Effects of Xylooligosaccharides on Plasma Biochemical Parameters, Muscle Amino Acid Content, and Muscle Fiber Type Composition in Growing-Finishing Pigs: Postprint

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Abstract

This experiment aimed to investigate the effects of xylo-oligosaccharides (XOS) on plasma biochemical parameters, muscle amino acid content, and muscle fiber type composition in growing-finishing pigs. Eighty Duroc × Landrace × Large White crossbred pigs at 70 days of age with an average body weight of approximately 30 kg were randomly divided into 8 groups, with 10 replicates per group (half male and half female), with one pig per replicate. The experiment consisted of a control group, an antibiotic group, 100, 250, and 500 g/t XOS groups for the 30–65 kg stage, and 100, 250, and 500 g/t XOS groups for the 30–100 kg stage. When the average body weight of the experimental pigs reached 100 kg, blood was collected from the anterior vena cava, plasma was separated by centrifugation, and biochemical parameters were measured; after slaughter, longissimus dorsi muscle samples were collected to determine amino acid content, muscle fiber type, and mRNA expression levels of muscle growth-related genes. The results showed that: 1) compared with the control or antibiotic group, dietary supplementation with different doses of XOS significantly increased plasma globulin content ($P<0.05$) and significantly decreased the albumin/globulin ratio ($P<0.05$); 2) compared with the control or antibiotic group, dietary supplementation with 250 g/t XOS during the 30–65 kg stage significantly decreased the contents of threonine (Thr), leucine (Leu), phenylalanine, and serine (Ser) in the longissimus dorsi muscle ($P<0.05$), while dietary supplementation with 500 g/t XOS during the 30–65 kg stage significantly increased the mRNA expression levels of myosin heavy chain IIx (MyHC IIx), myogenic determination factor (MyoD), myogenin (MyoG), and myocyte enhancer factor 2A (MEF2A) in the longissimus dorsi muscle; 3) compared with the control or antibiotic group, dietary supplementation with 100 or 500 g/t XOS during

the 30–100 kg stage significantly increased the contents of Thr, Leu, Ser, total amino acids, essential amino acids, and flavor amino acids, as well as the mRNA expression levels of MyHC IIx, MyoD, MyoG, MEF2A, and myostatin in the longissimus dorsi muscle ($P<0.05$). In conclusion, dietary supplementation with a certain dose of XOS can regulate body nitrogen metabolism and upregulate the expression of muscle fiber type- and muscle growth-related genes, with the best effect observed for supplementation with 100 g/t XOS during the 30–100 kg stage.

Full Text

Effects of Xylo-Oligosaccharide on Plasma Biochemical Indices, Amino Acid Contents and Fiber Type Composition of Muscle of Growing-Finishing Pigs

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Abstract: This study investigated the effects of xylo-oligosaccharide (XOS) on plasma biochemical indices, muscle amino acid contents, and muscle fiber type composition in growing-finishing pigs. Eighty Duroc × Landrace × Large White crossbred pigs at 70 days of age (approximately 30 kg initial body weight) were randomly allocated to eight groups with ten replicates per group (half male and half female), with one pig per replicate. The experimental groups included a control group, an antibiotic group, three groups receiving 100, 250, or 500 g/t XOS during the 30–65 kg stage, and three groups receiving 100, 250, or 500 g/t XOS during the 30–100 kg stage. When pigs reached approximately 100 kg body weight, blood samples were collected via anterior vena cava puncture and plasma was separated for biochemical analysis. After slaughter, longissimus dorsi muscle samples were collected to determine amino acid contents, muscle fiber types, and mRNA expression levels of muscle growth-related genes. The results demonstrated: (1) Compared with the control or antibiotic groups, dietary XOS supplementation at various doses significantly increased plasma globulin content and decreased the albumin/globulin ratio ($P < 0.05$); (2) During the 30–65 kg stage, 250 g/t XOS significantly decreased threonine (Thr), leucine (Leu), phenylalanine, and serine (Ser) contents in longissimus dorsi muscle, while 500 g/t XOS significantly increased mRNA expression of myosin heavy chain IIx (MyHC IIx), myogenic determination factor (MyoD), myogenin (MyoG), and

muscle enhancer factor 2A (MEF2A) ($P < 0.05$); (3) During the 30–100 kg stage, 100 or 500 g/t XOS significantly increased Thr, Leu, Ser, total amino acids, essential amino acids, and flavor amino acids, as well as mRNA expression of MyHC IIx, MyoD, MyoG, MEF2A, and myostatin in longissimus dorsi muscle ($P < 0.05$). In conclusion, dietary supplementation with appropriate XOS doses can regulate nitrogen metabolism and upregulate expression of muscle fiber type and muscle growth-related genes, with 100 g/t XOS during the 30–100 kg stage showing optimal effects.

Keywords: xylo-oligosaccharides; growing-finishing pigs; biochemical indices; amino acids; muscle fiber type

Introduction

With rising living standards and evolving dietary preferences, consumers increasingly demand safe, high-quality livestock products with superior taste, flavor, and nutritional value. However, long-term use of feed antibiotics in pig production has led to deteriorated pork quality and antibiotic residues. Consequently, developing green feed additives that can replace antibiotics while improving meat quality has become imperative. Xylo-oligosaccharide (XOS), a functional oligosaccharide composed of 2–7 xylose units linked by -1,4-glycosidic bonds, exhibits excellent acid and heat resistance and is not easily degraded by digestive enzymes. Previous research demonstrated that XOS promotes proliferation of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, increases short-chain fatty acids (acetate, propionate, and butyrate) from bacterial fermentation, reduces intestinal pH, and enhances absorption of calcium, magnesium, and zinc ions, thereby improving pork pH and reducing drip loss. XOS is considered the most promising prebiotic among all oligosaccharides. Furthermore, dietary supplementation with 100 g/t XOS significantly increased mRNA expression of myosin heavy chain (MyHC) IIx and MyHC IIa in weaned piglets, while 250 g/t XOS increased free amino acid content in longissimus dorsi muscle. Our previous studies found that 100 g/t XOS in diets for 30–100 kg growing-finishing pigs elevated intramuscular fat content and the oleic acid/linoleic acid ratio, and 500 g/t XOS increased muscle crude protein content, thereby enhancing pork nutritional value. Muscle fibers are the fundamental units of muscle, and their types and composition are closely associated with meat tenderness, flavor, and intramuscular fat content, while amino acid composition significantly influences pork palatability. Although research on XOS effects on livestock meat quality is expanding, few studies have examined its impact on amino acid composition and muscle fiber type transformation in growing-finishing pigs, and investigations on XOS supplementation strategies remain limited. Therefore, this study evaluated the effects of XOS supplementation at different doses and growth stages on plasma biochemical indices, muscle amino acid contents, and muscle fiber type composition in growing-finishing pigs to provide a basis for improving meat quality.

1.1 Experimental Materials

XOS was provided by Shandong Longli Biotechnology Co., Ltd., with main components of xylobiose, xylotriose, and xylotetraose (XOS content 35%).

1.2 Experimental Animals, Grouping, and Management

Eighty Duroc × Large White × Landrace crossbred pigs at 70 days of age (average initial weight ~30 kg), with equal sex distribution, were randomly assigned to eight groups with ten replicates per group (one pig per replicate) housed in individual pens (0.6 m × 1.1 m). The groups included: control (basal diet), antibiotic (basal diet + 0.2 kg/t colistin + 0.04 kg/t virginiamycin premix at 50% potency), three groups receiving 100, 250, or 500 g/t XOS during the 30–65 kg stage (followed by basal diet during 66–100 kg), and three groups receiving 100, 250, or 500 g/t XOS throughout the 30–100 kg stage. Pigs were slaughtered at approximately 100 kg body weight (~170 days of age). XOS supplementation levels were determined based on previous studies and manufacturer recommendations. The basal diet was formulated according to NRC (2012) nutrient requirements without antibiotics, with composition and nutrient levels as reported previously. The feeding trial was conducted from June to September 2015 at the Yongan Animal Experimental Station of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, following commercial farm management practices.

1.3 Sample Collection and Processing

At trial conclusion, eight pigs per group (equal sex distribution) were randomly selected after overnight fasting and weighed. Blood samples were collected via anterior vena cava puncture into heparinized tubes, centrifuged to separate plasma for nitrogen metabolism-related biochemical analysis. After slaughter, 2 g of longissimus dorsi muscle was collected and stored at -80 °C for gene expression analysis, and 200 g was stored at -20 °C for hydrolyzed amino acid determination.

1.4 Index Determination and Methods

1.4.1 Plasma Biochemical Indices Plasma total protein (TP), albumin (ALB), globulin (GLB), and alkaline phosphatase (ALP) activity were measured using a CX4 automatic biochemical analyzer (Beckman) with assay kits provided by Beijing Leadman Biochemistry Co., Ltd.

1.4.2 Muscle Amino Acid Content Hydrolyzed amino acids in longissimus dorsi muscle were determined using an L-8800 automatic amino acid analyzer (Hitachi) according to the method of Liu et al. Contents of total amino acids (TAA), essential amino acids (EAA), non-essential amino acids (NEAA), and flavor amino acids (FAA) were calculated.

1.4.3 mRNA Expression of Muscle Fiber Types and Muscle Growth-Related Genes Total RNA was extracted from longissimus dorsi muscle using an RNA Isolation Solvent kit. After concentration determination with a Nanodrop 2000 micro-UV spectrophotometer, RNA was reverse-transcribed to cDNA using a PrimeScript RT kit. Using cDNA as template and -actin as the reference gene, mRNA expression levels of MyHC I, MyHC IIa, MyHC IIb, MyHC IIx, MyoD, MyoG, MSTN, and MEF2A were quantified by real-time PCR using an ABI 7900HT system (Applied Biosystems). Relative expression was calculated using the 2^(-ΔΔCt) method. Primers were synthesized by Sangon Biotech (Shanghai) with sequences and parameters shown in .

1.5 Statistical Analysis

Data were initially processed using Excel 2010 and analyzed by one-way ANOVA using SAS 9.2. LSD multiple comparisons were performed with results expressed as least squares means. Differences were considered significant at $P < 0.05$ and trends at 0.05 $P < 0.10$.

Results

2.1 Effects of XOS on Plasma Biochemical Indices in Growing-Finishing Pigs

As shown in , compared with the control group, plasma GLB content was significantly increased in groups receiving 250 or 500 g/t XOS during the 30–65 kg stage and 250 or 500 g/t XOS during the 30–100 kg stage ($P < 0.05$). Compared with the antibiotic group, plasma GLB content was significantly increased in the 30–100 kg stage 250 g/t XOS group ($P < 0.05$). The plasma ALB/GLB ratio was significantly decreased in groups receiving 250 or 500 g/t XOS during the 30–65 kg stage and 100, 250, or 500 g/t XOS during the 30–100 kg stage compared with both control and antibiotic groups ($P < 0.05$). No significant differences were observed in plasma TP or ALB contents among groups ($P > 0.05$).

2.2 Effects of XOS on Muscle Amino Acid Contents in Growing-Finishing Pigs

As shown in , during the 30–65 kg stage, the 250 g/t XOS group exhibited significantly lower contents of threonine (Thr), leucine (Leu), phenylalanine (Phe), serine (Ser), TAA, EAA, NEAA, and FAA compared with the antibiotic group ($P < 0.05$). During the 30–100 kg stage, the 100 g/t XOS group showed significantly higher contents of Thr, Leu, Ser, TAA, EAA, and FAA compared with the control group ($P < 0.05$), while the 500 g/t XOS group had significantly higher Thr, Ser, and FAA contents ($P < 0.05$). The 30–100 kg stage 100 and 250 g/t XOS groups demonstrated significantly higher Phe contents compared with their 30–65 kg stage counterparts ($P < 0.05$), and the 100 g/t XOS group

showed significantly higher glutamic acid (Glu) content compared with the 30–65 kg stage 250 g/t XOS group ($P < 0.05$).

2.3 Effects of XOS on mRNA Expression of Muscle Fiber Types and Muscle Growth-Related Genes in Longissimus Dorsi Muscle

As shown in , compared with the control or antibiotic groups, the 30–65 kg stage 500 g/t XOS group significantly increased mRNA expression of MyHC IIx, MyoD, MyoG, and MEF2A ($P < 0.05$). During the 30–100 kg stage, the 100 g/t XOS group significantly increased mRNA expression of MyoD, MyoG, MEF2A, and MSTN compared with control or antibiotic groups ($P < 0.05$), while the 500 g/t XOS group significantly increased MyHC IIx expression ($P < 0.05$). The 30–100 kg stage 100 g/t XOS group also showed significantly higher MyoD, MyoG, MEF2A, and MSTN expression compared with the 30–100 kg stage 250 or 500 g/t XOS groups and the 30–65 kg stage 100 g/t XOS group ($P < 0.05$).

Discussion

Plasma biochemical indices reflect nutrient metabolism, tissue function, and pathological changes. Plasma TP content indicates protein absorption, synthesis, and catabolism capacity. Plasma ALB is an important indicator of nutritional status and liver function, playing a crucial role in hepatic protein metabolism. Plasma GLB, secreted by plasma cells, increases with antibody levels and primarily reflects immune capacity. The ALB/GLB ratio indicates immune status; when serum protein synthesis is impaired, ALB decreases while GLB increases, reducing the ALB/GLB ratio and enhancing immune function. In this study, dietary XOS supplementation significantly increased plasma GLB and decreased the ALB/GLB ratio, suggesting enhanced immunity, possibly through increased beneficial intestinal bacteria and improved gut function. These findings align with previous studies by Tan et al. and Wang et al.

Amino acid content, composition, and proportion in muscle are critical indicators of pork nutritional value and quality. EAA content determines muscle protein quality, while FAA serves as essential precursors for meat flavor formation. In this study, 100 g/t XOS throughout the 30–100 kg stage significantly increased EAA, FAA, and TAA contents in muscle tissue, while 500 g/t XOS increased FAA content, indicating that XOS can improve pork protein quality and flavor, with 100 g/t XOS during the entire period being most effective. This may be attributed to XOS serving as a fermentation substrate for hindgut beneficial bacteria, with increased microbial protein regulating amino acid metabolism. Studies in pigs demonstrated that colonic infusion of protein and amino acids improves nitrogen balance. Glu and Phe are important umami and sweet flavor factors in pork. The significant increase in Phe or Glu content with XOS supplementation throughout the experimental period suggests improved meat flavor. Thr and Leu regulate protein metabolism and participate in oxidative energy supply during special periods, while Ser regulates muscle growth and

lipid metabolism. The significant increase in Thr, Leu, or Ser contents with 100 or 500 g/t XOS throughout the period indicates enhanced protein and lipid metabolism, consistent with previous research.

Muscle fibers are the basic units of muscle, and muscle fiber type composition and muscle growth-related gene expression play important roles in meat quality regulation. In livestock, mRNA expression of MyHC I, MyHC IIa, and MyHC IIx positively correlates with meat tenderness, color, and intramuscular fat content, but negatively correlates with shear force, whereas MyHC IIb fiber type shows the opposite relationships. This study demonstrated that 500 g/t XOS promoted MyHC IIx expression, suggesting improved pork quality. Among muscle growth-related genes, MyoD can convert other cell types into myoblasts and promote differentiation into mature muscle fibers; MyoG is a positive regulator of skeletal muscle development that modulates expression of muscle-specific proteins and regulates myoblast fusion; MEF2A promotes muscle-specific gene expression. The upregulation of MyoD, MyoG, and MEF2A expression by 100 g/t XOS throughout the period, which was significantly higher than other XOS treatments, indicates that XOS can regulate myocyte differentiation and muscle fiber formation, thereby promoting muscle growth. MSTN expression positively correlates with intramuscular fat content but negatively correlates with muscle fiber diameter. The significant upregulation of MSTN expression by 100 g/t XOS throughout the period may explain the increased intramuscular fat content in longissimus dorsi muscle.

Conclusion

Dietary supplementation with appropriate XOS doses can regulate nitrogen metabolism and upregulate expression of muscle fiber type and muscle growth-related genes, with 100 g/t XOS during the 30–100 kg stage showing optimal effects.

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