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INFLUENCE OF LITTER SIZE ON GROWTH AND STRUCTURE OF *M. SEMITENDINOSUS* IN NEWBORN PIGLETS AND SLAUGHTER PIGS

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Abstract: Modern meat production has to meet the requirements of profitability, while meeting the demands of consumers in terms of meat quality at the same time. Huge scientific work has been done in order to find balance between quantity and quality of meet. Most authors agree that piglets of lower birth weight have less muscle fibers within muscle, grow slower, compensating the muscle growth with increase of muscle fiber diameter and accumulating carcass fat. In recent years, selection in pig production has been directed towards increase of piglet number per litter. Since the inverse relation of litter size and birth weight has been well documented, the purpose of this work was to investigate the possible effects of litter size as a factor on pig growth and *m. semitendinosus* characteristics. Except the statistically significant difference (p=0,05) in number of primary fibers among piglets from small litter (15053) and large litter (11347), litter size did not influence birth weight, or other observed morphological and histological characteristics of the muscle significantly. Similarly, results of this research show that litter size as a factor did not affect final weight, morphological characteristics or fiber type distribution within the muscle in slaughter pigs.

Key words: litter size, muscle structure, muscle fibers, growth, pigs

Introduction

Quantity and quality of pig meat are largely depending on structure and growth of skeletal muscles (*Picard et al., 2002*). In adult animals, skeletal muscles are built of elongated muscle cells – muscle fibers. Based on speed of contraction

and type of metabolism, muscle fibers can be classified as: slow twitch oxidative (STO), fast twitch oxidative (FTO) and fast twitch glycolitic (FTG) (*Lefaucheur and Gerrard, 2000)*. During myogenesis, primary fibers occur first, followed by the development of huge number of secondary fibers and some tertiary fibers. After birth in pigs, primary fibers transform into STO fibers, while secondary and tertiary fibers will transform into fibers of fast contractile type (*Brameld et al., 2008*). After birth, the number of muscle fibers increases only during first weeks in pigs (*Rehfeldt et al., 2000; Rehfeldt et al., 2008; Berard et al., 2011*). In adult pigs, muscle weight increases based on hypertrophy – enlargement of girth and length of a muscle fiber (*Te Paas et al., 2004*). Therefore, the final weight of the muscle is determined by the number of muscle fibers given at birth, and growth of fibers during production.

Different factors are influencing growth and development of muscle fibers (*Vitorović and Adamović, 2012*). During last years, selection in pig production was directed towards increasing the number of piglets per litter, which led to a big variation of body weight and general decrease of piglets birth weight and higher number of piglets born dead in larger litters (*Quiniou et al., 2002; Sorensen et al, 2000*). A possible explanation could be a weaker supply of nutrients to fetuses in large litters (*Perre and Etienne, 2000*), or much stronger competition for nutrients *in utero* (*Quiniou et al., 2002*), leading to decrease of fetus weight and piglet birth weight with increase of litter size. Large number of authors agrees that piglets of higher birth weight have a higher total number of muscle fibers (*Wigmore and Stickland, 1983; Dwyer and Stickland, 1991; Rehfeldt et al., 2001; Nissen et al., 2004; Rehfeldt and Kuhn, 2006*). At the same time, newer research (*Rehfeldt and Kuhn, 2006; Gondret et al., 2006; Berard et al., 2008*) have shown that piglets of lower birth weight have slower growth, ending with larger diameter muscle fibers (*Gondret et al., 2006*). Such animals have higher percent of carcass fat, higher percent of undesirable giant fibers, and poor meat quality (*Rehfeldt and Kuhn, 2006). Berard et al. (2008)* suggest that litter size affects growth, carcass characteristics and meat quality only indirectly, and that this impact is realized through an inverse correlation with birth weight. On the other hand, *Beaulieu et al. (2010)* have found litter size influencing piglet birth weight, and somewhat slower growth of smaller litter mates, but the authors did not determine any significant influence of litter size on carcass composition and meat quality.

The aim of this paper was to examine the possible effects of litter size on growth, morphological and histological characteristics of *m. semitendinosus* muscle in piglets at birth and animals at the end of fattening period.

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 German Landrace breed were used for the investigation. All procedures including 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). During the experiment two sows had to be excluded, due to bone desease and premature farrowing. 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, as well as the litter size of each sow. Mediane value for number of piglets per litter was 14, and therefore litters were classified as small (<14 piglets) or large (>14 piglets). 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 proportion 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, right side *m. semitendinosus* was used in piglets, and 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 (Romeis, 1989) and used for 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 (Guth and Samaha, 1970), and used for 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 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 by hematoxylin/eosin (Romeis, 1989), or exposed to a combined reaction for NADH-tetrazolium reductase (NADH-TR) (Novikoff et all, 1961) and acid preincubated ATPase at pH 4,2 (Guth and Samaha, 1970), 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 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, tested with Students T-test (SAS System for Windows Release 8e, SAS Institute Inc., Cary, NC 27513, USA).

Results and discussion

Piglets

Besides the birth weight, most important morphological characteristics (weight, length, girth and cross section) and histological characteristics of *m. semitendinosus* (total fiber number, number of primary and secondary fibers, percent of primary fibers and secondary:primary fibers ratio) were analysed in this experiment. The results are shown in the Table 1.

Table 1. Influence of litter size on birth weight, morphological characteristics and histological structure of *m. semitendinosus* in piglets, LSM±SE

Parameter	Small litter	Large litter	P value
Number of animals, n	8	4	
Birth weight, kg	1.32±0.06	1.13±0.08	0.106
Morphological characteristics			
- weight of *m. semitendinosus*, g	2.98±0.20	2.54±0.25	0.218
- length of *m. semitendinosus*, cm	4.79±0.21	4.68±0.25	0.734
- girth of *m. semitendinosus*, cm	3.78±0.16	3.53±0.19	0.357
- cross section of *m. semitendinosus*, cm^2	1.14±0.09	1.00±0.11	0.382
Histological characteristics			
- total fiber number	379248±23716	325343±29046	0.201
- number of primary fibers	15053±964.55	11347±1181.33	0.051
- number of secondary fibers	364196±22876	313996±28018	0.201
- primary fibers, %	3.97±0.18	3.56±0.22	0.197
- secondary:primary ratio	24.33±1.25	27.47±1.53	0.164

Body weight of newborn piglets was higher in animals from small litters (1,32 kg compared to 1,13 kg), but no statistically significant difference was determined. P value for this parameter (p=0.106) could be interpreted as a tendency. Similar situation was registered for all morphological characteristics of the muscle. Numerically higher values were found for weight, length, girth and cross section of the muscle in small litter piglets, but the differences were not statistically significant.

The influence of litter size on histological structure of the muscle was monitored through total fiber number, number of different types of fibers, percentage of primary fibers and secondary:primary fibers ratio. Although all characteristics were higher in small litter piglets, only for the number of primary fibers statistical significance (p=0.05) was determined. Significantly higher number of primary fibers was found in piglets from small litters (15053) compared to piglets from large litters (11347).

Slaughter animals

In animals at the end of fattening period, numerous muscle characteristics besides the final weight have been analyzed, and the results are summarized in Table 2.

Table 2. Influence of litter size on final weight, morphological characteristics and histological structure of *m. semitendinosus* in pigs at the end of fattening period, LSM±SE

Parameter	Small litter	Large litter	P value
Number of animals, n	n=40	n=22	
Body weight, kg	108.16±1.96	103.28±2.57	0.18
Morphological characteristics	n=23	n=8	
- weight of *m. semitendinosus*, g	477.80±21.37	465.03±36.03	0.78
- length of *m. semitendinosus*, cm	23.65±0.47	23.34±0.83	0.76
- girth of *m. semitendinosus*, cm	21.52±0.40	21.27±0.68	0.77
- cross section of *m. semitendinosus*, cm^2	37.00±1.31	36.04±2.21	0.73
Histological characteristics	n=22	n=7	
- total fiber number	953127±42791	937878±72637	0.87
- STO fiber area	4518.94±373.13	4451.11±632.22	0.93
- FTO fiber area	4137.42±245.74	4001.44±417.30	0.79
- FTG fiber area	3673.07±138.26	3679.65±246.95	0.98
- Average fiber area	3972.92±173.66	3928.01±301.27	0.90
- STO fibers, %	18.82±0.88	22.09±1.57	0.15
- FTO fibers, %	30.81±1.45	26.20±2.49	0.19
- FTG fibers, %	49.66±1.44	50.31±2.50	0.83

Although all characteristics were numerically higher in animals from small liters, no statistically significant differences were determined neither for slaughter weight nor for morphological characteristics of *m. semitendinosus* between animals from small and large litters. Accordingly, analysis of live weight gain (data not shown) showed no statistically significant impact of litter size. Also, litter size did not affect total fiber number and area of different fiber types. Histological structure of *m. semitendinosus* was different in slaughter animals from small and large litters, but the differences were again not statistically significant. The percent of STO fibers was higher in large litter animals, while percent of FTO fibers was higher in animals from small litters compared to animals from large litters (31% and 26% respectively). The percent of FTG fibers was eqal in both groups, about 50%.

The data on litter size influence on muscle morphological and histological characteristics are very scarce in literature, especially for piglets. Our results have shown no statistically significant impact of litter size on muscle characteristics in pigs. However, since all investigated muscle characteristics had higher numerical values in small litter piglets compared to large litter animals, and having in mind that due to complexity of this research only two average weight male piglets per litter were used for analysis, further investigations would be required. Results monitored in slaughter pigs in this experiment are in accordance with findings of *Beaulieu et al. (2010),* showing no significant affects of litter size on *m. semitendinosus* characteristics.

Conclusion

Pig meat production has been directed towards the increase of number of piglets per litter in the past decades. In recent years, increased need of consumers for meat of better quality focused the attention of researchers on examination of possible influence of piglet birth weight on muscle structure. Previous studies have shown that lower birth weight piglets are growing slower, by increasing muscle fiber diameter (hypertrophy) and accumulation of carcass fat. Some authors reported that litter size is affecting muscle characteristics indirectly, through an inverse correlation with birth weight. Although values for all investigated parameters in this research were numerically higher in piglets from small litters, statistically significant differences among piglets from small and large litters were determined neither for birth or final body weight, nor for *m. semitendinosus* morphological and histological characteristics, with exception of number of primary fibers in piglets. However, data on the influence of litter size on the growth and muscle properties are rare in the literature. Therefore, further studies would be necessary to examine the possible impact of litter size either as independent factor or in combination with other factors that are influencing pig meat production.

Uticaj veličine legla na porast i strukturu *m. semitendinosus*-a kod novorođene prasadi i tovljenika

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Rezime

Savremena proizvodnja mesa usmerena je na profitabilnost sa jedne strane uz istovremeno odgovaranje zahtevima potrošača u pogledu kvaliteta mesa sa druge. Isrcpna naučna istraživanja obavljaju se u cilju pronalaženja balansa između količine i kvaliteta mesa. Najveći broj autora slaže se da prasad manje porođajne mase ima manji broj mišićnih vlakana u skeletnim mišićima, sporije raste, kompenzujući mišićni porast povećanjem prečnika mišićnih vlakana i deponovanjem veće količine masti u trupu. Poslednjih godina selekcija u svinjarstvu bila je usmeravana u pravcu povećanja broja prasadi u leglu. Obzirom da je utvrđena inverzna korelacija između veličine legla i mase prasadi na rođenju, cilj ovog rada bio je da se prouče mogući uticaji veličine legla kao faktora na porast svinja i karakteristike *m. semitendinosus*-a. Osim utvrđene statistički značajne razlike (p=0,05) u broju primarnih vlakana kod prasadi iz malog legla (15053) u odnosu na prasad iz velikog legla (11347), analiza nije pokazala uticaj veličine legla na masu prasadi pri rođenju i druge posmatrane morfološke i

histološke osobine mišića. Slično tome, ni kod tovljenika nije utvrđen uticaj veličine legla kao faktora na klaničnu masu, morfološke karakteristike ispitivanog mišića ili na zastupljenost pojedinih tipova mišićnih vlakana u mišiću.

Ključne reči: veličina legla, struktura mišića, mišićna vlakna, porast, svinje

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