGMA (http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/learning-center/assay-library.html) as described by Huber, Petzold, Rehfeldt, Ender, and Fiedler (2007). All assays were adapted to microplate and the optical density was measured using a Spectramax Plus 384 plate reader (Molecular Devices Corporation, Sunnyvale, CA, USA). The activities of CK, LDH and ICDH were determined by measuring the change in the molecular concentrations of NADH or NADPH at 340 nm. The coloured complex of the Biuret reaction in the protein assay was measured at 750 nm.

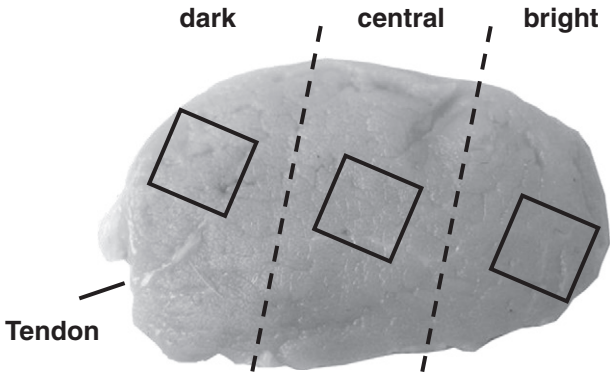

Fig. 1. Cross-section of *semitendinosus* muscle of a 28-day old piglet. The tendon was used to identify the dark region and the dashed lines separate the dark, central, and bright regions. The frames indicate the sampling sites.

2.3. Histochemistry and microscopy

Muscle cross-sectional area (**MCSA**) was calculated from the circumference of the muscle mid-belly. From the samples of each region, serial transverse sections of 10 µm were cut at −20 °C in a Jung Frigocut 2800 N cryostat (Leica, Nussloch, Germany). One section was stained for cytoplasm with eosin (Romeis, 1989) and for alkaline phosphatase (Spannhof, 1967) to visualize capillaries. Another section was exposed to the reaction for NADH-tetrazolium reductase (NADH-TR; Novikoff, Shin, & Drucker, 1961), which enables classification into red oxidative, intermediate, and white glycolytic fibres. Muscle micro-structure traits were determined on 900 muscle fibres (300 each in the dark, in the central, and in the bright region of the ST) by image analysis (TEMA v1.00, Scan Beam APS, Hadsund, Denmark). The estimated total fibre number was obtained by multiplying the fibre number per unit area by the MCSA of ST muscle (585 ± 109 mm²). Analysis of fibre type distribution revealed two values: first, the relative number of fibre types (%), and second the relative area occupied by each fibre type (%), the latter being calculated from the sums of the individual fibre areas of each type. Microscopic analyses were conducted by one and the same person.

2.4. Statistical analyses

For the comparison of the three regions, data were analyzed as repeated measures using the PROC MIXED procedure in SAS (SAS/STAT, Version 9.1, SAS Inst. Inc., Cary, NC, USA, 2004) with region and repeat as fixed factors and gilt within repeat as random factor. The repeated variable was region, and compound symmetry was used as covariance structure. In the case of a significant effect of region, differences between regions were tested by the Tukey test. Values in tables are given as least squares means ± standard error (SE) unless stated otherwise. In addition, a special contrast [central− (bright + central + dark)/3] was estimated.

To characterize the relationships among biochemical muscle traits, fibre type distribution, and fibre cross-sectional area (FCSA) within each region Pearson correlation analyses were performed.

Various methods were used to investigate whether the central region would represent the mean of the three regions [region mean = (dark + central + bright)/3]. The Pearson correlation coefficient between central and mean of three regions and contrast estimates 'central–mean' (ANOVA) are suitable measures to evaluate the concordance of mean values. The concordance correlation coefficient (CCC) by Lin (1989) is suitable for the concordance of individual measurements. The degree of concordance between two traits X and Y is measured by CCC as follows:

$$CCC = \frac{2\mathrm{cov}(x,y)}{\sigma_X^2 + \sigma_Y^2 + (\mu_X - \mu_Y)^2}$$

with μ_X, μ_Y = mean of X, Y; σ_X^2, σ_Y^2 = variances of X, Y; and cov(x,y) = covariance between X and Y. The CCCs are estimated by the corresponding sample values:

$$CCC_{est} = \frac{\frac{2}{n} * \sum_{i=1}^{n} \left(x_i - \overline{x}\right) * \left(y_i - \overline{y}\right)}{\frac{1}{n-1}\sum_{i=1}^{n}\left(x_i - \overline{x}\right)^2 + \frac{1}{n-1}\sum_{i=1}^{n}\left(y_i - \overline{y}\right)^2 + \left(\overline{x} - \overline{y}\right)^2}.$$

Li and Chow (2005) pointed out that the concordance correlation coefficient strikes a balance between a correlation measure insensitive to location differences and a measure of location differences. The estimated concordance correlation coefficient (CCC_{est}) was assessed according to Koch and Spörl (2007) who considered an almost complete concordance if CCCs are greater than 0.81, a strong