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Research article

HISTOLOGICAL AND HISTOCHEMICAL PROPERTIES OF *M. SEMITENDINOSUS* IN GERMAN LANDRACE PIGS AT BIRTH AND MARKET WEIGHT

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Current intensive pig meat production conditions impose the need to expand the knowledge about skeletal muscle characteristics, with the aim to improve both production of pig lean meat and meat quality. Histological and biochemical characteristics of the muscle highly influence the quality of meat, with muscle fiber number, size and fiber type distribution being important constituents. The objective of this study was to examine the structure of *m. semitendinosus* of piglets at birth, and slaughter pigs at the end of fattening. Total muscle fiber number was 350 x 10^3 in newborn piglets and increased up to nearly 900 x 10^3 in slaughter pigs. At birth, the muscle consisted of 3.76% primary fibers and 96.24% secondary fibers. At slaughter, slow-twitch oxidative fibers represented 21% of the total muscle fiber number, fast-twitch oxidative fibers represented 28 % while the majority of fibers (52%) in *m. semitendinosus* were of fast twitch glycolitic type. Obtained results indicate that postnatal muscle growth is accomplished mainly by muscle fiber hypertrophy.

Key words: age, fiber types, muscle fiber, pig

INTRODUCTION

The main constituent of skeletal muscle tissue is the muscle fiber – an elongated, multinucleated cell. The number and size of muscle fibers within the muscle largely determine the final weight of the muscle [1]. In pigs, the process of forming muscle fibers, myogenesis, takes place during fetal life and shortly after birth, within three successive stages [2]. The first wave of differentiation, in which primary (P) fibers are formed, appears in period 25-50 days of gestation [3]. In the second stage (50-80 days of gestation) about 20-25 secondary (S) fibers are formed around each primary fiber [3]. During the last days of gestation, and first days or weeks of postnatal life,

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the third stage of differentiation occurs and gives rise to tertiary (T) fibers between the secondary [2]. After birth, the number of muscle fibers increases only during first weeks in pigs [4-6]. Further on, muscle weight increases based on hypertrophy – enlargement of girth and length of muscle fibers [1]. At the same time, morphological and biochemical transformations of fibers occur, resulting in adult muscle fiber types: slow twitch oxidative (STO), fast twitch oxidative (FTO) and fast twitch glycolitic fibers (FTG) being formed. The importance and relations between muscle lineages (primary and secondary) in forming the adult types are not yet fully clarified [1]. It is thought, however, that primary fibers will give rise to adult STO fibers, secondary fibers which surround primary neonatal fiber will also transform to STO or FTO fibers, and the majority of secondary neonatal fibers will transform into FTG fibers in mixed muscles of the adult animal. The distribution of different metabolic types of muscle fibers, and hence the level of ATP within a muscle, will affect the development of *rigor mortis* after slaughter and consequently the quality of meat [7]. The aim of this study was to determine the differences in histological and histochemical characteristics of *m. semitendinosus* at birth and at market age in German Landrace pigs representing a modern meat-type pig breed.

MATERIAL AND METHODS

Animals and feeding

The research was conducted at the experimental station of the Leibnitz Institute for Farm Animal Biology - FBN Dummerstorf, Rostock, Germany. Pigs of the German Landrace breed were used for the investigation. All procedures including the use and treatment of animals 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. Eight multiparous sows were bred to the same German Landrace boar. Sow pregnancy was confirmed at day 28 of gestation by ultrasound. The sows were housed individually, under controlled environmental conditions (temperature 19°C, relative humidity 60-80%). All animals had free access to water, and were manually fed twice daily with standard soy based concentrate (Denkavit, Trede&Pein GmbH&Co. KG, Itzehohe, Germany). To induce farrowing, on day 114 of pregnancy all sows were injected intramuscularly with 1 ml of a synthetic prostaglandin (cloprostenol, 75 mg/ml: AniMedica West, Chemische Produkte GmbH, Senden, Germany). After birth, the body weight of piglets was recorded. From each litter two male piglets with body weight closest to the average for that litter were sacrificed for further analysis by injection of 1 ml mixture of Ursotamin (Ursotamin, Serumwerk Bernburg AG, Germany) and Combelen (Combelen, Bayer AG, Leverkusen, Germany) in the ratioo 1:1. The remaining male piglets were castrated at day 5 after birth, and all piglets were weaned at day 28 of age. During the whole growing-finishing period the offspring was fed *ad libitum*, with standard commercial

starter, grower and finisher feed mixtures. The growing period lasted until 180 days of age, and average market weight of slaughter pigs at the end of fattening was 108.35 kg.

Muscle histology and histochemistry

For histological and histochemical analysis, the right side *m. semitendinosus* was used in piglets, and the left side *m. semitendinosus* in slaughter pigs. In newborn piglets, muscle cross sectional area (MCSA) was estimated from the circumference of the muscle mid belly. Pieces of the mid belly from the neonatal muscle were mounted on cork-chucks and snap frozen in isopentane cooled in liquid nitrogen. Whole muscle serial transverse sections of 10 and 16 μm were cut at -20°C in a cryostat (Reichert-Jung, Leica, Nussloch, Germany). Muscle tissue sections of 10 μm were stained with eosin [8] and used for the determination of the total muscle fiber number per cross section, and sections of 16 μm were stained for myosin ATPase after acid preincubation at pH 4.2 [9], and used for the determination of primary and secondary fibers. Since in the pig muscle the central slow fiber in each cluster developed as primary fiber, the number of primary fibers corresponds to the number of dark, central fibers of the largest diameter in the cluster. The number of secondary fibers was calculated by difference.

In adult pigs, two samples were taken from *m. semitendinosus* of each individual: one sample was taken from the deep dark portion of the mid belly, and the second sample was taken from the superficial bright portion of the mid belly. Pieces of the muscle were mounted on cork-chucks and snap frozen in liquid nitrogen. Serial sections were cut at 10 μm in a cryostat, and stained for cytoplasm and nuclei with hematoxylin/eosin [8], or exposed to a combined reaction for NADH-tetrazolium reductase (NADH-TR) [10] and acid preincubated ATPase at pH 4,2 [9], which enables to classify STO, FTO and FTG muscle fibers. Further image analysis of sections of adult pig muscle was done by AMBA software (AMBA, IBSB, Berlin, Germany). On hematoxylin/eosin stained sections the number of fibers and cross sectional area (FCSA) of individual fibers were determined first, and immediately afterwards the sections stained for fiber types were analyzed. Average values for the investigated parameters calculated from both regions of the muscle were taken for further analysis. TFN was calculated by multiplying the number of fibers/unit area with FCSA.

Statistical analysis

Data were subjected to analysis of variance using the GLM and mixed classification models of SAS (SAS System for Windows Release 8e, SAS Institute Inc., Cary, NC 27513, USA).

RESULTS

An image of piglet *m. semitendinosus* section after staining for different muscle fiber types is shown in Figure 1. A clear difference in color intensity can be observed:

primary (P) fibers are of the most intense and dark color and centrally positioned in a clusters, surrounded by a number of lighter stained secondary (S) fibers. Values for histological parameters of *m. semitendinosus* in newborn piglets are summarized in Table 1.

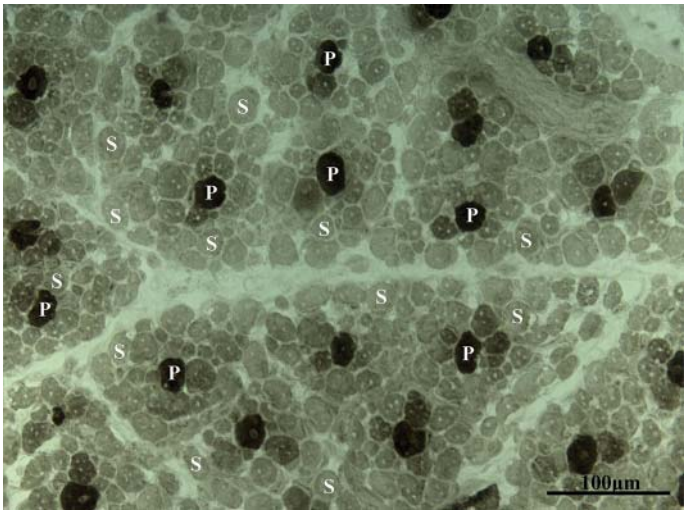

Figure 1. Image of *m. semitendinosus* cross section of a newborn piglet, stained for myosin ATPase after acid preincubation at pH 4.2, objective 20× (P – primary fibers, S – secondary fibers)

Table 1. Histological characteristics of *m. semitendinosus* in male newborn German Landrace piglets, n = 12 (LSM ± SE)

Histological trait	LSM ± SE
Total muscle fiber number	352.296 ± 18.749
Number of primary fibers	13.200 ± 762.55
Number of secondary fibers	339.096 ± 18.085
Percentage of primary fibers	3.76 ± 0.141
Secondary:primary ratio	25.90 ± 0.989

Microscopic images of deep and superficial portions of *m. semitendinosus* of one adult pig at the end of the fattening period are shown in Figure 2. Fibers of the most intense, dark color are STO fibers, the color of medium intensity is typical for FTO fibers, while the least color concentration and the lightest appearance are characteristics of FTG fibers. It was also observed that STO fibers are more numerous in the deep part of the muscle than in the superficial part, which is in opposite to the number of FTG fibers (Figure 2). Total fiber number, fiber type distribution and the area of individual fiber types within *m. semitendinosus* were monitored in slaughter pigs and presented in Table 2.

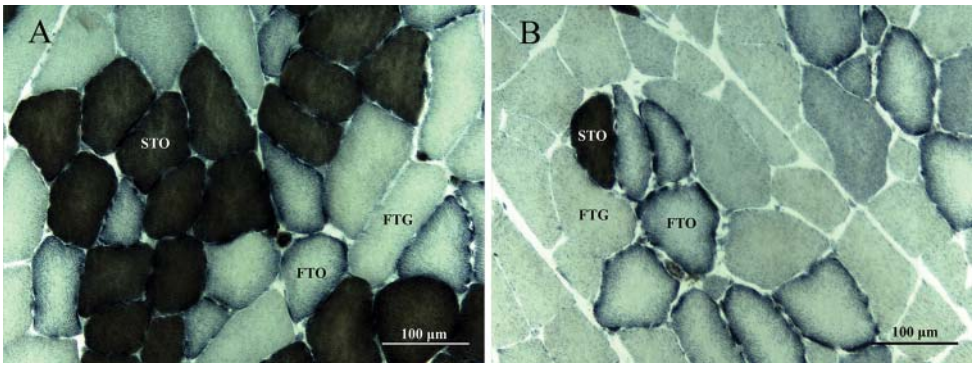

Figure 2. Image of the deep (A) and superficial (B) portions of *m. semitendinosus* from one slaughter pig after staining with combined reaction for NADH-tetrazolium reductase and acid preincubated ATPase at pH 4.2, objective 40× (STO – slow twitch oxidative, FTO – fast twitch oxidative, FTG – fast twitch glycolytic)

Table 2. Histological characteristics of *m. semitendinosus* in German landrace pigs at market age, n = 15 (LSM ± SE) (STO – slow twitch oxidative muscle fibers, FTO – fast twitch oxidative muscle fibers, FTG – fast twitch glycolytic muscle fibers

Histological trait	LSM ± SE
Total fiber number	882.165 ± 60.162
STO fibers, %	20.38 ± 1.32
FTO fibers, %	27.29 ± 2.07
FTG fibers, %	51.02 ± 2.09
STO fiber area, μm^2	4654.45 ± 521.77
FTO fiber area, μm^2	4360.03 ± 345.76
FTG fiber area, μm^2	3794.77 ± 207.82

DISCUSSION

The observed number of primary fibers in piglets was $13x10^3$, and the number of secondary fibers $339x10^3$, being in accordance with previously published results [11-13]. The percentage of primary fibers is relatively constant in newborn piglets, ranging from 3-5% [11,12,14]. The percentage of primary fibers determined in *m. semitendinosus* of newborn piglets in this research was 3.76%. The secondary:primary fibers ratio reported by [11] and [12] was 24.6 and 22.93, respectively, while in this study we found a ratio of 25.90.

Due to the appearance of new muscle fibers within the muscle during the first weeks of postnatal life in pigs, which is most probably the consequence of a combined elongation of fibers and development of a new generation of tertiary fibers [6], the total number of muscle fibers in *m. semitendinosus* increases from approximately 300-

- $400x10^3$ in piglets [11,13,15] up to 650 – $1.000x10^3$ muscle fibers in slaughter pigs [5,12,13,16]. In this research, the total fiber number in *m. semitendinosus* increased from approximately $350x10^3$ observed in newborn piglets, up to nearly $900x10^3$ in slaughter pigs, which is in accordance with findings of the aforementioned authors.

The results on the distribution of different muscle fiber types in *m. semitendinosus* of slaughter pigs are variable among authors. [5] determined about 25% STO fibers, 30% FTO fibers and 45% FTG fibers. In the same muscle, [17] reported 19 % STO fibers, 26% FTO fibers and 55% FTG fibers, while [13] determined 16-17 % STO fibers, 17-21 % FTO fibers and 61-66 % FTG fibers in *m. semitendinosus* of slaughter pigs. In this research, in *m. semitendinosus* of slaughter pigs it were determined about 21 % of STO fibers, 28 % of FTO fibers and 52 % of FTG fibers. The cross sectional area of different fiber types in *m. semitendinosus* of slaughter pigs obtained in this study was 3800-4650 μm^2, which is in accordance with results of [5] and [18].

The aim of this investigation was to examine the muscle structure of *m. semitendinosus* of piglets at birth, and slaughter pigs at the end of fattening. Total muscle fiber number determined in newborn piglets was $350x10^3$, and increased up to $900x10^3$ in slaughter pigs, which is an 2,5 fold increase, indicating that postnatal muscle growth is accomplished mainly by muscle fiber hypertrophy.

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REFERENCES

1. Te Paas MFW, Everts ME, Haagsman HP: Muscle Development of Livestock Animals. Physiology, Genetics and Meat Quality. Oxfordshire, United Kingdom: CABI Publishing, Wallingford 2004, 2-38.
2. Brameld J, Zoe T, Daniel R: In utero effects on livestock muscle development and body composition, Aust J Exp Agr 2008, 48:921-929.
3. Wigmore PM, Stickland NC: Muscle development in large and small pig fetuses, J Anat 1983, 137:235-245.
4. Rehfeldt C, Fiedler I, Dietl G, Ender K: Myogenesis and postnatal skeletal muscle cell growth as influenced by selection, Livest Prod Sci 2000, 66:177-188.
5. Rehfeldt C, Henning M, Fiedler I: Consequences of pig domestication for skeletal muscle growth and cellularity, Livest Sci 2008, 116:30-41.
6. Berard J, Kalbe C, Losel D, Tuchscherer A, Rehfeldt C: Potential sources of early-postnatal increase in myofibre number in pig skeletal muscle, Histochem Cell Biol 2011,136:217-225.

7. Dokmanović M, Baltić Ž M, Marković R, Bošković M, Lončina J, Glamočlija N, Đorđević M: Relationships among pre-slaughter stress, rigor mortis, blood lactate, and meat and carcass quality inpigs, Acta Veterinaria (Beograd) 2014, 64 (1), 124-137.
8. Romeis B: Mikroskopische Technik. P. BÖCK (Ed.), Urban & Schwarzenberg (696pp), München 1989, 110-120.
9. Guth L, Samaha FJ: Procedure for the histochemical demonstration of actomyosin ATPase, Exp Neurol 1970, 28:365-367.
10. Novikoff AB, Shin W, Drucker J: Mitochondrial localization of oxidative enzymes staining results with two tetrazolium salts, J Biophys Biochem Cytol 1961, 9:47-61.
11. Stickland NC, Handel SE: The number and types of muscle fibers in large and small breeds of pigs, J Anat1986, 147:181-189.
12. Rehfeldt C, Kuhn G: Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis, J Anim Sci 2006, 84:113-124.
13. Rehfeldt C, Adamovic I, Kuhn G: Effects of dietary supplementation of pregnant sows on carcass and meat quality and skeletal muscle cellularity of the progeny, Meat Sci 2007, 75:103-111.
14. Dwyer CM, Fletcher JM, Stickland NC: Muscle cellularity and postnatal growth in the pig, J Anim Sci 1993, 71:3339-3343.
15. Dwyer CM, Stickland NC, Fletcher JM: The influence of maternal nutrition on muscle fibre number development in the porcine fetus and on subsequent postnatal growth, J Anim Sci 1994, 72:911-918.
16. Nissen PM, Danielsen VO, Jorgensen PF, Oksbjerg N: Increased maternal nutrition of sows has no beneficial effects on muscle fiber number or postnatal growth and has no impact on the meat quality of the offspring, J Anim Sci 2003, 81:67-76.
17. Bee G: Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. J Anim Sci 2004, 82:826-836.
18. Velotto S, Vitale C, Stassi T, Crasto A: New Insights into Muscle Fibre Types in Casertana Pig, Acta Vet Brno 2010, 79:169-176.

HISTOLOŠKE I HISTOHEMIJSKE OSOBINE *M. SEMITENDINOSUS*-A SVINJA RASE NEMAČKI LANDRAS NA ROĐENJU I NA ZAVRŠETKU TOVA

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Savremena intenzivna proizvodnja svinjskog mesa nameće potrebu stalnog proširivanja znanja o karakteristikama skeletnih mišića, sa ciljem kako povećanja obima proizvodnje mesa, tako i poboljšanja kvaliteta mesa. Histološke i biohemijske osobine mišića u visokoj meri utiču na kvalitet mesa, pri čemu su od najvećeg značaja: broj i prečnik mišićnih vlakana, i distribucija pojedinih tipova mišićnih vlakana u okviru mišića. Cilj ovog istraživanja bio je da se ispita struktura *m. semitendinosus*-a prasadi na rođenju, i

tovljenika po završetku tova. Kod novorođene prasadi utvrđeno je ukupno 350 x 10^3 mišićnih vlakana u posmatranom mišiću, a taj se broj kod tovljenika uvećao na 900 x 10^3. Na rođenju, mišić se sastojao od 3,76 % primarnih vlakana i 96,24 % sekundarnih vlakana. Po završetku tova u *m. semitendinosus*-u su sporo okidajuća oksidativna vlakna činila 21 % ukupnog broja vlakana, brzo okidajuća oksidativna 28 %, a najveći deo vlakana (52 %) su bila tipa brzo okidajućih glikolitičkih vlakana. Dobijeni rezultati ukazuju da se postnatalni porast mišića svinja odvija uglavnom po osnovu hipertrofije mišićnih vlakana.