EFFECT OF MATERNAL DIETARY PROTEIN LEVEL ON OFFSPRING MYOFIBER CHARACTERISTICS AND MEAT QUALITY IN FINISHING MEISHAN PIGS

Jinquan Wang[1,2], Xiao Li[1], Xiaojing Yang[1], Qinwei Sun[1], Ruihua Huang[3], Jun Xing[4], Yingdong Ni[1], Ruqian Zhao[1*]

[1]Key Laboratory of Animal Physiology & Biochemistry, Nanjing Agricultural University, Nanjing 210095, P. R. China

[2]College of Animal Medicine, Xinjiang Agricultural University, Urumqi 830052, P. R. China

[3]Laboratory of Animal Heredity & Breeding, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, P. R. China

[4]Jiangsu Polytechnic College of Agriculture and Forestry, Jurong 212400, P. R. China

[*]Corresponding author (phone: +86-25-84395047; fax: +86-25-84398669; e-mail: Zhao.ruqian@gmail.com)

***Abstract*—the aim of this study was to investigate whether feeding sows with different dietary protein levels throughout gestation and lactation may affect properties of skeletal muscle and meat quality of their offspring at finishing stage. Sixteen primiparous purebred Meishan sows were assigned randomly into low (LP) and high protein (HP) groups. The HP sows were fed on diets containing 12% and 14% crude protein (CP), while LP sows were fed on diets containing 6% and 7% CP during gestation and lactation, respectively. Piglets were fed on same standard diets after weaning. Male offspring pigs were sacrificed at finishing stage, the longissimus dorsi (LD) and psoas major (PM) muscles were sampled to determine the morphological features, meat quality traits, as well as myosin heavy chain (MyHC) type composition. The body weight and LD and PM muscle weight did not differ between two groups. Meat quality traits, including $pH_{24\,h}$, shear force, water holding capacity, cooking loss, drip loss and meat color, were not affected regardless of muscle type. Nevertheless, HP pigs demonstrated higher myofiber cross-sectional area (CSA) yet decreased myofiber number with higher percentage of type I fibers in LD detected with myosin ATPase staining. Moreover, decreased MyHC II protein expression was observed in both LD and PM of the HP pigs, coinciding with decreased MyHC IIb mRNA in LD, and MyHC IIb and IIx mRNA in PM. These results suggest that maternal dietary protein level may program the postnatal functional differentiation of myofibers of skeletal muscles in the pig without affecting meat quality traits.**

***Index Terms*—maternal dietary protein level, porcine skeletal muscle, meat quality**

I. INTRODUCTION

Maternal malnutrition is reported to program offspring health in human and growth performance in animals. Previous studies have shown that maternal nutrition restriction during gestation increased type IIb proportion and intermuscular fat in adult offspring of lamb (Daniel, Brameld, Craigon, Scollan, and Buttery, 2007; Zhu, Ford, Means, Hess, Nathanielsz, and Du, 2006). Conversely, extra feed supplemented during midgestation led to fewer muscle fibers and a smaller percentage of type IIB fibers, causing changes on some meat quality traits in adult pigs (Cerisuelo, Baucells, Gasa, Coma, Carrion, and Chapinal, et al., 2009). Protein-energy malnutrition is the most prevalent form of maternal nutritional disorder. During the past decade, many experimental studies have shown that maternal protein restriction leads to a low birth weight and development of type 2 diabetes in the offspring (Bayol et al., 2009; Chen et al., 2009; Garcia-Souza et al., 2008; Martin-Gronert et al., 2008; Schoknecht et al., 1993). Compared with the brain and heart, skeletal muscle has a lower priority in nutrition partitioning, rendering it particularly vulnerable to maternal malnutrition. However, effect of maternal dietary protein level throughout gestation and lactation on offspring muscle development has not been documented in pigs. The aim of this study was to investigate whether and how maternal dietary protein level throughout gestation and lactation would affect postnatal muscle growth and myofiber characteristics in finishing stage using Chinese Meishan (MS) pigs as model.

II. MATERIALS AND METHODS

A. Animals and sampling

This study was carried out in a MS breeding feedlot at Jiangsu Polytechnic College of Agriculture and Forestry, Jurong, Jiangsu Province, P. R. China. Sixteen primiparous purebred MS sows (body weight (BW): 36.1 ± 1.8 kg) were assigned randomly into low (LP) and high protein (HP) groups. The HP sows were fed on diets containing 12% and 14% CP, while LP sows were fed on diets containing 6% and 7% CP during gestation and lactation, respectively. The dietary treatment started 2 months before artificial insemination at the first observation of estrus. After parturition, newborn piglets were allowed free access to their mother and fed with a creep diet from 2 weeks of age. Piglets were weaned at 35 days of age and fed on standard grower and finisher diets thereafter. Male pigs were sacrificed at 8 months of age when they reached the market body weight of 75 kg in average. Longissimus dorsi (LD) and psoas major

(PM) muscles were taken from the right half of the carcass within 20 min postmortem, snap-frozen in liquid nitrogen and stored at -80˚C until further analysis.

B. Pig Performance and meat quality

Pigs were weighed before slaughter and the weight of LD and PM muscles were recorded after dissection. The cross-sectional area of LD was measured at the last rib. At 24 h postmortem, pH ($pH_{24\,h}$) of LD and PM were measured using a handheld pH meter (Cat. No. HI 9025, HANNA, Italy) equipped with a glass electrode inserted into the muscle. Meat color was measured using a Minolta Chroma Meter CR300 (Minolta, Osaka, Japan) calibrated according to the instructions of the manufacturer. Meat color determination was made in the transverse cut of the LD and PM muscle using the Comission Internationale de l'Eclairage values (L*, lightness; a*, redness; and b*, yellowness). Drip loss was measured over 24 h on approximately 80 g of LD muscle maintained at 4˚C. The water-holding capacity, shear force and cooking loss were also measured and calculated.

C. Myofiber cross sectional area (CSA) and myofiber type composition

Myosin ATPase staining was applied to identify myofiber types and to measure myofiber size. A portion of frozen LD muscle samples were used for serial sectioning (10 μm) in a cryostat at -20˚C. Sections were stained following a method described previously (Li, Yuan, Yang, Ni, Xia, Barth et al., 2007), using 1% $(NH_4)_2S$ instead of 1% Azure Stain. This allowed clear classification of type I or II. The fiber type was determined as described by Moreno-Sanchez, Diaz, Carabano, Rueda, and Rivero (2008). Type I fibers are stained dark black, whereas type II fibers are non-stained.

D. RNA isolation, cDNA synthesis and Real-time PCR

Total RNA was isolated from muscle using RNAzol (TianGene Biotech Co, Ltd., Beijing, China). Aliquots of 4 μg RNA were subjected to electrophoresis through a 1.4% agarose-formaldehyde gel to verify their integrity. cDNA was made by incubating a 25 μL mixture of 2 μg RNA, 21 μmol/L random hexamers (TaKaRa Biotechnology Co. Ltd., Dalian, China), 8 U RNase inhibitor (Promega, Madison, WI, USA), 100 U moloney murine leukemia virus reverse transcriptase (M-MLV) (Promega), 0.8 mmol/L each dNTP (TaKaRa) in the accessory reverse transcription buffer. The reaction was terminated by heating at 95˚C for 5 min and quickly cooling on ice. RT was performed in a DNA Engine® Peltier Thermal Cycler PTC0200 (MJ, USA). Mock RT and No Template Controls were set to monitor the possible contamination of genomic and environmental DNA both at the stage of RT and RCR. The pooled sample made by mixing equal quantity of cDNA from all samples was used for optimizing the PCR condition and tailoring the standard curve. Real-time PCR was performed in Mx3000P (Stratagene, USA). Forty cycles of amplification were performed, consisting of 20 s at 95ºC, 20 s at 64˚C, 30 s at 72˚C, followed by a final 1 min extension at 72˚C. 18s was used as internal control to normalize the technical variations.

E. Tissue extraction and western blot analysis of MyHC II

Frozen muscle samples (ca. 200 mg) were homogenised in 2 mL of ice-cold lysis buffer (20 mmol/L Tris–HCl, pH 8.0, 137 mmol/L NaCl, 10% glycerol, 1% Nonidet P-40, 2 mmol/L EDTA, 1 mmol/L DTT, 10 mmol/L NaF, 1 mmol/L PMSF, 1 μmol/L pepstatin A). The homogenate was then centrifuged at 10,000 rpm for 10 min at 4˚C to remove all insoluble material. The supernatant was collected and the protein concentration was determined with a Bradford assay kit purchased from Nanjing Jiancheng Biotechnology Institute (Nanjing, China). 10 μg of protein extract was mixed with loading buffer and denatured by boiling for 5 min before loaded on a 7.5% SDS-PAGE gel. After electrophoresis proteins were transferred to nitrocellulose membranes and the latter were then blocked with 5% fat-free milk in Tween-Tris-buffer saline (TTBS) for 30 min at room temperature. After repeated washing with TTBS, the membranes were incubated with monoclonal antibodies against MyHC II (A4.1025, Millipore, Temecula, CA, diluted 1:5000), followed by corresponding horseradish peroxidase-conjugated secondary antibodies (ab6721-1, abcam, UK, diluted 1:4000). Finally, the membrane was washed and the specific signals were detected by chemiluminescence using the LumiGlo substrate (Super Signal West Pico Trial Kit, PIERCE, USA). Enhanced chemiluminescence (ECL) signals recorded on x-ray film were scanned and analyzed with Kodak 1D Electrophoresis Documentation and Analysis System 120 (Kodak Photo Film Co. Ltd., USA). The MyHC II content in tested samples was presented as the band density values of MyHC II relative to that of GAPDH (KC-5G4, Kangchen, diluted 1:10000).

F. Statistical analysis

All data are presented as means ± SEM and were analyzed using Independent-Samples T Test with SPSS 13.0 for Windows. The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time PCR data expressed as the fold change relative to the control group. Differences were considered significant when $P < 0.05$.

III. RESULTS AND DISCUSSION

In the present study, no significant differences in BW and muscle weight between two groups were observed at finishing stage (Table 1). This is similar to the findings that increasing maternal nutrition during gestation did not affect BW of the offspring at slaughter (Cerisuelo, et.al, 2009; Nissen, Danielsen, Jorgensen, and N. Oksbjerg, 2003). HP pigs demonstrated lower total myofiber number with increased myofiber CSA in LD, which may explain why the area and the weight of LD, as well as the body weight were not affected (Table 1).

Table 1 body weight, muscle weight, muscle fiber characteristics in finishing pigs

Parameters	LP	HP	p-value
Body weight, kg	64.5 ± 2.5	67.9 ± 4.4	0.502
Carcass weight, kg	42.7 ± 2.1	43.6 ± 3.1	0.810
LD weight, g	1363 ± 107	1255 ± 88	0.465
PM weight, g	296 ± 18	280 ± 38	0.674
LD area, cm^2	18.50 ± 0.99	17.38 ± 0.82	0.410
Total number of muscle fibers, $\times 10^3$	696.3 ± 17.3	501.0 ± 20.1	<0.001
Mean myofiber CSA, μm^2			
Total	2734.1 ± 55.9	3521.4 ± 180.3	0.002
Type Ⅰ	2397.2 ± 143.2	3337.7 ± 198.1	0.001
Type Ⅱ	2775.7 ± 62.2	3527.2 ± 219.8	0.003
Fiber type composition, %			
Type Ⅰ	9.0 ± 0.8	17.4 ± 2.5	0.004
Type Ⅱ	91.0 ± 0.8	82.6 ± 2.5	0.004

In the present study, maternal high protein dietary significantly increased area of both type I and type II fibers and decreased total number of muscle fibers (Table 1). A higher percentage of type I fibers (Table 1) with down-regulated expression of MyHC IIb mRNA were observed in LD of HP pigs (Figure 1 A). PM showed similar changes with significant down-regulation of MyHC IIb and IIx mRNA expression (Figure 1 B). However, these changes in fiber type composition were not associated with meat quality traits, regardless of muscle type. The effects of maternal nutrition on offspring muscle growth and characteristics are inconsistent. Some studies have shown that protein restriction on pigs (Schoknecht, Pond, Mersmann, and Maurer, 1993) or dietary restriction on lamb (Daniel, et al., 2007) during gestation did not cause significant alterations in offspring body weight or carcass quality. Increased feed intake in sows during early to midgestation did not affect meat quality traits in the offspring either (Bee, 2004; Nissen et al., 2003; Dwyer, Stickland, and J. M. Fletcher, 1994). However, a recent study showed that over-feeding during midgestation led to fewer muscle fibers and a smaller percentage of type IIB fibers, associated with changes in some meat quality traits in adult pig (Cerisuelo et al., 2009). Multiple factors may contribute to such divergence among different studies. Breed, method of nutritional intervention, as well as the stage when the sample were taken for analysis. Meishan pig used in the present study was small in size and traditionally raised on low-protein diet comparable to that used in the LP group. Therefore, the pigs in LP group may be considered as control, whereas those in HP group may have received excess quantity of protein. Our results are in good agreement with previous findings that maternal overfeeding did not affect body weight or meat quality traits, but affected the characteristics of muscle fibers in a similar fashion reported by Cerisuelo et al., 2009. Greater proportion of type IIb fibers is desired in terms of higher muscle mass (Ruusunen and Puolanne, 2004), while higher percentage of type I fiber is in favour of better meat quality. Since the changes in fiber type proportion detected in the present study are not associated with either muscle mass or meat quality traits, feeding Meishan sows with HP diet, which is the standard for most commercial pig breeds, does not seem beneficial. It was known that type I fibres in skeletal muscle are rich in mitochondria with higher oxidative capacity and greater insulin sensitivity. Whether this II to I myofiber type shift in muscle may causes changes in glucose metabolism and insulin sensitivity awaits further investigation.

IV. CONCLUSION

Maternal dietary high protein during gestation and lactation in Meishan sows did not have beneficial effects on muscle growth or meat quality in adult offspring. The major finding of the current study was that alterations in maternal dietary protein level significantly modified the phenotype of myofibers in offspring, demonstrating a II to I myofiber type shift in HP group.

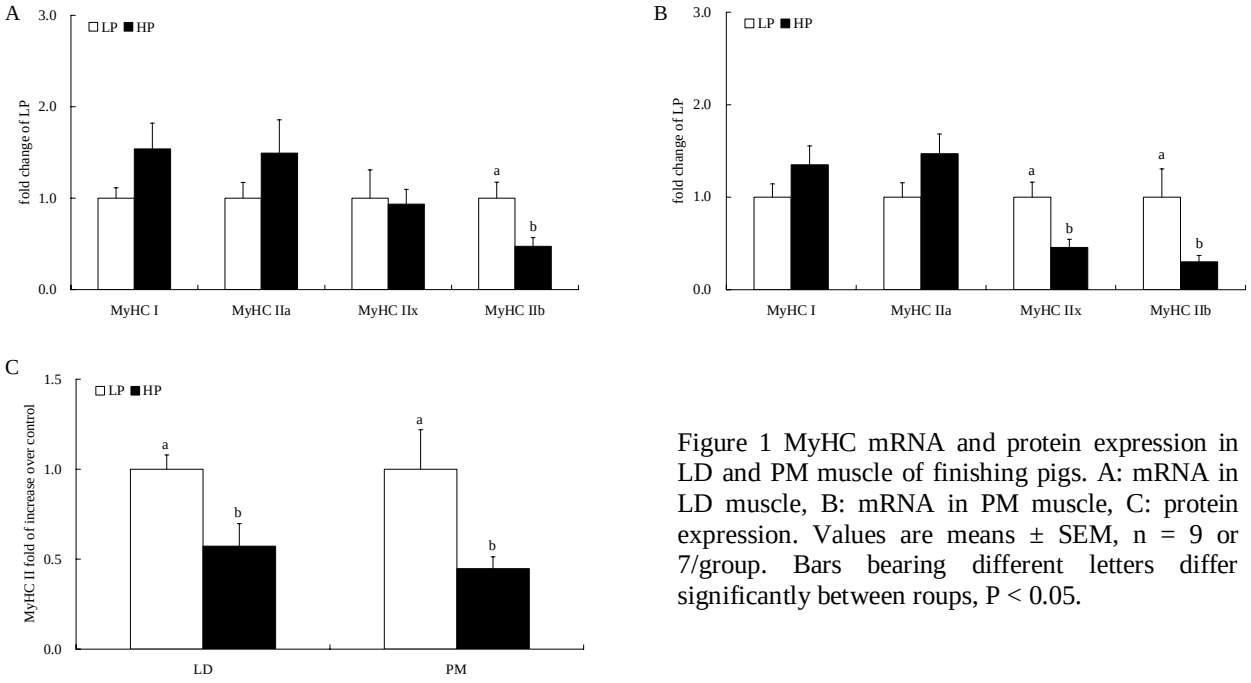

Figure 1 MyHC mRNA and protein expression in LD and PM muscle of finishing pigs. A: mRNA in LD muscle, B: mRNA in PM muscle, C: protein expression. Values are means ± SEM, n = 9 or 7/group. Bars bearing different letters differ significantly between roups, $P < 0.05$.

ACKNOWLEDGEMENT

This work was supported by the National Basic Research Program of China (2004CB117505).

REFERENCES

Bayol, S. A., R. Macharia, S. J. Farrington, B. H. Simbi, and N. C. Stickland. 2009. Evidence that a maternal "junk food" diet during pregnancy and lactation can reduce muscle force in offspring. Eur. J. Nutr. 48:62-65.

Bee, G. 2004. Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. J. Anim Sci. 82:826-836.

Cerisuelo, A., M. D. Baucells, J. Gasa, J. Coma, D. Carrion, N. Chapinal, and R. Sala. 2009. Increased sow nutrition during midgestation affects muscle fiber development and meat quality, with no consequences on growth performance. J. Anim Sci. 87:729-739.

Chen, J. H., M. S. Martin-Gronert, J. Tarry-Adkins, and S. E. Ozanne. 2009. Maternal protein restriction affects postnatal growth and the expression of key proteins involved in lifespan regulation in mice. PLoS. One. 4:e4950.

Daniel, Z. C., J. M. Brameld, J. Craigon, N. D. Scollan, and P. J. Buttery. 2007. Effect of maternal dietary restriction during pregnancy on lamb carcass characteristics and muscle fiber composition. J. Anim Sci. 85:1565-1576.

de, W. J., R. Mohren, S. van den Berg, M. Boekschoten, K. W. Dijk, G. P. de, M. Muller, E. Mariman, and E. Smit. 2008. Short-term high fat-feeding results in morphological and metabolic adaptations in the skeletal muscle of C57BL/6J mice. Physiol Genomics 32:360-369.

Dwyer, C. M., N. C. Stickland, and J. M. Fletcher. 1994. The influence of maternal nutrition on muscle fiber number development in the porcine fetus and on subsequent postnatal growth. J. Anim Sci. 72:911-917.

Garcia-Souza, E. P., S. V. da Silva, G. B. Felix, A. L. Rodrigues, M. S. de Freitas, A. S. Moura, and C. Barja-Fidalgo. 2008. Maternal protein restriction during early lactation induces GLUT4 translocation and mTOR/Akt activation in adipocytes of adult rats. Am. J. Physiol Endocrinol. Metab 295:E626-E636.

Li, Y., L. Yuan, X. Yang, Y. Ni, D. Xia, S. Barth, R. Grossmann, and R. Q. Zhao. 2007. Effect of early feed restriction on myofibre types and expression of growth-related genes in the gastrocnemius muscle of crossbred broiler chickens. Br. J. Nutr. 98:310-319.

Martin-Gronert, M. S., J. L. Tarry-Adkins, R. L. Cripps, J. H. Chen, and S. E. Ozanne. 2008. Maternal protein restriction leads to early life alterations in the expression of key molecules involved in the aging process in rat offspring. Am. J. Physiol Regul. Integr. Comp Physiol 294:R494-R500.

Moreno-Sanchez, N., C. Diaz, M. J. Carabano, J. Rueda, and J. L. Rivero. 2008. A comprehensive characterisation of the fibre composition and properties of a limb (flexor digitorum superficialis, membri thoraci) and a trunk (psoas major) muscle in cattle. BMC. Cell Biol. 9:67.

Nissen, P. M., V. O. Danielsen, P. F. Jorgensen, and N. Oksbjerg. 2003. 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. 81:3018-3027.

Ruusunen, M. and E. Puolanne. 2004. Histochemical properties of fibre types in muscles of wild and domestic pigs and the effect of growth rate on muscle fibre properties. Meat Science 67:533-539.

Schoknecht, P. A., W. G. Pond, H. J. Mersmann, and R. R. Maurer. 1993. Protein restriction during pregnancy affects postnatal growth in swine progeny. J. Nutr. 123:1818-1825.

Zhu, M. J., S. P. Ford, W. J. Means, B. W. Hess, P. W. Nathanielsz, and M. Du. 2006. Maternal nutrient restriction affects properties of skeletal muscle in offspring. J. Physiol 575:241-250.