Effects of Nutritional Level During Sow Gestation on Muscle Growth and Development in Offspring Piglets: Postprint

Authors: Su Guoqi, Wang Jun, Cao Meng, Che Lianqiang, Fang Zhengfeng, Lin Yan, Xu Shengyu, Wu De

Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment was conducted to investigate the effects of nutritional levels during sow gestation on muscle growth and development in offspring piglets. Thirty-three Landrace × Yorkshire (LY) crossbred replacement gilts with similar body weight and age were randomly assigned to three groups based on body weight [(150±9) kg] and age (255±10): low nutritional level group (LN group), adequate nutritional level group (AN group), and high nutritional level group (HN group). Each group had 11 replicates, with one sow per replicate. During early gestation (1-30 d), the nutritional intake of each group was 0.75, 1.0, and 1.5 times the maintenance requirement, respectively; during mid gestation (30-90 d), it was increased by 20% based on the early gestation level; and during late gestation (91-112 d), it was increased by 25% based on the mid gestation level. The results showed: 1) Compared with the LN group, the AN and HN groups exhibited significant or extremely significant increases in piglet birth weight and weaning weight, as well as newborn piglet muscle weight (except for the soleus muscle) ($P<0.05$ or $P<0.01$); the relative weight of semitendinosus muscle and longissimus dorsi muscle in piglets from the AN group was extremely significantly higher than that in the LN group ($P<0.01$), and the muscle protein concentration in newborn piglets from the AN group was significantly or extremely significantly higher than that in the LN and HN groups ($P<0.05$ or $P<0.01$). 2) The mRNA expression level of myosin heavy chain IIx in the longissimus dorsi muscle of newborn piglets from the AN group was extremely significantly lower than that in the LN group ($P<0.01$). 3) The mRNA expression level of myogenic regulatory factor 4 in the longissimus dorsi muscle of newborn piglets from the AN group was significantly higher than that in the LN and HN groups ($P<0.05$). In conclusion, nutritional levels during sow gestation can significantly affect piglet birth and weaning weights and muscle

weight; however, both excessively high and low nutritional levels can reduce offspring muscle weight and muscle protein concentration, which may be related to differential expression of the myogenic regulatory factor 4 gene.

Full Text

Effects of Nutrient Levels of Sows During Pregnancy on Muscle Growth and Development of Offspring Piglets

SU Guoqi, WANG Jun, CAO Meng, CHE Lianqiang, FANG Zhengfeng, LIN Yan, XU Shengyu, WU De*

Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu 611130, China

Abstract: This experiment was conducted to investigate the effects of nutrient levels during sow pregnancy on muscle growth and development in offspring piglets. Thirty-three crossbred Landrace × Yorkshire (LY) gilts with similar body weight [(150±9) kg] and age (255±10 days) were randomly assigned to three groups: low nutrient level (LN), appropriate nutrient level (AN), and high nutrient level (HN), with 11 replicates per group and one sow per replicate. During early pregnancy (Days 1-30), nutrient intake was set at 0.75, 1.0, and 1.5 times maintenance requirements for LN, AN, and HN groups, respectively. During mid-pregnancy (Days 31-90), intake was increased by 20% above early pregnancy levels, and during late pregnancy (Days 91-112), it was increased by 25% above mid-pregnancy levels. The results showed: (1) Compared with the LN group, birth weight, weaning weight, and muscle weight (except soleus) of piglets in AN and HN groups were significantly or extremely significantly increased ($P<0.05$ or $P<0.01$). The relative weights of semitendinosus and longissimus dorsi muscles in the AN group were extremely significantly higher than in the LN group ($P<0.01$), and muscle protein concentration in newborn piglets of the AN group was significantly or extremely significantly higher than in LN and HN groups ($P<0.05$ or $P<0.01$). (2) The mRNA expression of myosin heavy chain IIx (MyHC IIx) in the longissimus dorsi of newborn piglets in the AN group was extremely significantly lower than in the LN group ($P<0.01$). (3) The mRNA expression of myogenic regulatory factor 4 (MRF4) in the longissimus dorsi of newborn piglets in the AN group was significantly higher than in LN and HN groups ($P<0.05$). In conclusion, nutrient levels during sow pregnancy significantly affect piglet birth weight, weaning weight, and muscle weight, but both excessive and deficient nutrition reduce offspring muscle weight and muscle protein concentration, which may be related to differential expression of the MRF4 gene.

Keywords: sows; nutrient level; piglets; muscle

Mammalian skeletal muscle accounts for 40-50% of the body’ s dry weight and constitutes a vital component of the organism. Consequently, skeletal muscle growth and development are closely associated with animal production performance in livestock operations. Myofibers are the fundamental units of muscle, and previous research has demonstrated that greater total myofiber number correlates with higher growth potential, while increased myofiber density and smaller diameter contribute to better meat quality. In pigs, primary myofibers form within 38 days of gestation, while secondary myofibers develop between days 46 and 95, after which myofiber number no longer increases. During developmental stages, skeletal muscle occupies a lower priority in nutrient allocation, with maternal nutrients preferentially distributed to the nervous system, visceral organs, and bone, making fetal skeletal muscle particularly vulnerable to fluctuations in maternal nutrient supply.

As early as 1994, Dwyer et al. reported that increasing sow nutrition during days 25–50 of gestation increased secondary myofiber number per litter, a finding subsequently confirmed by Harrison et al. However, Gatford et al. found that elevated nutrition during days 25–50 of gestation only increased total and secondary myofiber density in the semitendinosus muscle. Other researchers observed that nutrient restriction during days 28–78 of gestation reduced fetal plasma amino acid and glucose concentrations, downregulated the mammalian target of rapamycin (mTOR) signaling pathway in skeletal muscle, decreased skeletal muscle protein synthesis rate, and reduced secondary myofiber number. Conversely, Cerisuelo et al. found that increased feed intake during mid-gestation (days 45–85) decreased primary, secondary, and total myofiber numbers while reducing type IIb myofibers, without significantly affecting performance.

Collectively, these studies demonstrate that gestational nutrition critically influences fetal muscle development, with increased nutrient levels enhancing myofiber number and exerting permanent effects on offspring muscle growth. However, reported results remain inconsistent, and no studies have examined the effects of nutrition throughout the entire gestation period on offspring muscle growth and myofiber development. Therefore, this experiment was designed to investigate how different nutrient levels during gestation affect offspring growth performance and muscle development, providing scientific evidence for precision feeding management of pregnant sows.

1.1 Experimental Animals and Design

Thirty-three crossbred Landrace × Yorkshire (LY) replacement gilts with similar body weight and age were fed the same diet until their fourth estrus cycle, then randomly assigned to three groups based on body weight [(150±9) kg] and age (255±10 days): low nutrient level (LN), appropriate nutrient level (AN, control), and high nutrient level (HN). Each group comprised 11 replicates with one sow per replicate. During early pregnancy (Days 1–30), nutrient intake was set at 0.75, 1.0, and 1.5 times maintenance requirements (M) for LN, AN, and HN groups, respectively. During mid-pregnancy (Days 31–90), intake increased by 20% above early pregnancy levels, and during late pregnancy (Days 91–112), it increased by 25% above mid-pregnancy levels . Maintenance digestible energy (DEm) was calculated as DEm = 110BW^0.75 kJ/d, where body weight (BW) = mating weight + 1/2 expected weight gain. With an expected weight gain of 55 kg, gestating sow DEm (kJ/d) = 110 × (mating weight + 55/2)^0.75. Feed intake at different gestational stages was determined by maintenance requirement multiples, with maintenance feed intake calculated as the ratio of DEm to dietary energy level.

1.2 Experimental Diets

Sow diets were formulated for two periods: Days 1–90 of gestation and Days 91 to parturition. Amino acid patterns followed Kim et al.' s recommended ideal amino acid profile, with other nutrient levels based on Johnston. Lactation diet amino acid levels followed Dourmad et al., with other nutrients based on NRC (1998). Diet composition and nutrient levels are presented in .

1.3 Feeding Management

All experimental sows were housed at the Teaching and Research Farm of the Institute of Animal Nutrition, Sichuan Agricultural University. Before mating, all sows received the same diet. After mating at the fourth estrus, sows were weighed and randomly allocated to treatment groups. During gestation, sows were fed twice daily (08:00 and 16:00) at fixed quantities. During lactation, sows were fed ad libitum four times daily (08:00, 12:00, 16:00, and 24:00) with free access to water, and daily feed intake was recorded accurately. On Day 110 of gestation, sows were moved to farrowing crates (2.0 m × 3.0 m slatted floor) with good ventilation and clean, dry conditions. On the day of parturition, sows were fed according to their condition. From Day 2 postpartum, feed was gradually increased: 2.5 kg on Days 1–2, then increased by 1 kg daily until ad libitum intake began around Day 5. Piglets were weighed before colostrum intake. On Day 3 postpartum, piglets underwent teeth clipping, tail docking, iron supplementation, and health care procedures. Castration was performed at 7 days, and creep feeding began. Piglets were weaned at 28 days of lactation. During the trial, pens were kept clean and disinfected weekly. Temperature and humidity were recorded daily, and sow feed intake and health status were monitored.

1.4 Sample Collection and Preparation

After farrowing, one piglet near the average litter weight was selected from each litter for slaughter (n=6). Remaining piglets were not cross-fostered and were reared until weaning. After an 8-hour fast, one piglet near the average litter weight was selected from each litter at weaning for slaughter (n=6). Following exsanguination, the right longissimus dorsi muscle was immediately collected, snap-frozen in liquid nitrogen, and stored at -80°C for analysis. The left longissimus dorsi, semitendinosus, psoas major, soleus, and gastrocnemius muscles were dissected and weighed. Longissimus dorsi samples (2 cm × 1 cm × 1 cm) were collected parallel and perpendicular to muscle fiber orientation, fixed in 4% paraformaldehyde at a 1:20 sample-to-fixative ratio for subsequent analysis.

1.4.1 Piglet Growth Performance and Muscle Weight

Within 12 hours of farrowing, individual birth weights were recorded. At 28 days of lactation, litter size and individual weights were recorded. After slaughter, the left-side longissimus dorsi, semitendinosus, psoas major, soleus, and gastrocnemius muscles were dissected and weighed. Muscle relative weight was calculated as muscle weight divided by body weight.

1.4.2 Myofiber Characteristics

Longissimus dorsi samples from newborn and 28-day-old piglets were fixed in 4% paraformaldehyde for 24 hours, then trimmed to 1 cm × 0.5 cm × 0.3 cm blocks, embedded in paraffin, and sectioned continuously at 5 µm thickness. Sections were stained with hematoxylin-eosin (HE), mounted with neutral balsam, and examined microscopically to determine myofiber size, density, and cross-sectional area (FCSA).

1.4.3 Determination of Muscle Fiber Development-Related Enzyme Activities

Muscle protein concentration in newborn and 28-day-old piglets was determined using the Coomassie brilliant blue method. Calcineurin (CaN) and creatine kinase (CK) activities in longissimus dorsi muscle were measured using absorbance spectrophotometry with a microplate reader (BIO-RAD Model 680, Beijing Chengzhikewei Biotechnology Co., Ltd.) according to kit instructions (Nanjing Jiancheng Bioengineering Institute).

1.4.4 Myofiber Type and Gene Expression Related to Muscle Fiber Development

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer instructions. RNA quality and purity were assessed using 1% agarose gel electrophoresis and a nucleic acid auto-analyzer (Beckman DU-800, Los Angeles, CA, USA). Target and reference genes were analyzed by real-time quantitative PCR (RT-PCR) using SYBR one-step reaction kit (Catalog no. DRR086A, Takara, Japan) on an ABI 7900HT system (Applied Biosystems). Primer sequences are listed in . The 10 µL RT-PCR reaction mixture contained 5.6 µL one-step RT-PCR reaction mix (with enzyme), 0.4 µL each of forward and reverse primers, and 100 ng DNA template. Reverse transcription parameters were: 42°C for 5 min, 95°C for 10 s, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s. PCR amplification was performed at 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s, followed by melting curve analysis. Gene expression was calculated using the $2^\frown(-\Delta\Delta Ct)$ method with -actin as the reference gene.

2 Data Processing and Statistical Analysis

All data were initially processed using Excel 2010, then analyzed by one-way ANOVA using SPSS 20.0. Duncan' s multiple range test was used for post-hoc comparisons. Data are expressed as means ± standard error. $P<0.05$ was considered significant, and $P<0.01$ was considered highly significant.

3.1 Effects of Sow Gestational Nutrition on Piglet Body Weight, Muscle Weight, and Muscle Weight/Body Weight

As shown in , sow gestational nutrition level extremely significantly affected piglet birth weight and weaning weight ($P<0.01$), with both parameters increasing as nutrition level increased. Gestational nutrition also extremely significantly affected muscle weights of longissimus dorsi, psoas major, semitendinosus, and gastrocnemius ($P<0.01$), and significantly affected relative weights of longissimus dorsi and semitendinosus ($P<0.05$). The longissimus dorsi relative weight in the AN group was extremely significantly higher than in the LN group ($P<0.01$). The semitendinosus relative weight in the LN group was extremely significantly lower than in the AN group ($P<0.01$) and significantly lower than in the HN group ($P<0.05$).

3.2 Effects of Sow Gestational Nutrition on Offspring Myofiber Characteristics

As shown in , different gestational nutrition levels had no significant effects on myofiber size, density, or cross-sectional area in the longissimus dorsi of newborn or weaned piglets ($P>0.05$).

3.3 Effects of Sow Gestational Nutrition on Longissimus Dorsi Protein Concentration and Enzyme Activity

As shown in , sow gestational nutrition level extremely significantly affected muscle protein concentration in newborn piglets ($P<0.01$), with the AN group being extremely significantly higher than the LN group ($P<0.01$) and significantly higher than the HN group ($P<0.05$). Gestational nutrition had no significant effects on intramuscular fat content or CaN and CK activities in the longissimus dorsi of newborn or weaned piglets ($P>0.05$).

3.4 Effects of Sow Gestational Nutrition on Offspring Myofiber Types

As shown in [Figure 1: see original paper], the mRNA expression of myosin heavy chain (MYHC) IIx in the longissimus dorsi of newborn piglets in the AN group was extremely significantly lower than in the LN group ($P<0.01$). Gestational nutrition had no significant effects on mRNA expression of MYHC I, MYHC IIa, or MYHC IIb in newborn piglets, nor on any MYHC isoforms in weaned piglets ($P>0.05$).

3.5 Effects of Sow Gestational Nutrition on Genes Regulating Muscle Growth and Myofiber Development

As shown in [Figure 2: see original paper], the mRNA expression of myogenic regulatory factor 4 (MRF4) in the longissimus dorsi of newborn piglets in the AN group was significantly higher than in LN and HN groups ($P<0.05$), while expression of insulin-like growth factor 1 (IGF-1), mTOR, and peroxisome proliferator-activated receptor coactivator-1 (PGC-1) was not significantly affected ($P>0.05$). Sow gestational nutrition tended to affect MRF4 mRNA expression in weaned piglets, with lower expression in the AN group compared to LN and HN groups, but had no significant effects on IGF-1, mTOR, or PGC-1 expression ($P>0.05$).

Fetal nutrition is initially provided by the ovum during early zygote formation, but as development progresses, nutrients can only be supplied by the dam. The dam acquires nutrients through feed intake, allocating portions for maternal tissue growth and transporting the remainder to the fetus via the placenta. This study demonstrated that increasing sow gestational nutrition elevated offspring birth weight, weaning weight, and muscle weight, consistent with findings by Dwyer et al. and Zhu et al. However, Cerisuelo et al. reported no significant effects of sow nutrition on offspring birth weight or 18-day weight, and Quigley et al. found no significant impact of ewe nutrition on fetal size or organ development. These discrepancies may relate to differences in animal species, dietary nutrient levels, and management practices. For instance, Quigley et al. used sheep, which have vastly different litter sizes than pigs, potentially altering outcomes. Additionally, our nutrition levels (0.75×, 1.0×, and 1.5× maintenance) differed from Cerisuelo et al., and sows possess strong self-regulatory capacity to prioritize fetal development, which may explain divergent results across studies.

The fetus is sensitive to maternal nutritional changes, and because skeletal muscle has lower nutrient priority, its development is readily affected by maternal nutrition. Interestingly, this study found that muscle weight relative to body weight did not increase linearly with nutrition level; instead, the AN group exhibited higher values than both LN and HN groups. This suggests that both excessive and deficient maternal nutrition impair muscle development, consistent with Zhu et al.' s findings in sheep. Maternal undernutrition reduces fetal plasma amino acids and glucose, downregulates skeletal muscle mTOR signaling, decreases protein synthesis rates, and reduces expression of genes related to nutrient transport and protein synthesis. Conversely, elevated maternal nutrition may be allocated to maternal tissues rather than fetal growth, limiting offspring development. While increased maternal nutrition raises nutrient supply to the fetus, this is not unlimited, as placental nutrient transport requires energy and carriers that have finite capacity.

Myofibers are the basic units of muscle, and their composition and type transitions directly affect muscle growth and metabolism. Myofibers can be classified by metabolic characteristics, contractile function, and MyHC polymorphism.

Our study of MyHC polymorphism revealed that newborn piglets in the AN group had lower MYHC IIx mRNA expression. Since previous research indicates myofiber transition follows the pathway MYHC I → MYHC IIa → MYHC IIb → MYHC IIx, and lower MYHC IIx proportions are associated with better meat quality, appropriate maternal nutrition may promote development toward superior meat quality. Multiple signaling pathways regulate myofiber type transition, with calcineurin (CaN) modulating fiber type-specific gene expression and promoting transition from fast to slow fibers. However, this study found no significant differences in CaN activity among groups, warranting further investigation.

This study also found that MRF4 mRNA expression in the longissimus dorsi of newborn piglets was higher in the AN group than in LN and HN groups, though differences were not significant at weaning. Myofiber development is regulated by myogenic regulatory factors (MRFs), including Myf5, MyoD, MRF4, and myogenin (MyoG). MyoD and Myf5 promote myogenic progenitor differentiation into myoblasts, MyoG is crucial for myoblast fusion into myotubes, and MRF4 is essential for maintaining muscle cell density and skeletal muscle integrity. MRF4 is expressed transiently during early myogenesis, followed by MyoD and Myf5, while MyoG is expressed later and persists throughout fetal development. MRF4 is re-expressed at later stages and becomes the dominant MRF family member after birth. Therefore, upregulated MRF4 mRNA expression in newborn piglets may be a key factor contributing to higher muscle protein concentration in the AN group, though the specific mechanisms require further study.

In conclusion, sow gestational nutrition level influences piglet birth weight, weaning weight, and muscle development by regulating MRF4 mRNA expression. Both excessive and deficient gestational nutrition reduce newborn piglet muscle relative weight, muscle mass, and muscle protein concentration, impairing normal fetal skeletal muscle development.

References

[1] Lü JH, Liu HZ, Ma LB, et al. Research progress on effects of nutrition on broiler meat quality[J]. Animal Science and Animal Medicine, 2004, 21(8): 37-39.

[2] WIGMORE PM, STICKLAND NC. Muscle development in large and small pig fetuses[J]. Journal of Anatomy, 1983, 137(Pt 2): 235-245.

[3] ZHU MJ, FORD SP, MEANS WJ, et al. Maternal nutrient restriction affects properties of skeletal muscle in offspring[J]. The Journal of Physiology, 2006, 575(1): 241-250.

[4] DWYER CM, STICKLAND NC, FLETCHER JM. The influence of maternal nutrition on muscle fiber number development in the porcine fetus and on subsequent postnatal growth[J]. Journal of Animal Science, 1994, 72(4): 911-917.

[5] HARRISON AP, ROWLERSON AM, DAUNCEY MJ. Selective regulation of myofiber differentiation by energy status during postnatal development[J]. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1996, 270(3 2): R667-R674.

[6] GATFORD KL, EKERT JE, BLACKMORE K, et al. Variable maternal nutrition and growth hormone treatment in the second quarter of pregnancy in pigs alter semitendinosus muscle in adolescent progeny[J]. British Journal of Nutrition, 2003, 90(2): 283-293.

[7] CERISUELO A, BAUCELLS MD, GASA J, et al. Increased sow nutrition during midgestation affects muscle fiber development and meat quality, with no consequences on growth performance[J]. Journal of Animal Science, 2009, 87(2): 729-739.

[8] POWELL SE, ABERLE ED. Skeletal muscle and adipose tissue cellularity in runt and normal birth weight swine[J]. Journal of Animal Science, 1981, 52(4): 748-756.

[9] WU D. Effects of nutrition level on performance and protein metabolism in pregnant and non-pregnant sows[D]. PhD thesis. Ya' an: Sichuan Agricultural University, 2003.

[10] KIM SW, WU GY. Regulatory role for amino acids in mammary gland growth and milk synthesis[J]. Amino Acids, 2009, 37(1): 89-95.

[11] JOHNSTON L. Gestating swine nutrient recommendations feeding management[Z]//National Swine Nutrition Guide. Des Moines, Iowa, US: Pork Center of Excellence, 2010.

[12] VAN MILGEN J, VALANCOGNE A, DUBOIS S, et al. InraPorc: a model and decision support tool for nutrition of growing pigs[J]. Animal Feed Science and Technology, 2008, 143(1/2/3/4): 387-405.

[13] LIVAK KJ, SCHMITTGEN TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method[J]. Methods, 2001, 25(4): 402-408.

[14] ZHU MJ, FORD SP, NATHANIELSZ PW, et al. Effect of maternal nutrient restriction in sheep on fetal skeletal muscle development[J]. Biology of Reproduction, 2004, 71(6): 1968-1973.

[15] QUIGLEY SP, KLEEMANN DO, KAKAR MA, et al. Myogenesis in sheep is altered by maternal intake during the peri-conception period[J]. Animal Reproduction Science, 2005, 87(3/4): 241-251.

[16] COSTELLO PM, ROWLERSON A, ASTAMAN NA, et al. Peri-implantation and late gestation maternal undernutrition differentially affect fetal sheep skeletal muscle development[J]. The Journal of Physiology, 2008, 586(9): 2371-2379.

[17] PARDI G, MARCONI AM, CETIN I. Placental-fetal interrelationship in IUGR fetuses–a review[J]. Placenta, 2002, 23(Suppl): S136-S141.

[18] PETTE D, STARON RS. Myosin isoforms, muscle fiber types, and transitions[J]. Microscopy Research and Technique, 2000, 50(6): 500–509.

[19] LEE SH, JOO ST, RYU YC. Skeletal muscle fiber type and myofibrillar proteins in relation to meat quality[J]. Meat Science, 2010, 86(1): 166–170.

[20] TALMADGE RJ, OTIS JS, RITTLER MR, et al. Calcineurin activation influences muscle phenotype in a muscle-specific fashion[J]. BMC Cell Biology, 2004, 5: 28.

[21] OLSON EN. MyoD family: a paradigm for development?[J]. Genes & Development, 1990, 4(9): 1454–1461.

[22] DU M, TONG J, ZHAO J, et al. Fetal programming of skeletal muscle development in ruminant animals[J]. Journal of Animal Science, 2010, 88(13 Suppl): E51-E60.

[23] KASSAR-DUCHOSSOY L, GAYRAUD-MOREL B, GOMÈS D, et al. Mrf4 determines skeletal muscle identity in Myf5:MyoD double-mutant mice[J]. Nature, 2004, 431(7007): 466–471.

[24] TAPSCOTT SJ. The circuitry of a master switch: MyoD and the regulation of skeletal muscle gene transcription[J]. Development, 2005, 132(12): 2685–2695.

Note: Figure translations are in progress. See original paper for figures.