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Effects of Dietary Energy and Nitrogen Levels on Meat Quality Traits and Related Gene Expression in Different Pig Breeds (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary energy and nitrogen levels on meat quality traits, muscle fiber types, and cytokine-related gene expression in different pig breeds. A total of 96 pigs (48 per breed) of Bama Xiang pigs and Landrace pigs were selected and randomly divided into 4 groups: Landrace pigs fed NRC diet group, Landrace pigs fed Chinese local pig standard (GB) diet group, Bama Xiang pigs fed NRC diet group, and Bama Xiang pigs fed GB diet group. The experiment commenced at 5 weeks of age and concluded at slaughter. At the end of the nursery, growing, and finishing phases, 8 pigs with similar body condition were selected from each group for slaughter, and muscle samples were collected for analysis. The results showed that growth stage significantly affected the cooked meat percentage of longissimus dorsi muscle and the mRNA expression levels of myosin heavy chain (MyHC) IIx, MyHC IIb, and interleukin-15 (IL-15) in experimental pigs ($P<0.05$), and exhibited an increasing trend with growth stage. Compared with Landrace pigs, Bama Xiang pigs had significantly higher cooked meat percentage of longissimus dorsi muscle and MyHC I mRNA expression level ($P<0.05$), while drip loss was significantly lower ($P<0.05$). Compared with the NRC diet, the GB diet significantly increased pH45min in growing Landrace and Bama Xiang pigs ($P<0.05$), and significantly upregulated MyHC I mRNA expression level in longissimus dorsi muscle of pigs during the nursery and finishing phases ($P<0.05$). Compared with the GB diet, the NRC diet significantly upregulated MyHC I mRNA expression level in longissimus dorsi muscle of growing pigs ($P<0.05$). Thus, both breed and growth stage could significantly affect meat quality traits and related gene expression, while dietary energy and nitrogen levels mainly affected meat quality traits and muscle fiber type-related gene expression through interaction effects with growth stage.

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Full Text

Effects of Dietary Energy and Nitrogen Levels on Meat Traits and Related Gene Expression in Different Strains of Pigs

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Abstract

This study investigated the effects of dietary energy and nitrogen levels on meat quality traits, muscle fiber type composition, and cytokine-related gene expression in different pig breeds. A total of 96 pigs (48 per breed) consisting of Bama mini-pigs and Landrace pigs were randomly allocated to four treatment groups: Landrace pigs fed NRC diets, Landrace pigs fed Chinese feeding standard (GB) diets, Bama mini-pigs fed NRC diets, and Bama mini-pigs fed GB diets. The experiment commenced at 5 weeks of age and continued until market weight. At the conclusion of each growth phase (nursery, growing, and finishing), eight pigs with similar body condition from each group were slaughtered, and muscle samples were collected for analysis. The results demonstrated that growth stage significantly affected cooking yield percentage and mRNA expression levels of myosin heavy chain (MyHC) IIx, MyHC IIb, and interleukin-15 (IL-15) in the longissimus dorsi muscle (LDM) ($P<0.05$), with all parameters showing an increasing trend across growth stages. Compared with Landrace pigs, Bama mini-pigs exhibited significantly higher cooking yield percentage and MyHC I mRNA expression in LDM ($P<0.05$), while drip loss was significantly lower ($P<0.05$). Relative to NRC diets, GB diets significantly increased pH at 45 minutes postmortem (pH_{45}) in LDM during the growing stage for both breeds ($P<0.05$) and upregulated MyHC I mRNA expression in LDM during nursery and finishing stages ($P<0.05$). Conversely, NRC diets significantly upregulated MyHC I mRNA expression in LDM during the growing stage compared with GB diets ($P<0.05$). These findings indicate that both breed and growth stage significantly influence meat quality traits and related gene expression, while dietary energy and nitrogen levels primarily affect meat quality and muscle fiber type gene expression through interactions with growth stage.

Keywords: meat traits; muscle fiber type; cytokine; Bama mini-pigs; dietary energy and nitrogen levels

Pork quality is fundamentally determined by the pig' s biological characteristics and genetic background, with normal nutritional metabolism serving as the platform for nutrient deposition into muscle tissue during growth and development. Muscle fibers, the basic structural units of skeletal muscle, not only govern muscle growth and development but also influence the physiological and biochemical properties, postmortem metabolic activity, and sensory quality of pork. Key factors affecting the formation of superior meat quality include muscle fiber hypertrophy, fiber type composition and transformation, and intramuscular fat deposition. Regulating muscle fiber type and its transformation represents an important approach to improving meat quality. Based on enzyme systems, enzymatic activities, and contractile functions, muscle fibers can be classified into slow oxidative (Type I), fast oxidative (Type IIa), fast glycolytic (Type IIb), and intermediate (Type IIx) types. Type I fibers are characterized by smaller diameter, higher lipid content, and greater myoglobin concentration, thus a higher proportion of Type I fibers correlates with better meat color, tenderness, and flavor. In contrast, Type IIb fibers are larger in diameter with lower myoglobin and lipid content but higher glycogen content, resulting in coarser muscle texture, lighter color, and inferior tenderness and flavor, with increased susceptibility to pale, soft, and exudative (PSE) meat under stress conditions.

Muscle fiber type is regulated not only by intrinsic developmental patterns but also by neuroendocrine factors, nutritional status, and stress. Fiber type transformation occurs throughout the entire developmental period. During prenatal development, myosin heavy chain (MyHC) isoforms are expressed sequentially from embryonic to fetal to neonatal to mature forms (slow Type I and fast Type II), while in mature muscle, MyHC isoforms transform in the order of I IIa IIx IIb. Bama mini-pig is a renowned Chinese indigenous breed known for its thin skin, fine bones, delicious meat, and early sexual maturity, albeit with slow growth rate and low lean meat percentage. Landrace pigs represent an excellent exotic breed with rapid growth and high lean meat percentage but inferior meat quality. Consequently, these two breeds serve as an ideal model for comparative studies on production performance and meat quality differences. Our previous research demonstrated that Bama mini-pigs exhibit higher intramuscular fat content and elevated expression of lipid synthesis-related genes compared with Landrace pigs, and these traits are regulated by dietary nutrient levels. Therefore, this study investigated the effects of dietary energy and nitrogen levels on meat quality traits and the expression of genes related to muscle fiber type and cytokines in different pig breeds to provide a basis for pork quality regulation.

1.1 Experimental Design

Ninety-six 5-week-old pigs (48 per breed, all castrated males) were selected and housed individually in pens, then randomly assigned to four groups: Landrace

pigs fed GB diets, Landrace pigs fed NRC diets, Bama mini-pigs fed GB diets, and Bama mini-pigs fed NRC diets. Experimental diets were formulated according to the NRC (2012) Nutrient Requirements of Swine and the Chinese Feeding Standard of Swine (2004). Diet composition and nutrient levels are presented in Table 1 . A 7-day pre-trial period was implemented for ear tagging, deworming, and vaccination. During the experiment, pigs had ad libitum access to feed and water, with daily feed intake recorded and animal health status monitored. The trial concluded when pigs reached market weight.

1.2 Sample Collection

Based on the different growth patterns of the two breeds, pigs were slaughtered at three distinct weight stages (Landrace: 20, 50, and 90 kg; Bama mini-pigs: 15, 35, and 50 kg). At each stage, eight pigs with similar body weight were selected from each group and slaughtered according to the National Standard of Operating Procedures of Pig Slaughtering. Longissimus dorsi muscle samples were collected from the 10th–11th rib region for immediate meat quality analysis. Additionally, 2 g samples of longissimus dorsi and biceps femoris muscles were collected, snap-frozen in liquid nitrogen, and stored at -80°C for molecular biological analysis.

1.3 Meat Quality Trait Measurement

Longissimus dorsi muscle samples from the 10th–11th rib region were used for meat quality assessment. Muscle pH was measured at 45–60 minutes (pH_{45}) and 24 hours (pH_{24}) postmortem using a specialized pH meter (pH Star, Germany). Meat color parameters [lightness (L*), redness (a)*, and yellowness (b*)] were determined within 2 hours postmortem using a CR410 colorimeter (Konica Minolta Sensing, Inc., Tokyo, Japan). Drip loss was measured by the hanging method on meat samples (2 cm × 5 cm × 3 cm) cut parallel to the muscle fiber direction at 2–3 hours postmortem. Cooking yield percentage was determined by the cooking method on approximately 100 g meat samples within 2 hours postmortem.

1.4 Measurement of Muscle Fiber Type-Related and Cytokine Gene Expression

Total RNA was extracted from muscle samples according to reference methods. RNA integrity was assessed by agarose gel electrophoresis, and concentration was measured using a NanoDrop 2000 spectrophotometer (Nano-drop Technologies, Wilmington, DE) with absorbance ratios at 260 nm and 280 nm ($A_{260}/_{280}$) of 1.9–2.0. All RNA samples were diluted with diethylpyrocarbonate (DEPC)-treated water to approximately 1,000 ng/ L, then reverse-transcribed to cDNA using a reverse transcription kit (TaKaRa Biotechnology Co., Ltd., Dalian) following the manufacturer' s instructions.

The synthesized cDNA was used as template for quantitative real-time PCR

analysis of target genes (Table 2). Primers were designed using Primer Premier 5.0 software and synthesized by Shanghai Biotechnology Engineering Co., Ltd. The PCR reaction mixture (25 L) contained 5 L cDNA template and 0.5 L each of forward and reverse primers. PCR conditions were: initial denaturation at 95°C for 10 s, followed by 45 cycles of 95°C for 10 s, annealing at 54.7°C (optimized for specific primers) for 30 s, and extension at 72°C for 15 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal reference. Relative gene expression levels were calculated using the $2^{-}\Delta$Ct method, where ΔCt = Ct(target gene) -Ct(GAPDH).

1.5 Statistical Analysis

Data were analyzed using the GLM procedure of SAS 9.1 (SAS Institute Inc., Cary, NC) with breed, diet, and growth stage as main effects. Means were compared using Tukey' s multiple comparison test. Results are expressed as means and SEM, with $P<0.05$ considered statistically significant.

2.1 Meat Quality Traits

As shown in Table 3 , growth stage, breed, and diet all influenced meat quality. The pH_{45} , pH_{24} , and drip loss of longissimus dorsi muscle decreased significantly with advancing growth stage in both breeds ($P<0.05$), while meat color b* values and cooking yield percentage increased significantly ($P<0.05$). Landrace pigs exhibited significantly higher pH values and drip loss but lower meat color a* values and cooking yield percentage compared with Bama mini-pigs at all stages ($P<0.05$). The GB diet significantly increased pH_{45} in longissimus dorsi muscle during the growing stage for both breeds compared with the NRC diet ($P<0.05$).

2.2 MyHC Isoform Gene Expression

Table 4 shows that growth stage affected muscle fiber isoform gene expression. The mRNA expression levels of MyHC IIx and MyHC IIb in skeletal muscle increased with growth, with higher expression during the finishing stage compared with nursery and growing stages. Bama mini-pigs displayed significantly higher mRNA expression of MyHC I and MyHC IIx in longissimus dorsi muscle and MyHC I and MyHC IIa in biceps femoris muscle compared with Landrace pigs ($P<0.05$), while MyHC IIb expression in biceps femoris muscle was significantly lower ($P<0.05$). A significant interaction between breed and growth stage was observed for MyHC IIa expression in both muscles ($P<0.05$). The GB diet significantly upregulated MyHC I mRNA expression in longissimus dorsi muscle during nursery and finishing stages ($P<0.05$) but downregulated it during the growing stage compared with the NRC diet ($P<0.05$).

2.3 Muscle Cytokine Gene Expression

As presented in Table 5 , IL-15 mRNA expression in longissimus dorsi muscle increased gradually with growth stage. A significant interaction between breed and growth stage was observed for IL-15 mRNA expression in biceps femoris muscle ($P<0.05$), with expression increasing progressively in Landrace pigs but decreasing initially then increasing in Bama mini-pigs. Chemerin mRNA expression in biceps femoris muscle was significantly higher in Bama mini-pigs than in Landrace pigs ($P<0.05$). The NRC diet significantly upregulated Chemerin mRNA expression in biceps femoris muscle during the finishing stage compared with the GB diet ($P<0.05$).

Key meat quality indicators include pH, meat color, drip loss, and cooking yield percentage. In this study, Bama mini-pigs exhibited lower drip loss and higher meat color a* values and cooking yield percentage compared with Landrace pigs at the same growth stage, consistent with previous reports that indigenous Chinese pig breeds possess superior meat quality traits compared with exotic breeds. Postmortem physiological metabolism ceases, and glycogen stored in muscle fibers undergoes anaerobic glycolysis to produce lactic acid, causing pH decline that subsequently affects meat color, water-holding capacity, soluble protein concentration, and shelf life. Therefore, pH represents a critical indicator for meat quality evaluation. The observation that GB diets increased pH_{45} in longissimus dorsi muscle of growing pigs suggests beneficial effects on meat quality improvement.

Since different muscle fiber types specifically express distinct MyHC isoforms encoded by corresponding genes, MyHC gene expression analysis enables muscle fiber typing. Muscle fiber types undergo continuous transformation during growth and are influenced by nutritional and non-nutritional factors. Numerous studies have demonstrated that muscle fiber type is a crucial determinant of meat quality, with MyHC I, MyHC IIa, and MyHC IIx mRNA expression positively correlated with muscle color, pH, marbling, and intramuscular fat content, whereas MyHC IIb mRNA expression shows negative correlations with these traits. In this study, the upregulation of MyHC IIb and MyHC IIx mRNA expression in longissimus dorsi and biceps femoris muscles with advancing growth was unfavorable for superior meat quality formation. Wu et al. reported that MyHC IIb and MyHC IIx fiber proportions increased with growth and were significantly positively correlated with drip loss and negatively correlated with pH decline. The present study revealed distinct differences in muscle fiber composition between breeds, with Bama mini-pigs exhibiting higher proportions of MyHC I and MyHC IIa fibers (oxidative fibers) in skeletal muscle, which may explain their higher meat color a* values, whereas Landrace pigs contained more Type IIb fibers, indicating superior meat quality in Bama mini-pigs. Similar findings were reported by Yang et al., who observed that the difference in muscle fiber types between Large White and Erhualian pigs became apparent after 90 days of age, with the higher proportions of MyHC I and MyHC IIa fibers in Erhualian longissimus dorsi muscle associated with superior meat

quality. Additionally, GB diets upregulated MyHC I mRNA expression during nursery and finishing stages, while NRC diets upregulated expression during the growing stage, suggesting that high-nutrient diets during the metabolically active growing period favor the formation of superior meat quality.

Interleukin-15 (IL-15) secreted by skeletal muscle regulates proliferation and differentiation of various cell types and modulates muscle and adipose tissue metabolism through endocrine and paracrine mechanisms. Quinn demonstrated that IL-15 stimulates expression of contractile proteins in differentiated muscle cells in vitro, promoting muscle fiber growth in vivo, and enhances differentiation of murine skeletal muscle myoblasts while increasing human skeletal muscle myosin expression. Li et al. confirmed using co-culture techniques that IL-15 participates in regulating the "crosstalk" between porcine muscle and adipose tissues. IL-15 also inhibits skeletal muscle protein degradation and reduces adipose deposition by directly acting on adipocytes. The observed upregulation of IL-15 mRNA expression in skeletal muscle with growth suggests enhanced muscle fiber differentiation and growth. Chemerin, an adipokine secreted by adipocytes, acts on its receptor CMKLR1 through autocrine pathways to promote adipocyte differentiation and glucose transport, thereby influencing glucose and lipid metabolism. Studies have shown that Chemerin gene expression is closely associated with meat quality traits including loin eye area, water-holding capacity, and marbling score, and plays an important role in intramuscular fat deposition. The significantly higher Chemerin mRNA expression in biceps femoris muscle of Bama mini-pigs compared with Landrace pigs suggests greater capacity for lipid metabolism and deposition, leading to increased intramuscular fat and better meat quality.

Both breed and growth stage significantly affect pork quality traits and related gene expression, while dietary energy and nitrogen levels primarily interact with growth stage to influence meat quality and muscle fiber type gene expression. Bama mini-pigs possess more oxidative muscle fibers and superior meat quality. GB diets upregulate MyHC I mRNA expression in longissimus dorsi muscle during finishing stage, thereby improving pork quality. Elevating dietary energy and nitrogen levels during the growing stage favors the formation of superior meat quality.

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