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Research Article

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Probiotics or synbiotics addition in Bama Mini-pigs’ diets improves growth performance, carcass traits, and meat quality by altering plasma metabolites and related gene expression of offspring pigs

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Abstract

The present study evaluated the effects of maternal probiotics or synbiotics addition on growth performance, carcass traits, meat quality, plasma metabolites, and related gene expression of their offspring pigs. A total of 64 Bama mini-pigs were used and randomly divided into the control, antibiotic, probiotics, or synbiotics groups, and fed experimental diets during pregnancy and lactation. After weaning, two piglets per litter and eight piglets per group were selected and fed a basal diet. At 65-, 95-, and 125-day-old, eight pigs per group were selected for analysis. The results showed that probiotics addition increased the ADFI of pigs during the 66–95 day-old and the backfat thickness at 65- and 125-day-old, whereas synbiotics addition increased backfat thickness and decreased muscle percentage and loin-eye area at 125-day-old. In meat quality, probiotics or synbiotics addition increased cooking yield, pH45min, and meat color, while decreased drip loss and shear force at different stages. However, maternal antibiotic addition showed an increased shear force at 125-day-old. Dietary probiotics or synbiotics addition in sow diets increased several amino acids (AA) contents in the plasma and longissimus thoracis (LT) muscle, including total AA (TAA), His, Met, Asp, Arg, and Leu, while decreased Gly, Pro, Ile, α-AAA, α-ABA, β-Ala, and γ-ABA at different stages. In the LT muscle fatty acid (FA) analysis, the contents of saturated FA (SFA; including C16:0, C17:0, and C20:0) and C18:1n9t were decreased, and the contents of C18:2n6c, MUFA (C16:1 and C20:1) and UFA were increased in the probiotics group, while the contents of SFA (C10:0, C12:0, and C14:0 at 65-day-old)
were increased and the contents of C20:1 and C18:1n9t were decreased in the synbiotics group. Plasma biochemical analysis revealed that maternal probiotics or synbiotics addition decreased plasma AMM, UN, and GLU, while synbiotics addition increased plasma ALT, HDL-C, and TC at different stages. Moreover, maternal probiotics or synbiotics regulated muscle fiber-type, myogenic regulatory, and lipid metabolism-related gene expressions of offspring pigs. In conclusion, maternal probiotics or synbiotics addition, as a nutritional intervention strategy, improved feed intake and meat quality by altering the body's metabolism of offspring pigs and gene expressions related to meat quality.

Keywords: Bama mini-pigs; Carcass traits; Growth performance; Meat quality; Plasma metabolites; Probiotics; Synbiotics
Introduction

Microbes inhabited in the gut have strong metabolic activity and play critical roles in regulating the growth and development, nutrient metabolism, immunity response, and health of the host [1]. Previous studies have shown that the intestinal microbiota can affect muscle growth, nutrient metabolism, and muscle fiber type transformation [2], indicating that intestinal microbiota can influence the carcass traits and meat quality through multiple metabolic pathways. Increasing evidence has shown that maternal probiotics supplementation has beneficial effects on offspring piglet’s growth and development. For example, dietary Bacillus addition during late gestation and lactation has beneficial effects on growth performance and intestinal microbiota modulation of piglets [3]. In addition, dietary prebiotics such as xylo-oligosaccharides (XOS) intervention could also facilitate the proliferation of beneficial microbes and thus improve the growth performance of weaned piglets by regulating intestinal health [4]. However, it has remained unknown how maternal gut microbiota regulates the meat quality of offspring and whether the gut microbiota or their metabolites have positive effects on meat quality. Therefore, maternal gut microbiota intervention through probiotics, prebiotics, and synbiotics addition would be used as a strategy to improve the meat quality of the offspring.

Maternal health and nutritional status during gestation and lactation play a vital role in the growth and development of the fetuses and have potential long-term effects on offspring pigs. Studies have demonstrated that changing maternal nutrition during
gestation and lactation can affect meat color, muscle tenderness, intramuscular fat (IMF), water-holding capacity, muscle fiber type, and muscle metabolism of offspring pigs [5, 6]. Furthermore, fetal and neonatal periods are the most critical periods for skeletal muscle development, which has been completed in the embryonic and fetal stages [7]. The development of postnatal muscle fibers is dependent on the increased diameter and alteration of muscle fiber type composition [8]. Therefore, pregnancy and lactation stages are the window periods to regulate the development of fetal muscle fibers and meat quality. In other words, maternal nutrition intervention during pregnancy and lactation is an effective strategy to improve the growth and development of fetal skeletal muscle and meat quality of offspring pigs.

Our previous studies showed that the addition of maternal synbiotics (including \textit{Lactobacillus plantarum} $\geq 1.0 \times 10^8$ CFU/g, \textit{Saccharomyces cerevisiae} $\geq 0.2 \times 10^8$ CFU/g and XOS) during pregnancy and lactation improved piglet’s survival rate, nutrient metabolism, and intestinal barrier permeability, reduced oxidative stress, and modified colonic microbiota composition and metabolic activity in suckling piglets [9, 10]. Based on the above-mentioned findings, we hypothesize that the intervention in the intestinal microbial balance of sows by dietary probiotics or synbiotics might improve the growth performance and meat quality of offspring pigs. Bama mini-pig, as one of the most well-known native mini-pigs in China, has better meat quality and lower muscle growth than commercial pigs [11]. In addition, the Bama mini-pig is a useful animal model for studying high-quality meat. Therefore, the present study used Bama
mini-pig as a model to explore the effects of maternal antibiotic (inhibiting the gut microbiota), probiotics (increasing the beneficial gut microbiota), or synbiotics (increasing the beneficial gut microbiota and their fermenting substrate) addition during pregnancy and lactation on growth performance, carcass traits, meat quality, plasma metabolites, and related gene expression of offspring pigs.

Materials and methods

Animals, diets, and treatments

The present study was conducted at the mini-pig experimental base of Goat Chong located in Shimen Town, Changde City, Hunan Province, China. A total of 64 pregnant Bama mini-pigs during 3-5 parities with initial body weight (BW) of about 90 kg were selected and randomly divided into four groups (16 sows (pens) per group). The dietary treatment groups were represented the control group (fed antibiotic-free basal diet), antibiotic group (added 50 g/t virginiamycin with the basal diet), probiotics group (added 200 mL/d probiotics mixture per animal with the basal diet), and synbiotics group (added 500 g XOS per ton diet + 200 mL/d probiotics mixture per animal with the basal diet). The probiotics mixture was provided by Hunan Lifeng Biotechnology Co., Ltd. (Changsha, Hunan, China), containing \( Lactobacillus plantarum \geq 1 \times 10^8 \) CFU/mL and \( Saccharomyces cerevisiae \geq 0.2 \times 10^8 \) CFU/mL. The XOS was provided by Shandong Longlive Biotechnology Co., Ltd. (Shandong, China), containing xylobiose, xylotriose, and xylotetraose at \( \geq 35\%\). The supplementing probiotics mixture was mixed with diets before feeding the sows, and the XOS was added to diets during
feed production. The dose of the probiotics and synbiotics was the recommended dose by the manufacturers.

After insemination, the sows were housed individually in gestation crates (2.2 × 0.6 m) from day 1 to day 105 of pregnancy. The sows were then transferred to farrowing crates (2.2 × 1.8 m) on day 106 of pregnancy, where they were housed until weaning. The sows were fed 0.8, 1.0, 1.2, 1.5, and 2.0 kg of the pregnant diets during 1-15, 16-30, 31-75, 76-90, and 91-105 days of pregnancy, respectively; fed 1.0 kg pregnant diets before a week of parturition and ad libitum after three days of parturition; and then fed 2.4 kg lactation diets until weaning. The sows were fed twice daily at 8:00 a.m. and 5:00 p.m., respectively, and water was freely available at all times. The creep feed was provided to the suckling piglets from 7- to 28-day-old. After weaned at 28-day-old, one male piglet and one female piglet close to the average BW per litter were selected and transferred to the nursery house for the subsequent feeding trial. After one week of adaption, four piglets from two litters in the same group were merged into one pen. Four piglets were assigned to one pen with eight pens (replicates) per group. All the piglets were fed the basal diet of the remaining trial. Basal diet composition and nutrient levels for the sows and piglets are presented in Supplementary Table 1 and Supplementary Table 2, respectively. Feeding and management were carried out according to the standard operation of commercial pig farms.

Sample collection
The offspring pigs from each group were fasted for 12 h and weighed at 65-, 95-, and 125-day-old, respectively. Then one pig per pen (a total of eight pigs per group) was selected and sacrificed under commercial conditions using electrical stunning (120 V, 200 Hz) and exsanguination. Blood samples were collected via precaval vein in 10 mL heparinized tubes, centrifuged at 4°C and 3,500 × g for 10 min to obtain plasma, and immediately stored at −20°C for further analysis. After removing the head, legs, tail, and viscera, the carcass was split longitudinally for evaluating carcass traits and meat quality. Then the longissimus thoracis (LT) muscle samples were collected, and stored in sealed plastic bags at −20°C to analyze the routine nutrients. In addition, the LT muscle samples (~2 g) were also collected and immediately frozen in liquid nitrogen and stored at −80°C for mRNA analyses.

**Determination of growth performance**

Feed intake of offspring pigs per pen was recorded daily. The BW of all pigs per pen was weighed at 65-, 95-, and 125-day-old pigs, respectively. The average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio (F/G) were calculated during 35–65, 66–95, and 96–125 day-old, respectively.

**Measurement of carcass traits**

According to the Chinese pig-raising industry standards (GB8467-87, 1988), the left side of each carcass was dissected into the skin, skeletal muscle, bone, and fat. The skeletal muscle, bone, fat, and leaf fat were weighted for calculating their percentages of live BW [tissue weight × 2 (kg)/BW (kg) × 100%] or ratio to live BW [leaf fat weight
Backfat thickness (between the sixth and seventh ribs) was measured using a Vernier caliper. The width and height of the LT muscle cross-section were measured using a Vernier caliper for calculating the loin-eye muscle area (width × height × 0.7).

**Measurement of meat quality**

The subjective marbling and meat color scores of the LT muscle were determined by following the National Pork Producers Council (NPPC) guidelines [12]. Meat color, including L* (lightness), a* (redness), and b* (yellowness) values of the LT muscle were measured using the colorimeter (CR410, Konica Minolta Sensing, Inc., Tokyo, Japan) at 45 min post mortem. The pH at 45 min (pH\textsubscript{45min}) and 24 h (pH\textsubscript{24h}) post mortem was measured by using a portable pH meter (Russell CD700, Russell pH Limited, Germany). The drip loss was determined using the “bag method” as previously described [13]. Briefly, the LT muscle samples were stored at 4°C, and then the samples were placed in individual polyethylene vacuum bags and cooked to an internal temperature of 70°C detected by the thermocouple thermometer (Digital Thermometer TP101, Changzhou KB Instruments & Meter Co., Ltd., China) in a water bath at 75°C at 24 h post mortem [14]. Then the cooked samples were cooled to room temperature, dried with a paper towel, and re-weighted for calculating cooking yield [(cooked weight/fresh weight) × 100%]. After the cooking yield determination, the samples were trimmed into shaped stripes with a diameter of 1.27 cm and a length of 20 mm along the direction parallel to the muscle fiber. The stripes were then used to determine the
shear force (N) using a texture analyzer (FTC-TMS/PRO, FTC Corporation, United States) with a load cell of 15 kg and a 200 mm/min crosshead speed.

**Chemical composition analysis of the longissimus thoracis muscle**

The LT muscle samples were minced after weighing and dried in a vacuum-freeze dryer at (20 ± 5) Pa and −(45 ± 5) °C for 72 h (CHRIST RVC2-25 CDPIUS, Christ Company, Osterode, Germany) to calculate the dry matter (DM) content. The crude protein (CP) content (N × 6.25) was determined using the Kjeldahl method following the standards provided by the Association of Official Analytical Chemists’ (AOAC) methods [15]. The intramuscular fat (IMF) content was determined using the Soxhlet extraction method [16].

Approximately 0.1000 g of freeze-dried skeletal muscle powder was hydrolyzed in 10 mL of 6 mol/L HCl solution in a sealed ampoule bottle at 110°C for 24 h. The suspension was diluted to 100 mL using double-distilled water in the volumetric flask [17], and 1 mL of the supernatant was transferred to 1.5 mL centrifuge tubes and evaporated to dryness in a water bath at 65°C. The samples were then dissolved using 1 mL of 0.02 mol/L HCl solution and filtered through a 0.45-μm membrane prior to analysis of hydrolyzed amino acids contents by an automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan) [18].

Medium- and long-chain fatty acids (FA) were identified via the gas-liquid chromatography (7890A, Agilent, California, USA). Briefly, FA were analyzed by FID detector after extracting with the mixture of benzene and petroleum ether and methyl
esterification through potassium hydroxide methanol. The sample pretreatment method was described as previously [19]. The GC conditions were as follows: the injector and detector temperatures were held at 280°C; the initial column temperature held at 100°C for 13 min; increased at 10°C per min to 180°C, and held for 6 min; increased at 1°C per min to 200°C, and held for 20 min; increased at 4°C per min to 230°C, and held for 15 min. Nitrogen was used as the carrier gas and the flow rate was 0.8 mL/min. The inlet temperature was 270°C, the injection volume was 1 μL, and the split ratio was 20:1. The contents of FA were identified by comparison with the retention times of reference standard mixtures (99%, Sigma, St. Louis, MO, USA). Data are expressed as the percentage of the total FA.

Muscular RNA extraction and real-time quantitative PCR analysis

Total RNA of the LT muscle was extracted using the AG RNAex Pro reagent (Accurate Biology, Hunan, China) according to the manufacturers’ protocol. The concentration of the extracted RNA was measured using the NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States), and the purity was ascertained by the A260/A280 ratio. The quality of RNA was evaluated with agarose gel electrophoresis. The total RNA (1000 ng) was reversely transcribed into cDNA using an Evo M-MLV RT Kit with gDNA Clean for qPCR (Accurate Biology, Hunan, China). The RT-PCR analysis was performed on the LightCycler® 480II Real-Time PCR System (Roche, Basel, Swiss) with SYBR® Green Premix Pro Taq HS qPCR Kit (Accurate Biology, Hunan, China). The specific primers of target genes were designed
and synthesized by Sangon Biotech (Shanghai) Co., Ltd., China (Supplementary Table 3). The RT-qPCR was performed in a 10 μL reaction system, including 0.25 μL of each primer, 5.0 μL SYBR® Green Premix, 2.0 μL cDNA, and 2.5 μL of double-distilled water. The PCR cycling conditions were followed according to the instructions of SYBR Green Premix. The relative gene expression levels between different groups were calculated using the 2^-ΔΔCt method [20].

**Determination of plasma biochemical parameters**

The plasma levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), α-amylase (α-AMS), aspartate aminotransferase (AST), cholinesterase (CHE), lactate dehydrogenase (LDH), albumin (ALB), ammonia (AMM), total protein (TP), urea nitrogen (UN), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), and glucose (GLU) were determined using commercially available kits (F. Hoffmann-La Roche Ltd, Basel, Switzerland) and the Roche automatic biochemical analyzer (Cobas c311, F. Hoffmann-La Roche Ltd, Basel, Switzerland).

**Determination of plasma free amino acids**

The plasma samples (800 μL) were mixed with 8% sulfosalicylic acid in equal proportion, and then stored at 4°C for overnight to precipitate protein. The supernatants were obtained by centrifuging at 8,000 × g for 10 min. The processed samples were then filtered through a 0.22-μm membrane into sample bottles prior to analysis of free amino acids with an automatic amino acid analyzer (L8900, Hitachi, Tokyo, Japan).
Statistical analysis

The experimental unit was a pen for the ADFI, ADG, and F/G data, and the individual pig was for the other data. All data were analyzed using the SPSS software package (SPSS v. 25.0, SPSS Inc., Chicago IL, USA). The normal distribution of the data was confirmed by the Shapiro-Wilk test before assessing the differences among the four groups. The data were analyzed by one-way ANOVA, and differences among the four treatments were compared using the Tukey post-hoc test. Data are presented as means with their standard error of the mean (SEM). Differences were considered significant if $P < 0.05$.

Results

Growth performance

The effects of probiotics and synbiotics addition in sow’s diets on growth performance are shown in Table 1. Compared with the antibiotic group, dietary probiotics addition in sow diets increased ($P < 0.05$) the BW of 95-day-old offspring pigs. The ADFI was increased in the probiotics group while decreased in the antibiotic group during 66−95 day-old, when compared with the control group ($P < 0.05$). Moreover, the antibiotic group had higher F/G compared with the control group and higher ADFI compared with the other three groups during 96−125 day-old ($P < 0.05$).

Carcass traits

The carcass traits of offspring pigs are presented in Table 2. At 65-day-old, the probiotics group had a higher ($P < 0.05$) carcass weight compared with the antibiotic
and synbiotics groups, while the backfat thickness was increased \((P < 0.05)\) in the probiotics group compared with the control and antibiotic groups. At 95-day-old, the leaf fat ratio was lower \((P < 0.05)\) in the antibiotic and probiotics groups compared with the control and synbiotics groups, while the loin-eye area was higher \((P < 0.05)\) in the synbiotics group compared with the antibiotic group. Moreover, the antibiotic group had a lower fat percentage than the other three groups and lower carcass weight than the control group at 95-day-old \((P < 0.05)\). At 125-day-old, dietary antibiotic, probiotics, and synbiotics addition increased the fat percentage and backfat thickness, while dietary synbiotics addition decreased the muscle percentage of pigs compared with the pigs in the control group \((P < 0.05)\). Moreover, dietary synbiotics addition decreased \((P < 0.05)\) the loin-eye area of pigs compared with the three groups at 125-day-old.

**Meat quality**

The effects of dietary probiotics and synbiotics addition in sow diets on meat quality of offspring pigs are shown in Table 3. Compared with the control and antibiotic groups, drip loss in the probiotics and synbiotics groups was lower \((P < 0.05)\), while cooking yield in the probiotics group was higher \((P < 0.05)\) at 65-day-old. In addition, the \(L^*\) value was higher \((P < 0.05)\) in the probiotics group, and the shear force was lower \((P < 0.05)\) in the synbiotics group compared with the other three groups at 65-day old. At 95-day-old, the \(a^*\) value was lower \((P < 0.05)\) in the probiotics group but was higher \((P < 0.05)\) in the synbiotics group compared with the control and antibiotic groups, and \(\text{pH}_{45\text{min}}\) was higher \((P < 0.05)\) in the probiotics group compared with the control and
synbiotics group. Moreover, the L* value in the probiotics group was higher ($P < 0.05$) than the synbiotics groups at 95-day-old. At 125-day-old, the L* value was lower in the synbiotics group compared with the control and probiotics groups, while the b* value was higher in the probiotics group compared with the antibiotic and synbiotics groups ($P < 0.05$). Moreover, the shear force in the probiotics and synbiotics groups was lower than the control and antibiotic groups, while cooking yield and shear force were higher in the antibiotic group than the other three groups at 125-day-old ($P < 0.05$).

**Amino acid contents of longissimus thoracis muscle**

The contents of amino acids in the LT muscle are presented in Table 4. Compared with the control and antibiotic groups, His content in the LT muscle was higher in the probiotics and synbiotics groups, while asparagine (Asp) and glycine (Gly) contents were lower in the probiotics group at 65-day-old ($P < 0.05$). At 95-day-old, compared with the control group, arginine (Arg) content in the LT muscle was higher in the antibiotic, probiotics, and synbiotics groups, as well as the Asp and TAA contents in the probiotics and synbiotics groups, while the histidine (His) content in the LT muscle was lower in the probiotics group ($P < 0.05$). Moreover, isoleucine (Ile) content in the probiotics and synbiotics groups and lysine (Lys) content in the synbiotics group were higher compared with the control and antibiotic groups, while proline (Pro) content in the synbiotics groups was lower compared with the antibiotic group ($P < 0.05$). At 125-day-old, Asp and leucine (Leu) contents in the antibiotic and probiotics groups and the phenylalanine (Phe) content in the synbiotics group were higher ($P < 0.05$) in the LT
muscle compared with the control group. Moreover, serine (Ser) and His contents in
the LT muscle were higher \((P < 0.05)\) in the synbiotics group compared with the other
three groups at 125-day-old.

**Fatty acid contents of longissimus thoracis muscle**

The effects of dietary probiotics and synbiotics addition in sow diets during pregnancy
and lactation on amino acid contents in the LT muscle of offspring pigs are shown in
Table 5. At 65-day-old, compared with the control group, the contents of C18:2n6c and
PUFA in the antibiotic and probiotics groups and the contents of C10:0, C12:0, and
C14:0 in the synbiotics group were increased \((P < 0.05)\) in the LT muscle of pigs,
whereas the contents of C16:0 and C20:1 in the probiotics group were decreased \((P <
0.05)\) in the muscle of pigs. In addition, the content of C24:0 was increased \((P < 0.05)\)
in the probiotics group, while the contents of C18:1n9c, C20:0, and SFA in the
probiotics group were decreased \((P < 0.05)\) compared with the other three groups.
Moreover, the content of C10:0 was increased \((P < 0.05)\) in the synbiotics group
compared with the other three groups, and the content of IMF was decreased \((P < 0.05)\)
in the probiotics group compared with the antibiotic and synbiotics groups. At 95-day-
old, the content of C10:0 in the probiotics and synbiotics groups and the content of
C14:0 in the probiotic group were increased \((P < 0.05)\) in the LT muscle of pigs, while
the content of C20:1 in the antibiotic and synbiotics groups was decreased \((P < 0.05)\)
compared with the control group. In addition, the content of C12:0 was increased while
the content of C16:1 was decreased in the antibiotic, probiotics, and synbiotics groups
compared with the control group ($P < 0.05$). Moreover, the contents of UFA and MUFA were increased ($P < 0.05$) in the probiotics group compared with the other three groups at 95-day-old. At 125-day-old, the contents of C15:0 in the probiotics and synbiotics groups, C17:0 in the probiotics group, C18:2n6c in the synbiotics group, and C18:1n9t in the antibiotic, probiotics, and synbiotics groups were decreased in the LT muscle of pigs compared with the control group. In addition, the contents of C16:1 in the probiotics group and IMF in the synbiotics group were increased ($P < 0.05$) compared with the other three groups.

The mRNA expression of myosin heavy chain isoforms, myogenic regulatory factors, and lipid metabolism related genes in longissimus thoracis muscle

The effects of probiotics and synbiotics addition in sow diets on mRNA expression of MyHC isoforms, MRFs, and lipid metabolism related genes in the LT muscle of offspring pigs at 65-, 95-, and 125-days are shown in Figure 1. Compared with the control group, the mRNA expression of $MyHC$ IIa in in the antibiotic, probiotics, and synbiotics groups was up-regulated ($P < 0.05$), while the mRNA expressions of $MyHC$ IIx and $MyHC$ IIb in the probiotics group were down-regulated ($P < 0.05$) in the LT muscle of pigs at 65-day old (Figure 1 A–B). Moreover, the mRNA expression of $MyHC$ I was up-regulated ($P < 0.05$) in the probiotics group compared with the other three groups and the mRNA expression of $Myf6$ was down-regulated ($P < 0.05$) in the antibiotic group compared with the probiotics group at 65-day-old (Figure 1 A–B). At 95-day-old, the mRNA expression of $MyHC$ IIb and $Myf5$ in the LT muscle were up-
regulated ($P < 0.05$) in the probiotics and synbiotics groups compared with the control and antibiotics groups. The mRNA expression of MyHC I and Myf6 in the LT muscle were up-regulated ($P < 0.05$) in the synbiotics group, while the expression of MSTN was down-regulated ($P < 0.05$) when compared with the other three groups (Figure 1 A–B). At 125-day-old, the mRNA expression of Myf6 in the antibiotic, probiotics, and synbiotics groups and the mRNA expression of MyHC IIa in the antibiotic and synbiotics groups were up-regulated ($P < 0.05$) in the LT muscle compared with the control group. In addition, the mRNA expression of MyOG in the probiotics and synbiotics groups was down-regulated ($P < 0.05$) compared with the control and antibiotics groups. Moreover, the mRNA expression of MyHC I and Myf5 were up-regulated ($P<0.05$) in the antibiotic group compared with the control and probiotics groups (Figure 1 A–B).

As shown in Figure 1 C–D, at 65-day-old, the mRNA expression of stearoyl-CoA desaturase (SCD) in the LT muscle was down-regulated ($P < 0.05$) in the antibiotic, probiotics, and synbiotics groups compared with the control group, whereas the mRNA expression of lipoprotein lipase (LPL) was up-regulated ($P < 0.05$) in the probiotics group compared with the other three groups. At 95-day-old, the mRNA expression of LPL in the antibiotic, probiotics, and synbiotics groups and the mRNA expression of acetyl-CoA carboxylase (ACC) in the probiotics group were up-regulated ($P < 0.05$) compared with the control group. Moreover, the mRNA expression of fatty acid synthase (FASN) in the probiotics and synbiotics groups and the mRNA expression of
SCD in the synbiotics group were up-regulated ($P < 0.05$), compared with the control and antibiotic groups. At 125-day-old, the mRNA expression of LPL was up-regulated ($P < 0.05$) in the antibiotic, probiotics, and synbiotics groups compared with the control group. The mRNA expression of ACC was up-regulated in the antibiotic and synbiotics groups and down-regulated in the probiotics group compared with the control group. Moreover, the mRNA expressions of FASN and SCD were up-regulated ($P < 0.05$) in the antibiotic group compared with the other three groups, while the mRNA expression of hormone-sensitive triglyceride lipase (LIPE) was down-regulated compared with the control group.

**Plasma biochemical parameters**

To evaluate the effects of maternal probiotics or synbiotics addition in sows’ diets on offspring pigs, we measured the plasma biochemical parameters at 65-, 95-, and 125-day-old, and the results are presented in Table 6. At 65-day-old, compared with the control group, the plasma activity of α-AMS in the antibiotic, probiotics, and synbiotics groups, while the plasma activity of LDH was increased in the antibiotic group and decreased in the probiotic group ($P < 0.05$). The concentrations of LDL-C and TC were increased in the antibiotic group while decreased in the synbiotics group compared with the control group ($P < 0.05$). In the synbiotics group, the concentration of HDL-C was increased compared with the other three groups, while the concentration of Glu was decreased compared with the control and probiotics groups ($P < 0.05$). Moreover, the concentrations of ALB in the antibiotic group and CHE in the probiotics group were
decreased ($P < 0.05$) compared with the other three groups. At 95-day-old, the plasma activities of AST and LDH in the antibiotic and probiotics groups, the concentration of GLU in the antibiotic, probiotics, and synbiotics groups, and concentration of TG in the synbiotics group were decreased ($P < 0.05$) compared with the control group. Compared with the control and antibiotic groups, the plasma CHE activity and UN concentration were decreased ($P < 0.05$) in the probiotics and synbiotics groups. Moreover, the plasma concentration of AMM was decreased ($P < 0.05$) in the probiotics group compared with the other three groups. At 125-day-old, the plasma concentration of HDL-C was increased ($P < 0.05$), whereas the AST and LDH activities were decreased ($P < 0.05$) in the antibiotic and probiotics groups compared with the control group. The plasma concentration of AMM was decreased ($P < 0.05$) in the antibiotic, probiotics, and synbiotics groups, while the plasma activity of $\alpha$-AMS was increased ($P < 0.05$) in the antibiotic and synbiotics groups, when compared with the control group. Moreover, the plasma concentration of TP in the antibiotic group was decreased ($P < 0.05$), and the plasma concentration of TG in the synbiotics group was increased ($P < 0.03$) compared with the other three groups.

**Plasma free amino acid concentration**

Table 7 presents the effects of maternal probiotics or synbiotics addition in sow’s diets in offspring pigs’ plasma free amino acid concentration. At 65-day-old, the plasma histidine (His) concentration in the probiotics group, the hydroxy-proline (Hypro) concentration in the antibiotic, probiotics, and synbiotics groups, and the $\beta$-
aminoisobutyric acid (β-AiBA) concentration in the antibiotic and synbiotics groups were increased ($P < 0.05$), whereas the plasma Gly, Ile, sarcosine (Sar), and taurine (Tau) concentrations in the antibiotic and probiotics groups and the Leu concentration in the probiotics group were decreased ($P < 0.05$), compared with the control group. Compared with the control and antibiotic groups, the plasma citrulline (Cit) and cystathionine (Cysthi) concentrations were increased ($P < 0.05$) in the synbiotics group, while the plasma alanine (Ala) concentration was decreased ($P < 0.05$) in the probiotics group. The plasma γ-amino-n-butyric acid (γ-ABA) concentration was increased in the synbiotics group compared with the other three groups, while the plasma tyrosine (Tyr) concentration was decreased and the plasma hydroxy-lysine (Hylys) was increased in the probiotics group compared with the other three groups ($P < 0.05$). However, the antibiotic group had increased ($P < 0.05$) plasma threonine (Thr) and Pro concentrations compared with the other three groups.

At 95-day-old, the plasma Ile, Leu, Cit, Tau, α-amino adipic acid (α-AAA), α-ABA, β-alanine (β-Ala), and γ-ABA concentrations were decreased, and the plasma γ-amino-n-butyric acid (β-AiBA) was increased in the antibiotic, probiotics, and synbiotics groups, compared with the control group ($P < 0.05$). In addition, compared with the control group, the plasma β-AiBA concentration was increased, and the ethanolamine (EOHNH2) concentration was decreased in the probiotics and synbiotics groups ($P < 0.05$). Compared with the control and antibiotic groups, the plasma Sar concentration was increased, and the EOHNH2 was decreased in the probiotics and synbiotics groups,
as well the plasma Cysthi concentration was increased in the synbiotics group \( (P < 0.05) \). Moreover, the plasma Hyllys concentration was increased, and the plasma anserine (Ans) concentration was decreased in the synbiotics groups, compared with the other three groups. However, the antibiotic group had increased \( (P < 0.05) \) plasma 1-Methyl-histidine (1-Mehis), EOHNH2, and ornithine (Orn) concentrations compared with the other three groups.

At 125-day-old, the plasma Ans and methionine (Met) concentrations in the antibiotic and probiotics groups, the plasma valine (Val) and Leu concentrations in the antibiotic and synbiotics groups were increased, compared with the control group. The plasma \( \alpha \)-ABA concentration was increased, whereas the Orn concentration was decreased in the antibiotic, probiotics, and synbiotics groups compared with the control group \( (P < 0.05) \). Compared with control and antibiotic groups, the plasma Cysthi concentration was increased in the synbiotics group, while the plasma Ser concentration was decreased in the probiotics and synbiotics groups \( (P < 0.05) \). Moreover, the plasma Asp and His concentrations were increased in the probiotics group, and the plasma EOHNH2 concentration was decreased in the synbiotics group, when compared with the other three groups \( (P < 0.05) \). However, the antibiotic group had increased \( (P < 0.05) \) plasma Thr, Ala, Arg, Gly, Pro, Ser, Tau, Tyr, and \( \beta \)-AiBA concentrations compared with the other three groups.

**Discussion**
There is growing scientific and industrial interest for prebiotics, probiotics, and synbiotics addition in pig production [21, 22]. Numerous studies showed that dietary probiotics or synbiotics could improve the health status and production performance of pigs by modulating intestinal microbiota [23, 24]. Therefore, the present study aimed to determine the effects of maternal gut microbiota intervention via dietary probiotics or synbiotics addition on the growth performance, carcass traits, meat quality, and metabolites of their offspring pigs. Our findings indicated that dietary probiotics or synbiotics addition in Bama mini-pigs’ diets could improve growth performance, meat quality, and the body's metabolism of offspring pigs.

Dietary probiotics or synbiotics addition in diets have been widely used in livestock production. Recent studies have found that can improve the growth performance in growing-finishing pigs [25], such as dietary *Lactobacillus plantarum* ZLP001 addition could increase the ADFI of weaning piglets [26]. In the present study, maternal probiotics or synbiotics addition did not affect the growth performance of offspring pigs, except for an increase of ADFI in the probiotics group during the 66–95 day-old. A possible reason for the inconsistent growth performance of the present study might be related to the pig breeds. Moreover, Bergamaschi et al. (2020) demonstrated that three breed pigs present different ADFI due to their different gut microbiota composition [27]. Our previous study also showed that dietary synbiotics (including *Lactobacillus plantarum* ≥ 1.0 × 10^8 CFU/g, *Saccharomyces cerevisiae* ≥ 0.2 × 10^8 CFU/g and XOS) addition can alter the abundance, diversity, and composition of gut
microbiota in pregnant and lactating sows [10]. However, the present study also found that maternal antibiotic addition significantly decreased the ADFI of offspring pigs during 66–95 day-old and increased F/G during 96–125 day-old, which may be related to the adverse effects of antibiotic use during pregnancy on the offspring [28]. A previous study also indicated that maternal antibiotic addition could alter the maternal and fetus microbiome in utero and during the postnatal period through maternal-fetus interaction, thereby affecting the health of offspring [29]. Therefore, the adverse effect of maternal antibiotic addition on the growth performance of offspring needs to be further studied.

Carcass traits and meat quality are the major factors that influence the meat flavor, tenderness, juiciness, and overall consumer acceptance. In the present study, maternal probiotics or synbiotics addition increased the backfat thickness and fat percentage of offspring pigs at 125-day-old along with the antibiotic, while probiotics addition increased backfat thickness at 65-day-old, suggesting that these additives could improve body fat deposition and then improve the meat tenderness in the 125-day-old pigs. Generally, the loin-eye area is positively related to the lean meat rate and negatively related to backfat thickness. However, the present study showed a decrease in the loin-eye area and lean meat rate in the synbiotics group at 125-day-old, indicating that maternal synbiotics has no impact on lean meat. Previous studies also found that dietary XOS addition has no significant effect on lean meat [30, 31]. This inconsistency
may be correlated with the pig breed. Bama mini-pig belongs to the fatty breed of pigs, which has higher fat content than the Landrace pigs [19].

Meat color is an important sensory index because it affects consumers’ first impression of the meat, and it is generally evaluated by redness, lightness, and yellowness values [32]. The high-quality meat has higher redness values and lower lightness and yellowness values [33]. In the present study, maternal synbiotics addition increased the redness values at 95-day-old and decreased the lightness values at 125-day-old in the LT muscle of offspring pigs. Moreover, maternal probiotics decreased the lightness and redness values of LT muscle at 65- and 95-day-old, respectively. Previous studies have reported that dietary synbiotics (including yeast cell wall, XOS, Clostridium butyricum, Bacillus licheniformis, and Bacillus subtilis) had no effects on the redness and lightness values [34], while dietary probiotics addition could increase the redness values but not lightness values [32]. However, the present study suggesting that maternal synbiotics addition could improve the sense-impression of pork, while probiotics addition has no positive effect on meat color. These inconsistent findings might be related to the feeding stage and the type and dose of probiotics or synbiotics.

Additionally, the results of this study revealed that maternal probiotics or synbiotics addition also improved the meat quality by increasing pH, water holding capacity, and cooking yield and decreasing the shear force in the LT muscle of offspring pigs. After slaughter, due to glycolysis, the accumulation of lactic acid in the muscle leads to a decrease in pH, which is highly correlated with the drip loss and shear force
Drip loss can reflect the water-holding capacity of muscle and is also an important factor affecting meat quality [36], while shear force is correlated with meat tenderness (50). In the present study, maternal probiotics addition increased pH_{45min} at 95-day-old and cooking yield at 65-day-old, while maternal probiotics or synbiotics addition increased water holding capacity at 65-day-old and decreased shear force at 125-day-old. However, maternal antibiotic addition showed an increased shear force of the LT muscle at 125-day-old. A previous study reported that drip loss usually ranges from 2% to 10% when the meat is cut into slices [37], which is consistent with the present study (ranges 2.78%–6.32%). The results were also consistent with the previous studies by Suo et al. (2012), who demonstrated that *Lactobacillus plantarum* ZJ316 addition improved pH_{45min} [38], and Zhou et al. (2010), who reported that *Bacillus coagulans* could affect meat quality by decreasing drip loss [39]. Thus, the results of the present study indicated that maternal probiotics addition could decrease the lactic acid accumulation by improving muscle glycolysis, while maternal probiotics or synbiotics addition could increase water holding capacity and cooking by reducing drip loss, cooking loss, and shear force and thereby improve meat quality.

Changes in the nutrient contents of muscular tissue, especially the contents of IMF and CP, could directly affect the sensory properties and nutritional values of meat [40]. Moreover, the IMF is a major quality trait of meat, of which content influences the consumers’ perceptions of cooked pork palatability [41]. Fernandez et al. (1999) demonstrated that the tenderness, juiciness, color, and flavor of meat were substantially...
improved with the increase in IMF content [42]. In the present study, maternal synbiotics addition increased the IMF content of LT muscle at 125-day-old, indicating that the meat quality was improved by maternal synbiotics addition. The change in IMF content of LT muscle is consistent with the change of shear force in LT muscle because the IMF content has a negative correlation with the shear force [43]. Moreover, the higher IMF content can improve the taste of meat [44].

Amino acids play pivotal roles in the growth, development, reproduction, and health of animals. Moreover, the composition and contents of amino acids in muscle could represent the protein quality and nutritional value of meat [45]. In the present study, dietary probiotic or synbiotics addition significantly increased the TAA contents in the LT muscle at 95-day-old, suggesting that the nutritional value of pork of growing pigs was improved by increasing amino acid deposition. Several AA play key roles in the aroma and flavor profiles of muscle [46]. For example, Arg, Leu, Ile, Val, Phe, Met, and His present a bitter taste; Glu and Asp present a pleasant fresh taste; and Gly, Ala, and Ser present a sweet taste [47]. In the present study, maternal probiotics or synbiotics addition increased several AA contents, including His, Arg, Asp, Leu, Ser, and Phe, at different stages in the LT muscle. These changes could improve the flavor of LT muscle of offspring pigs. A previous study also indicated that dietary *Clostridium butyricum* addition improved the flavor of the duck meat through altering FAA content [48]. Free AA are the main direct source of amino acids for muscle protein synthesis and the key index of protein renewal [49]. Plasma free AA profile reflects the sum of metabolic
flow of nutrients and their metabolites from all tissues and organs [50]. Plasma free AA
profile also reflects the dynamics state of the metabolic flux of AAs absorbed from the
small intestine, as well as the rates of their utilization and intracellular protein turnover
in the whole body [51]. For example, Gly has crucial roles in nutrition and metabolism,
and protein synthesis accounts for 80% of whole-body glycine needs by growing
animals [52]. Branched-chain AA, including Leu, Ile, and Val, have an impact on the
regulation of energy homeostasis and nutrition metabolism [53]. Our results showed
that plasma Gly, Ile, Leu, and Tau concentrations were decreased in the probiotics or
synbiotics groups at 65- or 125-day-old, suggesting that maternal probiotics or
synbiotics addition attenuates the breakdown of protein in muscle and then promotes
the AA deposition in the muscle. In addition, the present study also showed that
maternal probiotics addition decreased the plasma Asp, Ala, Sar, Tyr, Car, and His
concentrations, while probiotics or synbiotics addition decreased the plasma Orn, Cit,
α-AAA, α-ABA, β-Ala, and γ-ABA concentrations at different stages of age, suggesting
that maternal probiotics and synbiotics addition could improve the protein synthesis of
the body.

Plasma biochemical parameters reflect the physiological and nutritional status of
the animal [54]. The ALT and AST activities can reflect the status of protein synthesis
and catabolism, and the increased activities were related to the improvement of amino
acid metabolism [55]. The AMM and UN concentrations accurately reflect the status
of protein metabolism and AA balance. The present study showed that maternal
probiotics or synbiotics addition increased ALT activity and decreased AMM and UN concentrations at different stages. These results indicated that maternal probiotics or synbiotics addition could promote the balance of AA and improve the utilization of protein and then the deposition of protein. The rate of lipid utilization could be reflected by the TC, TG, HDL-C, and LDL-C [56]. In the present study, maternal synbiotics addition increased HDL-C, LDL-C, and TC concentrations at 65-day-old and decreased TG concentration at 95-day-old. However, maternal antibiotic addition decreased the concentrations of LDL-C and TC at 65-day-old. These findings suggested that maternal synbiotics improved the lipid metabolism of offspring pigs, while maternal antibiotic addition had a negative effect on the lipid metabolism. The plasma levels of LDH and GLU can reflect energy and glucose metabolism, respectively. The present study showed that LDH activity was decreased in the probiotics group at 95- and 125-day-old, GLU concentration was decreased in the synbiotics group at 65-day-old, and in the probiotics and synbiotics group at 95-day-old. Therefore, the results indicated that maternal probiotics addition improved the energy and glucose metabolism of offspring pigs, while maternal synbiotics addition improved glucose metabolism.

The FA composition profoundly affects the nutritional value, organoleptic properties, and eating quality of pork [57, 58]. The C14:0, C16:0, C16:1n7, C18:0, C18:1n9, and C18:2n6 are the primary FA in pork [59]. In the present study, maternal probiotics addition increased the C18:2n6c, MUFA, and UFA contents in the LT muscle at different stages. Similarly, previous studies also found that dietary probiotics,
such as *Lactobacillus amylovorus* and *Enterococcus faecium* could improve the FA profiles of pork by enhancing the contents of C18:2n6c, MUFA, and PUFA [60]. These alterations indicated that maternal probiotics could improve the primary FA in the muscle of offspring pigs, which may be associated with the favorable changes in the gut microbiota after probiotics addition [61]. The FA profiles of pork can be improved by increasing UFA and decreasing SFA contents in the muscle [60]. Moreover, meat with lower SFA contents could reduce the risk of cardiovascular heart diseases [62]. In the present study, maternal probiotics addition decreased the SFA content at 65-day-old, suggesting that the nutritional value of meat was improved by maternal probiotics addition. Cameron et al. (2000) indicated that the C16:1 content was positively correlated with meat flavor [63]. In our study, maternal probiotics or synbiotics addition increased the C16:1 content at 95-day-old, suggesting that these additives could improve the pork flavor of offspring pigs. In addition, maternal probiotics or synbiotics addition decreased the C18:1n9t content at 125-day-old, which is a kind of trans FA and has adverse effects on human health [64]. Consequently, maternal probiotics or synbiotics addition could improve the meat quality and nutritional value of offspring pigs, which would have beneficial effects on human health.

The muscle fiber type is mainly defined by the myosin heavy chain (MyHC) isoforms [65], and the fiber type composition is largely responsible for the determination of meat quality [66]. The higher proportion of *MyHC I* and *MyHC IIa* fibers are related to the excellent meat quality [67]. In the present study, the expressions
of MyHC I and MyHC IIa were up-regulated in the probiotics or synbiotics groups at different stages. In addition, the expressions of MyHC IIb and MyHC IIx were down-regulated in the synbiotics group at 65-day-old. The MRF gene family, including MyoD, MyoG, Myf5, and Myf6, plays dominant roles in muscle fiber formation, muscle maturation, and functional perfection of muscle fibers [68]. Myostatin (MSTN), a negative regulator of myogenesis, can inhibit muscle growth and development [69].

Previous studies indicated that the expression of the MRF gene family affected the expression of MyHC gene isoforms [70], and the MSTN plays an important role in the muscle fiber-type conversion [71]. In the present study, maternal probiotics and synbiotics addition up-regulated the Myf5 and Myf6 mRNA levels at 95- and 125-day-old, respectively; whereas synbiotics down-regulated the MSTN mRNA level at 95-day-old. Therefore, these findings indicated that maternal probiotics or synbiotics addition had positive effects on enhancing muscle growth and altering muscle fiber type composition.

Lipogenesis and lipolysis are major factors affecting fat accumulation in adipose tissue and animal products. The FASN and ACC could modulate fat deposition in animals by regulating FA synthesis [19]. The SCD is involved in the FA biosynthesis and composition [72]. In the present study, maternal probiotics or synbiotics addition up-regulated the ACC, SCD, and FASN mRNA levels at different age stages of offspring pigs. These findings suggested that maternal probiotics or synbiotics addition improved the fat deposition and FA composition in the LT muscle of offspring pigs. The LPL, the
critical lipid uptake gene, encodes a protein that participates in the process of fatty acid flux into adipocytes clustered along myofiber fasciculi in the muscle and then provides the appropriate substrate for IMF synthesis [73]. A previous study also indicated that the expressions of FASN, SCD, and LPL have a positive association with the IMF deposition [74]. In the present study, the beneficial effect of maternal probiotics or synbiotics addition on the deposition of IMF could be elucidated by the up-regulation of LPL mRNA expression level at 95- and 125-day-old. This finding was also consistent with the increased IMF content of the LT muscle in the present study. Moreover, the LIPE was found to participate in the lipolysis and negatively correlated with the IMF deposition [74]. Our results also showed that maternal antibiotic addition not only up-regulated the expression levels of FASN, LPL, SCD, and ACC, but also up-regulated LIPE expression at 125-day-old. Thus, indicating that antibiotic addition does not have the positive effects on the lipid metabolism of LT muscle.

Conclusions

In summary, maternal synbiotics addition showed only the improvement in daily feed intake, while maternal antibiotics addition had adverse effects on the growth performance of offspring pigs. Dietary probiotics or synbiotics addition during pregnancy and lactation could improve offspring piglets’ body metabolism by promoting glucose, amino acids, and energy metabolism. Furthermore, maternal probiotics or synbiotics addition could increase meat quality and nutritional value of meat, including the increase of fat or protein deposition, the improvement of sensory
impression, and the alteration of amino acids and fatty acids profiles, by improving the composition of muscle fiber type and growth, and lipid metabolism. However, maternal antibiotic addition had no effects on the lipid metabolism of LT muscles of offspring pigs. Collectively, maternal probiotics or synbiotics could serve as a maternal nutritional intervention strategy to improve feed intake, the body's metabolism, and meat quality of offspring pigs, which are better than the antibiotic.

**Abbreviations**

1-Mehis, 1-methyl-histidine; 3-Mehis, 3-methyl-histidine; ACC, acetyl-CoA carboxylase; ADG, average daily gain; ADFI, average daily feed intake; Ala, alanine; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ALB, albumin; AMM, ammonia; Ans, anserine; Arg, arginine; Asp, aspartate; AST, aspartate aminotransferase; BW, body weight; Car, carnosine; CHE, cholinesterase; Cit, citrulline; Cysthi, cystathionine; Cys, cysteine; CP, crude protein; DM, dry matter; EOHNH2, ethanolamine; EAA, essential amino acids; FAA, flavor amino acids; FA, fatty acids; F/G, feed/gain ratio; FASN, fatty acid synthase; Glu, glutamate; GLU, glucose; Gly, glycine; HDL-C, high density lipoprotein-cholesterol; His, histidine; Hyllys, hydroxy-lysine; Hypro, hydroxy-proline; IMF, intramuscular fat; Ile, isoleucine; LDH, lactate dehydrogenase; LDL-C, low density lipoprotein-cholesterol; Leu, leucine; LIPE, hormone-sensitive triglyceride lipase; LT, *longissimus thoracis*; LPL, lipoprotein lipase; Lys, lysine; Met, methionine; MRFs, myogenic regulatory factors; MUFA, monounsaturated fatty acid; *Myf*5, myogenic factor 5; *Myf*6, myogenic factor 6; MyHC,
myosin heavy chain; *MyOG*, myogenin; *MSTN*, myostatin; NEAA, non-essential amino acids; Orn, ornithine; Phe, phenylalanine; Pro, proline; PUFA, polyunsaturated fatty acids; Sar, sarcosine; *SCD*, stearoyl-CoA desaturase; Ser, serine; SFA, saturated fatty acid; *SREBP-1*, sterol-regulatory element-binding protein-1; TAA, total amino acids; Tau, taurine; TC, total cholesterol; TG, triglyceride; Thr, threonine; TP, total protein; Tyr, tyrosine; UFA, unsaturated fatty acid; UN, urea nitrogen; Val, valine; XOS, xylo-oligosaccharides; α-AAA, α-amino adipic acid; α-ABA, α-amino-n-butyric acid; α-AMS, α-amylase; β-Ala, β-alanine; β-AiBA, β-aminoisobutyric acid; and γ-ABA, γ-amino-n-butyric acid.

**Authors’ contributions**

XK and YY conceived and design the experiment and revised the manuscript. QZ, MS, MA, and CM performed the animal feeding experiment, sample collection, and sample analysis. QZ analyzed the data and wrote the manuscript. All the authors read and approved the final manuscript.

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**Declarations**

**Ethics approval and consent to participate**

The animal procedures in the present study followed the Chinese Guidelines for Animal Welfare and experimental protocols approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Science.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declared that no conflict of interest.

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Fig. 1 Effects of probiotics or synbiotics addition in sows’ diets on mRNA expression of myosin heavy chain (MyHC) isoforms (A), myogenic regulatory factors (MRFs)(B), and lipid metabolism related genes (C and D) in the *longissimus thoracis* muscle of offspring pigs at 65-, 95-, and 125-day-old. 65 d, 65-day-old; 95 d, 95-day-old; 125-d, 125-day-old. ACC, acetyl-CoA carboxylase; FASN, fatty acid synthase; LIPE, hormone-sensitive triglyceride lipase; LPL, lipoprotein lipase; Myf5, myogenic factor 5; Myf6, myogenic factor 6; MyOG, myogenin; MSTN, myostatin; SCD, stearoyl-CoA desaturase; SREBP-1, sterol-regulatory element-binding protein-1.
Table 1

Effects of probiotics or synbiotics addition in sows’ diets on growth performance of offspring pigs.

| Items                          | Control group | Antibiotic group | Probiotics group | Synbiotics group | SEM   | P values |
|-------------------------------|---------------|------------------|------------------|------------------|-------|----------|
| Body weight (kg)              |               |                  |                  |                  |       |          |
| 35-day-old                    | 4.97          | 4.90             | 4.80             | 4.66             | 0.172 | 0.609    |
| 65-day-old                    | 9.37          | 8.99             | 9.54             | 9.26             | 0.190 | 0.243    |
| 95-day-old                    | 14.05<sup>ab</sup> | 12.37<sup>b</sup> | 14.61<sup>a</sup> | 13.32<sup>ab</sup> | 0.528 | 0.032    |
| 125-day-old                   | 22.67         | 26.19            | 25.36            | 27.19            | 1.289 | 0.097    |
| Average daily gain (kg/d)     |               |                  |                  |                  |       |          |
| 35–65 day-old                 | 0.15          | 0.14             | 0.15             | 0.16             | 0.005 | 0.079    |
| 66–95 day-old                 | 0.18          | 0.15             | 0.18             | 0.16             | 0.012 | 0.284    |
| 96–125 day-old                | 0.27          | 0.27             | 0.25             | 0.26             | 0.027 | 0.902    |
| Average daily feed intake (kg/d) |         |                  |                  |                  |       |          |
| 35–65 day-old                 | 0.41          | 0.41             | 0.44             | 0.41             | 0.012 | 0.330    |
| 66–95 day-old                 | 0.64<sup>b</sup> | 0.55<sup>c</sup> | 0.75<sup>a</sup> | 0.70<sup>ab</sup> | 0.024 | <.0001   |
| 96–125 day-old                | 0.92<sup>b</sup> | 1.32<sup>a</sup> | 0.91<sup>b</sup> | 1.10<sup>b</sup> | 0.065 | <.0001   |
| Feed/Gain ratio               |               |                  |                  |                  |       |          |
| 35–65 day-old                 | 3.02          | 2.86             | 2.68             | 2.78             | 0.102 | 0.136    |
| 66–95 day-old                 | 3.56          | 3.82             | 3.75             | 4.25             | 0.220 | 0.173    |
| 96–125 day-old                | 3.32<sup>b</sup> | 4.17<sup>a</sup> | 4.00<sup>ab</sup> | 3.90<sup>ab</sup> | 0.211 | 0.040    |

Data are presented as means with pooled SEM. Values in the same row with different superscript letters were significantly different (P < 0.05). The replicates per group during 35–65 and 66–95 day-old were 8; during 96–125 day-old, the replicates of the control group, antibiotic group, probiotics group, and synbiotics group were 8, 6, 8, and 6, respectively.
Table 2

Effects of probiotics or synbiotics addition in sows’ diets on carcass traits of offspring pigs.

| Items                        | Control group | Antibiotic group | Probiotics group | Synbiotics group | SEM  | P values |
|------------------------------|---------------|------------------|------------------|------------------|------|----------|
| Carcass weight (kg)          |               |                  |                  |                  |      |          |
| 65-day-old                   | 5.00<sup>a</sup> | 4.71<sup>b</sup> | 5.27<sup>a</sup> | 4.82<sup>b</sup> | 0.108| 0.006    |
| 95-day-old                   | 8.25<sup>a</sup> | 6.36<sup>b</sup> | 7.73<sup>ab</sup>| 7.32<sup>ab</sup>| 0.445| 0.037    |
| 125-day-old                  | 13.83         | 16.40            | 14.60            | 16.25            | 0.817| 0.091    |
| Muscle percentage (%)        |               |                  |                  |                  |      |          |
| 65-day-old                   | 21.56         | 21.09            | 22.49            | 21.17            | 0.491| 0.191    |
| 95-day-old                   | 21.23         | 20.67            | 22.00            | 21.51            | 0.389| 0.130    |
| 125-day-old                  | 23.87<sup>a</sup> | 23.11<sup>ab</sup>| 23.53<sup>a</sup> | 22.44<sup>b</sup> | 0.236| 0.002    |
| Fat percentage (%)           |               |                  |                  |                  |      |          |
| 65-day-old                   | 12.14         | 12.10            | 13.31            | 11.78            | 0.396| 0.052    |
| 95-day-old                   | 15.24<sup>a</sup> | 12.47<sup>b</sup> | 15.29<sup>a</sup> | 15.01<sup>a</sup> | 0.662| 0.011    |
| 125-day-old                  | 17.73<sup>b</sup> | 21.49<sup>a</sup> | 22.28<sup>a</sup> | 20.89<sup>a</sup> | 0.548| <.0001   |
| Bone percentage (%)          |               |                  |                  |                  |      |          |
| 65-day-old                   | 11.80         | 11.89            | 11.95            | 11.98            | 0.190| 0.915    |
| 95-day-old                   | 10.13         | 10.44            | 10.57            | 9.99             | 0.273| 0.424    |
| 125-day-old                  | 9.15          | 8.55             | 9.60             | 9.25             | 0.261| 0.069    |
| Leaf fat ratio (g/kg)        |               |                  |                  |                  |      |          |
| 65-day-old                   | 6.93          | 6.04             | 7.05             | 6.75             | 0.376| 0.248    |
| 95-day-old                   | 11.41<sup>a</sup> | 8.09<sup>b</sup> | 7.77<sup>b</sup> | 11.17<sup>a</sup> | 0.587| <.0001   |
| 125-day-old                  | 16.77<sup>b</sup> | 17.88<sup>b</sup> | 21.54<sup>a</sup> | 19.33<sup>ab</sup>| 0.925| 0.005    |
| Backfat thickness (mm)       |               |                  |                  |                  |      |          |
| 65-day-old                   | 13.27<sup>b</sup> | 12.78<sup>b</sup>| 14.65<sup>a</sup> | 13.90<sup>ab</sup> | 0.396| 0.014    |
| 95-day-old                   | 18.79         | 16.78            | 18.46            | 16.98            | 0.611| 0.060    |
| 125-day-old                  | 24.75<sup>b</sup> | 28.81<sup>a</sup> | 27.53<sup>a</sup> | 28.15<sup>a</sup> | 0.832| 0.009    |
| Loin-eye muscle area (cm²)   |               |                  |                  |                  |      |          |
| 65-day-old                   | 4.21          | 4.29             | 4.45             | 4.19             | 0.200| 0.788    |
| 95-day-old                   | 4.33<sup>ab</sup> | 4.22<sup>b</sup> | 4.39<sup>ab</sup> | 5.01<sup>a</sup>| 0.200| 0.038    |
| 125-day-old                  | 7.20<sup>a</sup> | 7.36<sup>a</sup> | 7.67<sup>a</sup> | 5.57<sup>b</sup>| 0.282| <.0001   |

Data are presented as means with pooled SEM. Values in the same row with different superscript letters were significantly different (P < 0.05). The replicates per group at 65- and 95-day-old were 8. At 125-day-old, the replicates of the control group, antibiotic group, probiotics group, and synbiotics group are 8, 6, 8, and 6, respectively.
### Table 3
Effects of probiotics or synbiotics addition in sows’ diets on meat quality of offspring pigs.

| Items                      | Control group | Antibiotic group | Probiotics group | Synbiotics group | SEM | P values |
|----------------------------|---------------|------------------|------------------|------------------|-----|----------|
| **Marbling score**         |               |                  |                  |                  |     |          |
| 65-day-old                 | 1.75          | 1.63             | 1.38             | 1.50             | 0.180 | 0.502    |
| 95-day-old                 | 1.88<sup>a</sup> | 1.38<sup>ab</sup> | 1.25<sup>ab</sup> | 1.38<sup>ab</sup> | 0.190 | 0.120    |
| 125-day-old                | 1.50          | 1.67             | 1.25             | 1.67             | 0.193 | 0.376    |
| **Meat color score**       |               |                  |                  |                  |     |          |
| 65-day-old                 | 3.13          | 2.88             | 2.75             | 2.50             | 0.336 | 0.620    |
| 95-day-old                 | 2.50          | 2.38             | 2.38             | 2.63             | 0.184 | 0.740    |
| 125-day-old                | 2.75          | 3.17             | 2.50             | 2.83             | 0.208 | 0.188    |
| **L* value**               |               |                  |                  |                  |     |          |
| 65-day-old                 | 51.01<sup>a</sup> | 50.66<sup>a</sup> | 48.11<sup>b</sup> | 50.75<sup>a</sup> | 0.623 | 0.008    |
| 95-day-old                 | 50.16<sup>ab</sup> | 49.67<sup>ab</sup> | 51.78<sup>a</sup> | 48.50<sup>b</sup> | 0.766 | 0.041    |
| 125-day-old                | 50.94<sup>a</sup> | 49.49<sup>ab</sup> | 52.35<sup>a</sup> | 47.33<sup>b</sup> | 0.993 | 0.011    |
| **a* value**               |               |                  |                  |                  |     |          |
| 65-day-old                 | 19.58         | 19.11            | 19.47            | 18.50            | 0.617 | 0.605    |
| 95-day-old                 | 18.44<sup>b</sup> | 18.01<sup>b</sup> | 17.12<sup>c</sup> | 19.86<sup>a</sup> | 0.226 | <.0001   |
| 125-day-old                | 18.14         | 17.89            | 17.17            | 17.16            | 0.499 | 0.377    |
| **b* value**               |               |                  |                  |                  |     |          |
| 65-day-old                 | 6.87          | 6.82             | 7.50             | 7.05             | 0.192 | 0.071    |
| 95-day-old                 | 6.72          | 6.59             | 6.06             | 6.47             | 0.213 | 0.171    |
| 125-day-old                | 7.07<sup>ab</sup> | 6.50<sup>b</sup> | 7.30<sup>a</sup> | 6.48<sup>b</sup> | 0.187 | 0.008    |
| **pH<sub>4.5 min</sub>**  |               |                  |                  |                  |     |          |
| 65-day-old                 | 6.55          | 6.55             | 6.50             | 6.52             | 0.035 | 0.744    |
| 95-day-old                 | 6.54<sup>b</sup> | 6.67<sup>ab</sup> | 6.74<sup>a</sup> | 6.55<sup>b</sup> | 0.037 | 0.002    |
| 125-day-old                | 6.49          | 6.63             | 6.66             | 6.52             | 0.054 | 0.069    |
| **pH<sub>24h</sub>**       |               |                  |                  |                  |     |          |
| 65-day-old                 | 5.46          | 5.49             | 5.61             | 5.45             | 0.053 | 0.134    |
| 95-day-old                 | 5.46          | 5.44             | 5.46             | 5.49             | 0.013 | 0.088    |
| 125-day-old                | 5.46          | 5.53             | 5.50             | 5.50             | 0.025 | 0.245    |
| **Drip loss (%)**          |               |                  |                  |                  |     |          |
| 65-day-old                 | 6.32<sup>a</sup> | 5.64<sup>a</sup> | 4.41<sup>b</sup> | 2.96<sup>c</sup> | 0.340 | <.0001   |
| 95-day-old                 | 3.42          | 3.35             | 2.98             | 2.78             | 0.313 | 0.437    |
| 125-day-old                | 4.82          | 3.31             | 3.82             | 3.94             | 0.485 | 0.182    |
| **Cooking yield (%)**      |               |                  |                  |                  |     |          |
| 65-day-old                 | 63.01<sup>bc</sup> | 60.94<sup>c</sup> | 67.02<sup>a</sup> | 65.22<sup>ab</sup> | 1.087 | 0.003    |
| 95-day-old                 | 70.92         | 69.26            | 70.99            | 68.53            | 0.806 | 0.098    |
| Age           | Shear force (N) |     |     |     |     |
|---------------|-----------------|-----|-----|-----|-----|
|               |                 | L*  | a*  | b*  |     |
| 125-day-old   | 68.57<sup>b</sup> | 78.41<sup>a</sup> | 68.50<sup>b</sup> | 66.94<sup>b</sup> | 2.331 | 0.012 |
| 65-day-old    | 61.50<sup>b</sup> | 62.96<sup>b</sup> | 58.73<sup>b</sup> | 75.28<sup>a</sup> | 2.311 | <.0001 |
| 95-day-old    | 73.25           | 64.61 | 65.74 | 70.82 | 2.936 | 0.143 |
| 125-day-old   | 91.94<sup>b</sup> | 101.74<sup>a</sup> | 77.14<sup>c</sup> | 84.41<sup>c</sup> | 2.516 | <.0001 |

Data are presented as means with pooled SEM. Values in the same row with different superscript letters were significantly different ($P < 0.05$). The replicates per group at 65- and 95-day-old were 8. At 125-day-old, the replicates of the control group, antibiotic group, probiotics group, and synbiotics group are 8, 6, 8, and 6, respectively. L*, lightness; a*, redness; b*, yellowness.
Table 4
Effects of probiotics or synbiotics addition in sows’ diets on amino acids content in the *longissimus thoracis* muscle of offspring pigs (g/100 g fresh muscle).

| Item | Control group | Antibiotic group | Probiotics group | Synbiotics group | SEM | *P* values |
|------|---------------|------------------|------------------|------------------|-----|------------|
| CP   | 65-day-old    | 23.39<sup>ab</sup> | 23.80<sup>ab</sup> | 24.50<sup>a</sup> | 23.17<sup>b</sup> | 0.318 | 0.032 |
|      | 95-day-old    | 22.12            | 22.65            | 22.83            | 22.26 | 0.316 | 0.365 |
|      | 125-day-old   | 22.21<sup>ab</sup> | 22.79<sup>a</sup> | 22.06<sup>ab</sup> | 21.44<sup>b</sup> | 0.252 | 0.016 |
| Ala  | 65-day-old    | 1.20             | 1.20             | 1.18             | 1.20  | 0.031 | 0.975 |
|      | 95-day-old    | 1.06             | 1.14             | 1.12             | 1.07  | 0.031 | 0.237 |
|      | 125-day-old   | 1.17             | 1.23             | 1.27             | 1.21  | 0.029 | 0.126 |
| Asp  | 65-day-old    | 1.80<sup>a</sup>  | 1.80<sup>a</sup>  | 1.63<sup>b</sup>  | 1.70<sup>ab</sup> | 0.038 | 0.009 |
|      | 95-day-old    | 1.51<sup>b</sup>  | 1.55<sup>ab</sup> | 1.68<sup>a</sup>  | 1.69<sup>a</sup> | 0.044 | 0.014 |
|      | 125-day-old   | 1.76<sup>b</sup>  | 1.91<sup>a</sup>  | 1.94<sup>a</sup>  | 1.77<sup>b</sup> | 0.041 | 0.004 |
| Glu  | 65-day-old    | 2.63             | 2.63             | 2.53             | 2.54  | 0.070 | 0.632 |
|      | 95-day-old    | 2.63             | 2.68             | 2.82             | 2.81  | 0.066 | 0.144 |
|      | 125-day-old   | 2.84             | 2.96             | 3.03             | 3.00  | 0.070 | 0.215 |
| Gly  | 65-day-old    | 1.09<sup>a</sup>  | 1.08<sup>a</sup>  | 0.96<sup>b</sup>  | 1.01<sup>ab</sup> | 0.034 | 0.047 |
|      | 95-day-old    | 0.95             | 1.01             | 1.05             | 1.06  | 0.035 | 0.118 |
|      | 125-day-old   | 0.98             | 0.96             | 1.01             | 0.96  | 0.040 | 0.817 |
| Pro  | 65-day-old    | 0.94             | 0.96             | 1.07             | 0.96  | 0.039 | 0.094 |
|      | 95-day-old    | 1.17<sup>ab</sup> | 1.33<sup>a</sup>  | 1.14<sup>ab</sup> | 0.98<sup>b</sup> | 0.061 | 0.006 |
|      | 125-day-old   | 1.04             | 0.90             | 0.97             | 1.07  | 0.050 | 0.136 |
| Ser  | 65-day-old    | 0.69             | 0.71             | 0.67             | 0.68  | 0.020 | 0.518 |
|      | 95-day-old    | 0.71             | 0.74             | 0.76             | 0.75  | 0.017 | 0.172 |
|      | 125-day-old   | 0.75<sup>b</sup>  | 0.75<sup>b</sup>  | 0.78<sup>b</sup>  | 0.84<sup>a</sup> | 0.019 | 0.012 |
| Tyr  | 65-day-old    | 0.71             | 0.67             | 0.70             | 0.70  | 0.016 | 0.341 |
|      | 95-day-old    | 0.63             | 0.70             | 0.71             | 0.70  | 0.038 | 0.428 |
|      | 125-day-old   | 0.75             | 0.70             | 0.75             | 0.80  | 0.026 | 0.176 |
| Arg  | 65-day-old    | 1.34             | 1.31             | 1.27             | 1.31  | 0.026 | 0.378 |
|      | 95-day-old    | 1.21<sup>b</sup>  | 1.30<sup>a</sup>  | 1.35<sup>a</sup>  | 1.34<sup>a</sup> | 0.032 | 0.017 |
|                | 125-day-old | 125-day-old | 125-day-old | 125-day-old | 125-day-old | 125-day-old | 125-day-old | 125-day-old | 125-day-old | 125-day-old | 125-day-old |
|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                |             | 1.34        | 1.37        | 1.43        | 1.38        | 0.031       | 0.256       |             |             |             |             |
| His            |             | 0.80b       | 0.75b       | 0.91a       | 0.79a       | 0.021       | <.0001      |             |             |             |             |
| 65-day-old     | 0.94a       | 0.90ab      | 0.82b       | 0.85ab      | 0.027       | 0.022       |             |             |             |             |
| 95-day-old     | 0.89b       | 0.95b       | 0.94b       | 1.08a       | 0.027       | <.0001      |             |             |             |             |
| Ile            |             | 0.90        | 0.90        | 0.83        | 0.87        | 0.021       | 0.056       |             |             |             |             |
| 65-day-old     | 0.82b       | 0.83b       | 0.91a       | 0.91a       | 0.021       | 0.002       |             |             |             |             |
| 95-day-old     | 0.99        | 1.04        | 1.06        | 0.98        | 0.024       | 0.075       |             |             |             |             |
| 125-day-old    |             |             |             |             |             |             |             |             |             |             |             |
| Leu            |             | 1.74        | 1.71        | 1.61        | 1.68        | 0.034       | 0.079       |             |             |             |             |
| 65-day-old     | 1.43        | 1.42        | 1.50        | 1.51        | 0.037       | 0.252       |             |             |             |             |
| 95-day-old     | 1.67b       | 1.80a       | 1.82a       | 1.71ab      | 0.040       | 0.033       |             |             |             |             |
| 125-day-old    |             |             |             |             |             |             |             |             |             |             |             |
| Lys            |             | 1.77        | 1.77        | 1.64        | 1.73        | 0.040       | 0.105       |             |             |             |             |
| 65-day-old     | 1.59b       | 1.58b       | 1.72ab      | 1.78a       | 0.047       | 0.014       |             |             |             |             |
| 95-day-old     | 1.89        | 1.96        | 2.02        | 1.91        | 0.045       | 0.194       |             |             |             |             |
| 125-day-old    |             |             |             |             |             |             |             |             |             |             |             |
| Met            |             | 0.53        | 0.54        | 0.50        | 0.49        | 0.016       | 0.278       |             |             |             |             |
| 65-day-old     | 0.54        | 0.52        | 0.54        | 0.53        | 0.013       | 0.612       |             |             |             |             |
| 95-day-old     | 0.62        | 0.65        | 0.66        | 0.66        | 0.019       | 0.362       |             |             |             |             |
| 125-day-old    |             |             |             |             |             |             |             |             |             |             |             |
| Phe            |             | 0.87        | 0.84        | 0.86        | 0.87        | 0.021       | 0.789       |             |             |             |             |
| 65-day-old     | 0.86        | 0.81        | 0.84        | 0.87        | 0.020       | 0.193       |             |             |             |             |
| 95-day-old     | 0.90b       | 0.91ab      | 0.96ab      | 1.00a       | 0.025       | 0.039       |             |             |             |             |
| 125-day-old    |             |             |             |             |             |             |             |             |             |             |             |
| Thr            |             | 0.93        | 0.93        | 0.87        | 0.90        | 0.018       | 0.051       |             |             |             |             |
| 65-day-old     | 0.84        | 0.88        | 0.92        | 0.89        | 0.024       | 0.120       |             |             |             |             |
| 95-day-old     | 0.96        | 0.97        | 1.00        | 0.99        | 0.024       | 0.493       |             |             |             |             |
| 125-day-old    |             |             |             |             |             |             |             |             |             |             |             |
| Val            |             | 0.97        | 0.97        | 0.90        | 0.95        | 0.023       | 0.107       |             |             |             |             |
| 65-day-old     | 0.96        | 0.93        | 0.98        | 1.00        | 0.023       | 0.130       |             |             |             |             |
| 95-day-old     | 1.08        | 1.11        | 1.14        | 1.12        | 0.027       | 0.447       |             |             |             |             |
| 125-day-old    |             |             |             |             |             |             |             |             |             |             |             |
| EAA            |             | 9.83        | 9.72        | 9.49        | 9.59        | 0.195       | 0.637       |             |             |             |             |
| 65-day-old     | 9.17        | 9.18        | 9.58        | 9.68        | 0.216       | 0.233       |             |             |             |             |
| 95-day-old     | 10.35       | 10.76       | 11.03       | 10.84       | 0.234       | 0.190       |             |             |             |             |
| NEAA           |             | 9.08        | 9.06        | 8.76        | 8.80        | 0.214       | 0.610       |             |             |             |             |
| 65-day-old     | 8.56        | 9.14        | 9.28        | 9.06        | 0.229       | 0.159       |             |             |             |             |
| 95-day-old     |             |             |             |             |             |             |             |             |             |             |             |
|         | Mean 1 | Mean 2 | Mean 3 | Mean 4 | Mean 5 | Mean 6 | Mean 7 | Mean 8 | Mean 9 | Mean 10 | Mean 11 |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| FAA     | 9.29   | 9.42   | 9.73   | 9.64   | 0.226  | 0.469  |
| 125-day-old | 8.06   | 8.03   | 7.57   | 7.76   | 0.181  | 0.211  |
| 65-day-old   | 7.39   | 7.68   | 8.01   | 7.97   | 0.180  | 0.075  |
| 95-day-old   | 8.09   | 8.43   | 8.66   | 8.31   | 0.188  | 0.176  |
| TAA      | 18.90  | 18.78  | 18.27  | 18.39  | 0.402  | 0.643  |
| 65-day-old   | 17.25^b| 18.32^ab| 18.85^a| 18.74^a| 0.403  | 0.035  |
| 95-day-old   | 19.64  | 20.18  | 20.76  | 20.4   | 0.451  | 0.303  |
| 125-day-old   | 19.64  | 20.18  | 20.76  | 20.4   | 0.451  | 0.303  |

Data are presented as means with pooled SEM. Values in the same row with different superscript letters were significantly different (P < 0.05). The replicates per group at 65- and 95-day-old were 8. At 125-day-old, the replicates of the control group, antibiotic group, probiotics group, and synbiotics group are 8, 6, 8, and 6, respectively.

CP, crude protein; EAA, essential amino acids; NEAA, non-essential amino acids; FAA, flavor amino acids; TAA, total amino acids. The EAA included Arg, arginine; His, histidine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; and Val, valine. The NEAA included Ala, alanine; Asp, aspartate; Glu, glutamate; Gly, glycine; Pro, proline; Ser, serine; and Tyr, tyrosine; The FAA, included Asp, Glu, Gly, Ala, and Arg.
Table 5

Effects of probiotics or synbiotics addition in sows’ diets on medium- and long-chain fatty acids content in *longissimus thoracis* muscle of offspring pigs (%).

| Item                | Control group | Antibiotic group | Probiotics group | Synbiotics group | SEM   | P values |
|---------------------|---------------|------------------|------------------|------------------|-------|----------|
| **Intramuscular fat** |               |                  |                  |                  |       |          |
| 65-day-old          | 2.72<sup>ab</sup> | 3.13<sup>a</sup> | 2.32<sup>b</sup> | 3.24<sup>a</sup> | 0.231 | 0.035    |
| 95-day-old          | 2.62<sup>ab</sup> | 2.44<sup>ab</sup> | 3.21<sup>a</sup> | 1.52<sup>b</sup> | 0.325 | 0.009    |
| 125-day-old         | 2.15<sup>b</sup> | 2.07<sup>b</sup> | 1.79<sup>b</sup> | 2.80<sup>a</sup> | 0.197 | 0.012    |
| 65-day-old          | 0.024<sup>b</sup> | 0.023<sup>b</sup> | 0.021<sup>b</sup> | 0.028<sup>a</sup> | 0.001 | 0.004    |
| 95-day-old          | 0.022<sup>b</sup> | 0.024<sup>ab</sup> | 0.027<sup>a</sup> | 0.026<sup>a</sup> | 0.001 | 0.028    |
| 125-day-old         | 0.024          | 0.023           | 0.026           | 0.029           | 0.002 | 0.145    |
| **C10:0**           |               |                  |                  |                  |       |          |
| 65-day-old          | 0.036<sup>b</sup> | 0.039<sup>ab</sup> | 0.038<sup>ab</sup> | 0.042<sup>a</sup> | 0.001 | 0.039    |
| 95-day-old          | 0.041<sup>a</sup> | 0.030<sup>b</sup> | 0.035<sup>b</sup> | 0.031<sup>b</sup> | 0.002 | <.0001   |
| 125-day-old         | 0.028<sup>a</sup> | 0.023<sup>b</sup> | 0.028<sup>a</sup> | 0.029           | 0.001 | 0.024    |
| **C12:0**           |               |                  |                  |                  |       |          |
| 65-day-old          | 0.447<sup>bc</sup> | 0.486<sup>a</sup> | 0.423<sup>c</sup> | 0.524<sup>a</sup> | 0.014 | <.0001   |
| 95-day-old          | 0.487<sup>b</sup> | 0.514<sup>ab</sup> | 0.559<sup>a</sup> | 0.536<sup>ab</sup> | 0.017 | 0.040    |
| 125-day-old         | 0.497          | 0.505           | 0.474           | 0.509           | 0.027 | 0.772    |
| **C14:0**           |               |                  |                  |                  |       |          |
| 65-day-old          | 0.024          | 0.029           | 0.026           | 0.027           | 0.001 | 0.152    |
| 95-day-old          | 0.022          | 0.024           | 0.023           | 0.026           | 0.001 | 0.156    |
| 125-day-old         | 0.022<sup>a</sup> | 0.019<sup>ab</sup> | 0.017<sup>b</sup> | 0.016<sup>b</sup> | 0.001 | 0.001    |
| **C16:0**           |               |                  |                  |                  |       |          |
| 65-day-old          | 8.137<sup>a</sup> | 8.176<sup>ab</sup> | 7.604<sup>b</sup> | 8.355<sup>a</sup> | 0.162 | 0.017    |
| 95-day-old          | 8.334          | 8.209           | 8.643           | 8.301           | 0.141 | 0.172    |
| 125-day-old         | 8.231          | 8.290           | 7.796           | 8.089           | 0.216 | 0.356    |
| **C16:1**           |               |                  |                  |                  |       |          |
| 65-day-old          | 1.177          | 1.169           | 1.091           | 1.254           | 0.055 | 0.253    |
| 95-day-old          | 0.728<sup>c</sup> | 0.906<sup>ab</sup> | 0.976<sup>a</sup> | 0.884<sup>b</sup> | 0.025 | <.0001   |
| 125-day-old         | 0.785<sup>b</sup> | 0.755<sup>b</sup> | 0.868<sup>a</sup> | 0.757<sup>b</sup> | 0.026 | 0.013    |
| **C17:0**           |               |                  |                  |                  |       |          |
| 65-day-old          | 0.103          | 0.116           | 0.100           | 0.110           | 0.005 | 0.183    |
| 95-day-old          | 0.091          | 0.094           | 0.094           | 0.100           | 0.004 | 0.514    |
| 125-day-old         | 0.082<sup>a</sup> | 0.078<sup>ab</sup> | 0.061<sup>b</sup> | 0.070<sup>ab</sup> | 0.006 | 0.045    |
| **C18:0**           |               |                  |                  |                  |       |          |
| 65-day-old          | 4.364          | 4.207           | 4.062           | 4.244           | 0.085 | 0.118    |
| 95-day-old          | 5.141          | 4.824           | 5.001           | 4.759           | 0.103 | 0.056    |
| 125-day-old         | 4.670          | 4.440           | 4.687           | 4.621           | 0.102 | 0.345    |
| Lipid          | Age            | Value 1     | Value 2     | Value 3     | Value 4     | Value 5     | Value 6     |
|---------------|----------------|------------|------------|------------|------------|------------|------------|
| C18:1n9c      | 65-day-old     | 8.753      | 8.813      | 8.023      | 8.805      | 0.198      | 0.021      |
|               | 95-day-old     | 8.820ab    | 8.592b     | 9.663a     | 8.894ab    | 0.256      | 0.035      |
|               | 125-day-old    | 9.722      | 9.520      | 9.567      | 10.171     | 0.358      | 0.610      |
| C18:1n9t      | 65-day-old     | 0.036      | 0.036      | 0.035      | 0.035      | 0.001      | 0.500      |
|               | 95-day-old     | 0.034      | 0.032      | 0.035      | 0.032      | 0.002      | 0.538      |
|               | 125-day-old    | 0.042a     | 0.035b     | 0.035b     | 0.036b     | 0.002      | 0.019      |
| C18:2n6c      | 65-day-old     | 4.135b     | 4.499a     | 4.626a     | 4.008b     | 0.107      | 0.001      |
|               | 95-day-old     | 3.179      | 3.503      | 3.242      | 3.165      | 0.104      | 0.101      |
|               | 125-day-old    | 2.603a     | 2.306ab    | 2.405ab    | 2.150b     | 0.105      | 0.037      |
| C20:0         | 65-day-old     | 0.063a     | 0.064a     | 0.051b     | 0.068a     | 0.003      | 0.001      |
|               | 95-day-old     | 0.070      | 0.074      | 0.075      | 0.068      | 0.003      | 0.190      |
|               | 125-day-old    | 0.063      | 0.057      | 0.061      | 0.064      | 0.003      | 0.387      |
| C20:1         | 65-day-old     | 0.204b     | 0.223ab    | 0.181c     | 0.241a     | 0.008      | <.0001     |
|               | 95-day-old     | 0.303a     | 0.223c     | 0.296a     | 0.263b     | 0.010      | <.0001     |
|               | 125-day-old    | 0.097      | 0.088      | 0.090      | 0.085      | 0.003      | 0.139      |
| C20:2         | 65-day-old     | 0.163      | 0.155      | 0.143      | 0.156      | 0.005      | 0.059      |
|               | 95-day-old     | 0.153      | 0.138      | 0.148      | 0.138      | 0.006      | 0.267      |
|               | 125-day-old    | 0.105      | 0.091      | 0.091      | 0.088      | 0.005      | 0.097      |
| C20:3n6       | 65-day-old     | 0.101      | 0.108      | 0.114      | 0.116      | 0.005      | 0.241      |
|               | 95-day-old     | 0.080      | 0.090      | 0.073      | 0.087      | 0.006      | 0.146      |
|               | 125-day-old    | 0.072      | 0.073      | 0.078      | 0.068      | 0.006      | 0.700      |
| C20:4n6       | 65-day-old     | 0.858      | 0.841      | 0.936      | 0.790      | 0.069      | 0.522      |
|               | 95-day-old     | 0.512b     | 0.762a     | 0.499b     | 0.614b     | 0.049      | 0.002      |
|               | 125-day-old    | 0.521      | 0.562      | 0.588      | 0.448      | 0.051      | 0.274      |
| C24:0         | 65-day-old     | 0.052b     | 0.056b     | 0.074a     | 0.054b     | 0.004      | 0.002      |
|               | 95-day-old     | 0.051a     | 0.055a     | 0.036b     | 0.046ab    | 0.004      | 0.010      |
|               | 125-day-old    | 0.048      | 0.054      | 0.057      | 0.057      | 0.004      | 0.401      |
| SFA           | 65-day-old     | 13.267a    | 13.245a    | 12.506b    | 13.271a    | 0.219      | 0.049      |
|               | 95-day-old     | 14.218     | 13.855     | 14.391     | 13.757     | 0.234      | 0.204      |
|               | 125-day-old    | 13.712     | 12.881     | 13.271     | 13.111     | 0.393      | 0.496      |
| UFA           | 65-day-old     | 15.777     | 15.921     | 15.444     | 15.608     | 0.226      | 0.482      |
|                  | 95-day-old  | 125-day-old | MUFA       | 65-day-old  | 95-day-old  | 125-day-old | PUFA       | 65-day-old  | 95-day-old  | 125-day-old | PUFA/SFA  |
|------------------|-------------|-------------|------------|-------------|-------------|-------------|------------|-------------|-------------|-------------|------------|
| Data are presented as means with pooled SEM. Values in the same row with different superscript letters were significantly different (*P* < 0.05) The replicates per group at 65- and 95-day-old were 8. At 125-day-old, the replicates of the control group, antibiotic group, probiotics group, and synbiotics group are 8, 6, 8, and 6, respectively. SFA, saturated fatty acid; UFA, Unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids. SFA, including C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, and C24:0. UFA, including C16:1, C18:1n9c, C18:1n9t, C18:2n6c, C18:3n3, C20:1, C20:2, C20:3n6, and C20:4n6. MUFA, including C16:1, C18:1n9c, C18:1n9t, and C20:1. PUFA, including C18:2n6c, C18:3n3, C20:2, C20:3n6, and C20:4n6. |
### Table 6
Effects of probiotics or synbiotics addition in sows’ diets on plasma biochemical parameters of offspring pigs

| Item       | Control group | Antibiotic group | Probiotics group | Synbiotics group | SEM     | P values |
|------------|---------------|------------------|------------------|------------------|---------|----------|
| ALT (U/L)  |               |                  |                  |                  |         |          |
| 65-day-old | 58.79         | 56.28            | 56.81            | 59.15            | 2.369   | 0.782    |
| 95-day-old | 54.74         | 54.22            | 59.60            | 78.33            | 4.811   | 0.004    |
| 125-day-old| 51.77         | 48.18            | 50.76            | 48.10            | 3.847   | 0.873    |
| ALP (U/L)  |               |                  |                  |                  |         |          |
| 65-day-old | 148.88        | 164.00           | 134.57           | 155.50           | 8.351   | 0.108    |
| 95-day-old | 123.63        | 127.17           | 118.50           | 122.83           | 10.524  | 0.951    |
| 125-day-old| 162.88        | 197.50           | 142.83           | 123.00           | 13.740  | 0.010    |
| α-AMS (U/L)|               |                  |                  |                  |         |          |
| 65-day-old | 2260.50       | 1851.40          | 1842.17          | 1852.33          | 76.887  | 0.001    |
| 95-day-old | 2186.71       | 2074.40          | 2176.80          | 2271.60          | 80.523  | 0.405    |
| 125-day-old| 2306.00       | 2783.00          | 2438.25          | 2806.00          | 121.075 | 0.016    |
| AST (U/L)  |               |                  |                  |                  |         |          |
| 65-day-old | 59.75         | 56.40            | 73.71            | 53.83            | 6.181   | 0.129    |
| 95-day-old | 73.30         | 53.80            | 47.33            | 63.75            | 3.606   | <.0001   |
| 125-day-old| 65.17         | 47.20            | 49.50            | 67.50            | 5.292   | 0.023    |
| CHE (g/L)  |               |                  |                  |                  |         |          |
| 65-day-old | 534.63        | 540.33           | 420.29           | 548.33           | 16.605  | <.0001   |
| 95-day-old | 516.25        | 542.83           | 427.60           | 457.71           | 15.986  | <.0001   |
| 125-day-old| 467.67        | 475.25           | 445.50           | 487.25           | 13.724  | 0.203    |
| LDH (U/L)  |               |                  |                  |                  |         |          |
| 65-day-old | 416.75        | 332.20           | 503.67           | 378.17           | 18.040  | <.0001   |
| 95-day-old | 465.71        | 374.43           | 322.67           | 437.67           | 19.825  | <.0001   |
| 125-day-old| 475.57        | 383.25           | 383.29           | 411.25           | 26.291  | 0.042    |
| ALB (g/L)  |               |                  |                  |                  |         |          |
| 65-day-old | 41.23         | 35.54            | 41.21            | 40.50            | 0.643   | <.0001   |
| 95-day-old | 40.45         | 39.46            | 40.58            | 40.48            | 0.657   | 0.596    |
| 125-day-old| 43.86         | 44.53            | 45.18            | 43.93            | 0.915   | 0.677    |
| AMM (μmol/L)|              |                  |                  |                  |         |          |
| 65-day-old | 167.93        | 159.24           | 203.50           | 189.87           | 14.293  | 0.137    |
| 95-day-old | 265.37        | 236.36           | 171.86           | 229.62           | 17.090  | 0.005    |
| 125-day-old| 332.90        | 128.30           | 198.58           | 269.55           | 21.299  | <.0001   |
| TP (g/L)   |               |                  |                  |                  |         |          |
| 65-day-old | 65.00         | 66.62            | 65.13            | 67.52            | 0.765   | 0.078    |
| 95-day-old | 73.68         | 73.68            | 74.63            | 70.82            | 0.939   | 0.043    |
### Data Table

|                | UN (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) | TC (mmol/L) | TG (mmol/L) | GLU (mmol/L) |
|----------------|-------------|----------------|----------------|-------------|-------------|--------------|
|                | 125-day-old | 65-day-old     | 95-day-old     | 125-day-old | 65-day-old  | 95-day-old   | 125-day-old |
|                |             | 72.76<sup>a</sup> | 3.10<sup>b</sup> | 3.87<sup>b</sup> | 5.30<sup>a</sup> | 5.04<sup>a</sup> | 4.48<sup>b</sup> |
|                | 68.90<sup>b</sup> | 3.20<sup>b</sup> | 3.18<sup>b</sup> | 4.66<sup>b</sup> | 5.12<sup>a</sup> | 4.66<sup>b</sup> | 4.92<sup>b</sup> |
|                | 73.78<sup>a</sup> | 2.87<sup>b</sup> | 2.42<sup>c</sup> | 3.05<sup>a</sup> | 4.66<sup>b</sup> | 3.50<sup>b</sup> | 4.66<sup>b</sup> |
|                | 73.10<sup>a</sup> | 0.833<sup>b</sup> | 3.14<sup>b</sup> | 2.78<sup>a</sup> | 0.176<sup>b</sup> | 4.66<sup>b</sup> | 4.80<sup>b</sup> |
|                | 0.002<sup>b</sup> | 0.901<sup>b</sup> | 0.127<sup>b</sup> | 0.034<sup>b</sup> | 0.176<sup>b</sup> | 0.246<sup>b</sup> | 0.161<sup>b</sup> |

Data are presented as means with pooled SEM. Values in the same row with different superscript letters were significantly different (P < 0.05). The replicates per group at 65- and 95-day-old were 8. At 125-day-old, the replicates of the control group, antibiotic group, probiotics group, and synbiotics group are 8, 6, 8, and 6, respectively.

ALT, alanine aminotransferase; ALP, alkaline phosphatase; α-AMS, α-amylase; AST, aspartate aminotransferase; CHE, cholinesterase; LDH, lactate dehydrogenase; ALB, albumin; AMM, ammonia; TP, total protein; UN, urea nitrogen; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride; GLU, glucose.
### Table 7
Effects of probiotics or synbiotics addition in sows’ diets on plasma free amino acid of offspring pigs (nmol/mL)

| Item | Control group | Antibiotic group | Probiotics group | Synbiotics group | SEM | P values |
|------|---------------|-----------------|-----------------|-----------------|-----|----------|
| 1-Mehis |              |                 |                 |                 |     |          |
| 65-day-old | 0.85 | 0.95 | 0.50 | 0.90 | 0.147 | 0.157 |
| 95-day-old | 6.13<sup>b</sup> | 8.96<sup>a</sup> | 5.06<sup>b</sup> | 6.70<sup>b</sup> | 0.626 | 0.001 |
| 125-day-old | 0.93 | 0.89 | 0.44 | 1.16 | 0.201 | 0.095 |
| 3-Mehis |              |                 |                 |                 |     |          |
| 65-day-old | 12.38 | 11.64 | 11.69 | 12.13 | 0.766 | 0.885 |
| 95-day-old | 14.09 | 13.74 | 12.71 | 15.27 | 0.730 | 0.123 |
| 125-day-old | 13.92 | 15.41 | 17.17 | 16.37 | 1.082 | 0.146 |
| Ala |              |                 |                 |                 |     |          |
| 65-day-old | 449.55<sup>a</sup> | 445.57<sup>a</sup> | 323.57<sup>b</sup> | 391.40<sup>ab</sup> | 24.723 | 0.011 |
| 95-day-old | 358.50<sup>a</sup> | 252.02<sup>b</sup> | 296.22<sup>ab</sup> | 347.86<sup>a</sup> | 18.048 | 0.001 |
| 125-day-old | 312.99<sup>b</sup> | 393.20<sup>a</sup> | 301.66<sup>b</sup> | 284.56<sup>b</sup> | 20.815 | 0.009 |
| Ans |              |                 |                 |                 |     |          |
| 65-day-old | 1.05 | 1.04 | 0.98 | 0.88 | 0.105 | 0.682 |
| 95-day-old | 0.48<sup>a</sup> | 0.46<sup>a</sup> | 0.51<sup>a</sup> | 0.36<sup>b</sup> | 0.028 | 0.005 |
| 125-day-old | 22.53<sup>c</sup> | 55.86<sup>a</sup> | 35.01<sup>b</sup> | 21.56<sup>c</sup> | 2.124 | <.0001 |
| Arg |              |                 |                 |                 |     |          |
| 65-day-old | 70.36 | 79.78 | 83.70 | 88.64 | 4.783 | 0.070 |
| 95-day-old | 89.22<sup>ab</sup> | 67.25<sup>b</sup> | 69.73<sup>b</sup> | 102.12<sup>a</sup> | 6.953 | 0.004 |
| 125-day-old | 84.84<sup>b</sup> | 105.52<sup>a</sup> | 85.15<sup>b</sup> | 88.29<sup>b</sup> | 3.540 | 0.001 |
| Asp |              |                 |                 |                 |     |          |
| 65-day-old | 7.88 | 9.41 | 8.84 | 7.39 | 1.256 | 0.665 |
| 95-day-old | 9.86 | 10.99 | 11.55 | 9.53 | 0.748 | 0.211 |
| 125-day-old | 10.85<sup>a</sup> | 12.44<sup>a</sup> | 6.99<sup>b</sup> | 10.34<sup>a</sup> | 0.679 | <.0001 |
| Car |              |                 |                 |                 |     |          |
| 65-day-old | 10.51 | 10.61 | 10.72 | 7.03 | 1.152 | 0.087 |
| 95-day-old | 10.93<sup>a</sup> | 8.97<sup>a</sup> | 3.30<sup>b</sup> | 8.24<sup>a</sup> | 0.985 | <.0001 |
| 125-day-old | 18.25 | 17.06 | 13.97 | 15.10 | 1.300 | 0.083 |
| Cit |              |                 |                 |                 |     |          |
| 65-day-old | 25.65<sup>c</sup> | 30.35<sup>b</sup> | 23.54<sup>c</sup> | 34.81<sup>a</sup> | 1.272 | <.0001 |
| 95-day-old | 40.77<sup>a</sup> | 31.80<sup>b</sup> | 30.24<sup>b</sup> | 34.47<sup>b</sup> | 1.829 | 0.002 |
| 125-day-old | 38.50 | 34.22 | 40.66 | 34.89 | 2.474 | 0.229 |
| Cysthi |              |                 |                 |                 |     |          |
| 65-day-old | 13.04<sup>b</sup> | 7.55<sup>c</sup> | 13.52<sup>b</sup> | 18.79<sup>a</sup> | 0.927 | <.0001 |
| 95-day-old | 6.05<sup>b</sup> | 6.69<sup>b</sup> | 7.17<sup>ab</sup> | 8.37<sup>a</sup> | 0.437 | 0.006 |
| Age        | Cys          | EOHNH2       | Glu          | Gly          | His          | Hyllys       | Hypro        | Ile          | Leu          | Lys          |
|------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 125-day-old| 9.97\(^b\)  | 10.88\(^{ab}\) | 13.89\(^a\) | 1.027        | 0.036        |              |              |              |              |              |
| 65-day-old | 9.56         | 8.58         | 12.69        | 1.062        | 0.052        |              |              |              |              |              |
| 95-day-old | 94.43        | 59.16        | 70.89        | 14.719       | 0.264        |              |              |              |              |              |
| 125-day-old| 12.55        | 7.17         | 12.87        | 1.872        | 0.115        |              |              |              |              |              |
| 65-day-old | 0.27         | 0.25         | 0.28         | 0.031        | 0.558        |              |              |              |              |              |
| 95-day-old | 24.52\(^b\) | 0.46\(^c\)   | 0.27\(^c\)   | 2.086        | <.0001       |              |              |              |              |              |
| 125-day-old| 5.09\(^a\)  | 5.90\(^a\)   | 2.95\(^b\)   | 0.522        | 0.006        |              |              |              |              |              |
| 65-day-old | 186.00       | 197.39       | 183.61       | 11.824       | 0.334        |              |              |              |              |              |
| 95-day-old | 207.52       | 213.62       | 215.32       | 11.319       | 0.066        |              |              |              |              |              |
| 125-day-old| 155.75\(^a\)| 159.41\(^a\) | 134.23\(^b\) | 4.571        | <.0001       |              |              |              |              |              |
| 65-day-old | 525.00\(^a\)| 417.84\(^a\) | 517.45\(^a\) | 22.405       | 0.003        |              |              |              |              |              |
| 95-day-old | 470.34       | 484.09       | 560.27       | 29.847       | 0.129        |              |              |              |              |              |
| 125-day-old| 566.31\(^b\)| 417.49\(^b\) | 582.66\(^b\) | 20.194       | <.0001       |              |              |              |              |              |
| 65-day-old | 36.13\(^bc\)| 39.58\(^a\)  | 35.05\(^c\)  | 0.834        | 0.003        |              |              |              |              |              |
| 95-day-old | 43.72\(^a\) | 37.98\(^b\)  | 40.04\(^ab\) | 1.406        | 0.026        |              |              |              |              |              |
| 125-day-old| 44.72\(^a\)| 39.29\(^b\)  | 44.59\(^a\)  | 1.074        | <.0001       |              |              |              |              |              |
| 65-day-old | 1.08\(^b\)  | 1.77\(^a\)   | 1.00\(^b\)   | 0.144        | 0.001        |              |              |              |              |              |
| 95-day-old | 0.55\(^b\)  | 0.31\(^b\)   | 17.46\(^b\)  | 0.545        | <.0001       |              |              |              |              |              |
| 125-day-old| 20.27        | 16.29        | 13.96        | 2.598        | 0.404        |              |              |              |              |              |
| 65-day-old | 80.45\(^a\) | 61.61        | 63.80\(^b\)  | 3.157        | 0.002        |              |              |              |              |              |
| 95-day-old | 37.70        | 31.12        | 40.87        | 3.707        | 0.062        |              |              |              |              |              |
| 125-day-old| 67.51        | 56.72        | 4.385        | 0.098        |              |              |              |              |              |              |
| 65-day-old | 100.01\(^a\)| 85.87\(^b\)  | 106.56\(^a\) | 3.376        | 0.001        |              |              |              |              |              |
| 95-day-old | 90.11\(^a\) | 75.76\(^b\)  | 3.811        | 0.002        |              |              |              |              |              |              |
| 125-day-old| 86.29\(^b\) | 95.59\(^ab\)| 101.40\(^a\) | 3.731        | 0.042        |              |              |              |              |              |
| 65-day-old | 164.89\(^a\)| 141.18\(^b\) | 157.55\(^ab\)| 4.946        | 0.015        |              |              |              |              |              |
| 95-day-old | 140.61\(^a\)| 108.49\(^b\) | 101.71\(^b\) | 6.546        | 0.001        |              |              |              |              |              |
| 125-day-old| 142.49\(^c\)| 145.71\(^c\)| 188.21\(^a\) | 5.000        | <.0001       |              |              |              |              |              |
| 65-day-old | 143.17       | 136.97       | 143.94       | 6.986        | 0.619        |              |              |              |              |              |
| 95-day-old | 125.61       | 107.98       | 137.62       | 9.034        | 0.167        |              |              |              |              |              |
|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
|     | Met |     |     |     |     |     |
| 65-day-old | 15.68 | 15.23 | 14.20 | 14.48 | 0.603 | 0.306 |
| 95-day-old | 12.76 | 10.83 | 10.76 | 11.79 | 0.641 | 0.113 |
| 125-day-old | 12.73<sup>c</sup> | 16.16<sup>a</sup> | 14.10<sup>b</sup> | 12.84<sup>c</sup> | 0.305 <.0001 |
|     | Orn |     |     |     |     |     |
| 65-day-old | 51.91 | 55.78 | 59.34 | 56.87 | 3.443 | 0.500 |
| 95-day-old | 56.21<sup>b</sup> | 40.61<sup>a</sup> | 53.07<sup>b</sup> | 64.72<sup>b</sup> | 4.032 | 0.002 |
| 125-day-old | 68.98<sup>a</sup> | 55.47<sup>b</sup> | 43.97<sup>b</sup> | 56.39<sup>b</sup> | 3.736 <.0001 |
|     | Phe |     |     |     |     |     |
| 65-day-old | 81.15 | 85.83 | 78.38 | 79.57 | 3.381 | 0.436 |
| 95-day-old | 81.64 | 79.95 | 73.05 | 83.18 | 2.810 | 0.077 |
| 125-day-old | 90.72<sup>ab</sup> | 92.96<sup>a</sup> | 85.38<sup>b</sup> | 88.08<sup>ab</sup> | 1.540 | 0.009 |
|     | Pro |     |     |     |     |     |
| 65-day-old | 173.12<sup>b</sup> | 214.35<sup>a</sup> | 163.76<sup>b</sup> | 188.85<sup>b</sup> | 7.593 <.0001 |
| 95-day-old | 157.10 | 143.26 | 154.65 | 148.96 | 8.298 | 0.650 |
| 125-day-old | 183.79<sup>b</sup> | 224.84<sup>a</sup> | 162.65<sup>b</sup> | 160.26<sup>b</sup> | 11.179 | 0.002 |
|     | Sar |     |     |     |     |     |
| 65-day-old | 8.53<sup>a</sup> | 3.72<sup>b</sup> | 4.13<sup>b</sup> | 6.10<sup>ab</sup> | 0.875 | 0.002 |
| 95-day-old | 0.41<sup>c</sup> | 0.66<sup>c</sup> | 6.67<sup>a</sup> | 2.08<sup>b</sup> | 0.381 <.0001 |
| 125-day-old | 2.25 | 2.38 | 2.28 | 2.28 | 0.115 | 0.855 |
|     | Ser |     |     |     |     |     |
| 65-day-old | 75.51 | 87.29 | 82.69 | 80.79 | 3.228 | 0.101 |
| 95-day-old | 81.57 | 75.69 | 78.11 | 75.35 | 2.589 | 0.318 |
| 125-day-old | 81.30<sup>b</sup> | 97.57<sup>a</sup> | 69.65<sup>c</sup> | 73.61<sup>c</sup> | 1.802 <.0001 |
|     | Tau |     |     |     |     |     |
| 65-day-old | 133.56<sup>a</sup> | 108.96<sup>bc</sup> | 100.33<sup>c</sup> | 121.25<sup>ab</sup> | 5.264 | 0.001 |
| 95-day-old | 138.40<sup>a</sup> | 116.37<sup>b</sup> | 116.01<sup>b</sup> | 116.55<sup>b</sup> | 5.698 | 0.022 |
| 125-day-old | 137.88<sup>b</sup> | 153.35<sup>a</sup> | 133.68<sup>b</sup> | 128.73<sup>b</sup> | 5.074 | 0.021 |
|     | Thr |     |     |     |     |     |
| 65-day-old | 116.70<sup>b</sup> | 151.48<sup>a</sup> | 108.88<sup>b</sup> | 117.32<sup>b</sup> | 7.145 | 0.001 |
| 95-day-old | 105.91 | 107.08 | 113.80 | 114.86 | 8.913 | 0.852 |
| 125-day-old | 119.87<sup>b</sup> | 147.49<sup>a</sup> | 111.62<sup>b</sup> | 113.26<sup>b</sup> | 4.004 <.0001 |
|     | Tyr |     |     |     |     |     |
| 65-day-old | 50.03<sup>a</sup> | 47.53<sup>a</sup> | 24.34<sup>b</sup> | 47.37<sup>a</sup> | 4.762 | 0.002 |
| 95-day-old | 44.93 | 53.06 | 48.90 | 52.60 | 2.562 | 0.112 |
| 125-day-old | 62.07<sup>b</sup> | 67.95<sup>a</sup> | 60.31<sup>b</sup> | 60.48<sup>b</sup> | 1.552 | 0.008 |
|     | Val |     |     |     |     |     |
| 65-day-old | 249.16<sup>ab</sup> | 222.48<sup>b</sup> | 220.34<sup>b</sup> | 264.19<sup>a</sup> | 12.257 | 0.047 |
| 95-day-old | 213.22 | 183.85 | 205.35 | 209.81 | 8.517 | 0.089 |
| Age (Days)       | 125-day-old | 65-day-old  | 95-day-old | 125-day-old | Control | Antibiotic | Probiotics | Synbiotics |
|-----------------|-------------|-------------|------------|-------------|---------|------------|------------|------------|
| α-AAA           | 237.37\(^b\) | 283.85\(^a\) | 257.01\(^ab\) | 284.97\(^a\) | 13.022  | 0.044      |            |            |
| 65-day-old      | 283.85\(^a\) | 57.40\(^b\)  | 47.63\(^b\)  | 45.07\(^b\)  | 4.768   | 0.247      |            |            |
| 95-day-old      | 60.05\(^a\)  | 56.64\(^a\)  | 67.93\(^a\)  | 57.68\(^a\)  | 3.673   | 0.049      |            |            |
| 125-day-old     | 55.04\(^ab\) | 67.93\(^a\)  | 75.15\(^a\)  | 75.78\(^a\)  | 4.061   | 0.013      |            |            |
| α-ABA           | 15.47\(^b\)  | 15.68\(^b\)  | 18.36\(^ab\) | 2.277\(^b\)  | 0.042   |            |            |            |
| 65-day-old      | 24.10\(^a\)  | 2.70\(^c\)   | 6.33\(^b\)   | 0.555\(^c\)  | <.0001  |            |            |            |
| 95-day-old      | 4.47\(^b\)   | 6.00\(^a\)   | 7.30\(^a\)   | 0.763\(^a\)  | 0.096   |            |            |            |
| 125-day-old     | 3.45\(^b\)   | 8.60\(^a\)   | 2.70\(^c\)   | 0.373\(^c\)  | 0.001   |            |            |            |
| β-Ala           | 8.88\(^a\)   | 3.91\(^c\)   | 2.70\(^c\)   | 6.33\(^b\)   | 0.555   | <.0001     |            |            |
| 65-day-old      | 6.91\(^b\)   | 6.97\(^b\)   | 7.85\(^ab\)  | 0.273\(^b\)  | <.0001  |            |            |            |
| 95-day-old      | 2.55\(^a\)   | 1.72\(^b\)   | 1.73\(^b\)   | 0.196\(^b\)  | <.0001  |            |            |            |
| 125-day-old     | 1.05\(^a\)   | 0.59\(^b\)   | 0.37\(^c\)   | 0.054\(^c\)  | <.0001  |            |            |            |
| γ-ABA           | 0.25\(^b\)   | 0.16\(^b\)   | 0.59\(^a\)   | 0.041\(^a\)  | <.0001  |            |            |            |
| 65-day-old      | 0.23\(^b\)   | 0.18\(^b\)   | 0.16\(^b\)   | 0.060\(^b\)  | <.0001  |            |            |            |
| 95-day-old      | 0.67\(^a\)   | 0.68\(^a\)   | 0.68\(^a\)   | 0.68\(^a\)   | <.0001  |            |            |            |
| 125-day-old     | 1.81         | 1.89         | 1.66        | 1.64        | 0.115   | 0.374      |            |            |

Data are presented as means with pooled SEM. Values in the same row with different superscript letters were significantly different (\(P < 0.05\)). The replicates per group at 65- and 95-day-old were 8. At 125-day-old, the replicates of the control group, antibiotic group, probiotics group, and synbiotics group are 8, 6, 8, and 6, respectively.

1-Mehis, 1-methyl-histidine; 3-Mehis, 3-methyl-histidine; Ala, alanine; Ans, anserine; Arg, arginine; Asp, aspartate; Car, carnosine; Cit, citrulline; Cysthi, cystathionine; Cys, cysteine; EOHNH2, ethanolamine; Glu, glutamate; Gly, glycine; His, histidine; Hyls, hydroxy-lysine; Hypro, hydroxy-proline; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Sar, sarcosine; Ser, serine; Tau, taurine; Thr, threonine; Tyr, tyrosine; Val, valine; α-AAA, α-amino adipic acid; α-ABA, α-amino-n-butyric acid; β-Ala, β-alanine; β-AIBA, β-aminoisobutyric acid; and γ-ABA, γ-amino-n-butyric acid.
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