Effect of Dietary Amylose/amylopectin Ratio on Antioxidant Ability and Amino Acid Metabolism of Liver in Weaned Pigs Challenged with Lipopolysaccharide

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Research

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Abstract

Background: Antioxidant function could be regulated by resistant starch supplementation in rats with kidney disease. It is controversial whether antioxidant ability could be regulated by changing dietary amylose/amylopectin ratio (DAR) when weaned piglets under lipopolysaccharide (LPS) stress.

Methods: Sixty male weaned piglets (Duroc × Landrace × Yorkshire, 21 day old, 6.51 ± 0.64 kg) were allotted to 5 dietary treatments with 12 cages per treatment, and fed ad libitum with diets different in DAR (0.00, 0.20, 0.40, 0.60 and 0.80). Feed transformation happened at day 15. On day 29, 12 h before slaughter, pigs were intraperitoneal injected with 100 µg/kg body weight LPS or sterile saline.

Results: Results showed that LPS stress caused increase in serum urea nitrogen and glucose concentration ($P < 0.05$). Serum immunoglobulin G concentration increased after LPS stress in diet 0.80 but decreased in other groups ($P < 0.05$). Serum serine concentrations decreased after LPS stress in diets 0.20 and 0.80 but increased in other groups ($P < 0.05$). All tested hepatic amino acids (AA) increased after LPS stress ($P < 0.05$) but hepatic isoleucine, leucine, threonine, aspartate, serine, glutamate, glycine concentrations decreased after LPS stress in diet 0.20 ($P < 0.05$). Piglets supplemented diet with DAR 0.00 had highest superoxide dismutase (SOD1), nuclear factor 2 (Nrf2) and glutathione peroxidase 1 (GPX1) mRNA expression in liver ($P < 0.05$). Total superoxide dismutase (T-SOD) activity of liver increased in diet 0.00 and 0.80 but decreased in diet 0.40 ($P < 0.05$). Total antioxidant capacity (T-AOC) activity of *longissimus dorsi* muscle (LM) increased in diets 0.20 and 0.80 but decreased in diet 0.40 ($P < 0.05$). Piglets in diet 0.20 had the highest hepatic glutathione peroxidase activity compared with groups 0.40, 0.60, 0.80 ($P < 0.05$). Mn concentration of LM and hepatic Cu decreased after LPS stress in diet 0.20 but increased in diet 0.80 ($P < 0.05$). Piglets in diet 0.80 had the highest hepatic Cu, Fe, Mn, Zn concentrations ($P < 0.05$). Cecal indol(e) concentration was higher in diet 0.00 than in diet 0.80 ($P < 0.05$).

Conclusions: Antioxidant ability could be improved by feeding diet with low or high amylose/amylopectin ratio when weaned piglets suffered LPS stress.

Background

Lipopolysaccharide (LPS) elicit a systemic inflammatory process through the release of proinflammatory mediators such as IL-1 β , IL-6 and TNF- α , as well as nitric oxide and reactive oxygen species (ROS) that are associated with oxidative stress [1]. Weaned piglets which have a weak mucosal barrier resistance to pathogens may increase the piglets' susceptibility to the infection of enterotoxigenic *E.coli*, which may even affect the intestinal disfunction [2], lead to oxidative stress in animals [3], then result in hepatic damage (80 µg/kg body weight LPS) [4]. Lipopolysaccharide-induced hepatotoxicity remains a leading cause of death [5]. Liver plays an important physiological role in LPS detoxification, clearing of endotoxin of intestinal derivation and exhibiting metabolic functions such as glycogenolysis and glycogen synthesis, protein metabolism [6].

The rate of starch digestion affects the level of digestion and absorption of nutrients, such as glucose, small peptides and amino acid [7]. Indigestible starch could be the source of nutrients for intestinal bacteria that convert the dietary fiber to short chain fatty acids (SCFA) [8]. SCFA are essential for the maintenance of the immunological self-tolerance and limitation of the inflammatory response [9]. Amelioration of oxidative stress, inflammation, restoration of Nrf2 activity was accompanied with consumption of 59% high amylose maize resistant starch HAMRS2 in Chronic Kidney Disease (CKD) rats [10]. C57BL/6J mice supplemented with 20% HAMRS2 by weight showed 15-58% reduction in all measured liver and plasma amino acids, liver transcriptomics revealed pathways related to cell proliferation, differentiation, and growth were affected by HAMRS2 feeding [11]. The beneficial effect of resistant starch (RS) could be driven by changes elicited at the hepatic which is associated with the oxidation reaction and hepatic inflammatory response in rats [12].

Starch is composed of amylose and amylopectin. Resistant starch content increased as amylose increased [7]. However, there is a dearth of information regarding the effect of dietary amylose/amylopectin ratio (DAR) on antioxidant activities of liver in piglets under LPS-challenge. Thus, this study was conducted to examine the antioxidant function of liver and its amino acid mechanism in weaned piglets under LPS stress.

Methods

Experimental procedure in this study was reviewed and approved by the Animal Care and Use Committee of Hunan Normal University.

Animals and diets

Sixty castrated male pigs (Landrace × Yorkshire) with an initial average body weight (BW) of 6.51 ± 0.64 kg were selected, blocked by BW and allotted to five dietary treatments with 12 cages per treatment and 1 pig per metabolic cage. The experimental diet was formulated on the basis of nutrient requirements established by the NRC [13] for 7–11 kg pigs. The A, B, C, D, E diets differed only in DAR, 0.00, 0.20, 0.40, 0.60 or 0.80 respectively (Supplementary Table 1). Pigs were fed ad libitum. Water was provided ad libitum. On day 15, feed was changed from phase I to phase II. On day 29, 12 h before slaughter, 6 pigs every treatment were challenged with lipopolysaccharides (LPS, from *Escherichia coli* O55:B5, Sigma Chemical Inc., St Louis, MO, USA, L2880) whereas an equivalent amount of sterile saline was administered to other 6 pigs. LPS was dissolved in sterile saline and administered intraperitoneal injection at 100 µg/kg body weight. One pig from A, B, D, E died 11 h after LPS stress.

Sample collection

At day 15 before feed transition and day 28, blood was collected from jugular vein and centrifuged at 3,000×g, 4°C for 10min to recover serum. Piglets were euthanized via an intravenous (jugular vein) injection of 40 mg/kg BW sodium pentobarbital solution. Then, tissues such as the middle right lobe of liver,

dorsal muscles (approximately 2 cm×2 cm) and chyme from cecal and colon were collected using the sterile scissors and tweezers. All of the samples were immediately stored at -80°C for the further study.

Analysis of serum biochemical variables

The collected serum samples were thawed and centrifuged at 3000 rpm for 10 min at 4°C, and collected 200µL supernatant for measuring the concentration of total protein (TP), albumin (ALB), alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), triglyceride (TG), total cholesterol (CHOL), amylase (AMS), hepatic lipase (LIPC), immunoglobulin G and M (IGG, IGM) using commercial kits in accordance with manufacturer instructions (Jiancheng Bioengineering Institute, Nanjing, China) and were identified using a TBA-120FR Automatic Biochemistry Radiometer (Hitachi Co., Tokyo, Japan).

Analysis of AA contents in serum, liver, and muscle

The serum samples were thawed on 4°C. Taking 600 µL of serum and 8% sulfosalicylic acid in 1.5 ML were mixed thoroughly, which stored at 4°C for 12 h. Then the mixed fluids were centrifuged at 10,000 rpm and 4°C for 10 min. The supernatant was filtered through a 0.22 µm membrane and analyzed for AA using an AA analyzer (Hitachi L-8900, Japan). When AA contents in liver and muscle were tested, about 0.1g ground freeze-dried sample was hydrolyzed in 10 mL of 6mol/L HCL at 110°C for 22 h. The solution was then adjusted to a volume of 100 mL and 1 mL of the settled solution was used for further analysis after 10-fold dilution. The diluent solution analyzed using an amino acid analyzer (L-8900, Hitachi, Japan) after filtered by a 0.22-µm membrane.

Assay of hepatic, muscular antioxidant enzyme activities

Hepatic and muscular tissue samples, were grounded in liquid nitrogen and homogenized in saline, and centrifuged at 3000×g, 4°C for 10 min. The activities of malonaldehyde (MDA), superoxide dismutase (SOD) which includes cooper-zinc SOD (CuZn-SOD) and manganese SOD (Mn-SOD), glutathione peroxidase (GSH-PX/GPx), and total antioxidant capacity (T-AOC) were analyzed using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

RNA Extraction and real-time quantitative PCR

Total RNA was isolated from liver using RNAiso Plus (TaKaPa) and then reverse transcription reactions were performed using RT reagent kit (TaKaPa). All the procedures were carried out as described by the manufacturer's protocol. The quantity and quality of RNA were determined using NanoDrop ND-2000 spectrophotometer system (Thermo Fisher Scientific). Real-time RT-PCR primers were designed to assay genes related to antioxidant function (supplemented Table 2). 18S were used as reference gene. Real-time RT-PCR for target genes were performed on MyIQ instrument (Bio-Rad, Hercules, California) using SYBR Green quantitative PCR mix (TaKaRa).

Analysis of mineral element contents in liver and muscle

Macro and micro elements (Ca, S, Mg, Cu, Fe, Mn, Zn) were analyzed using analytical method as described earlier [14]. Briefly, liver and muscle samples (5.00 ±0.20 g) were weighed in triplicate and digested using a mixture acid (25 mL of HNO₃: HClO₄ in volume ratio of 4:1) following heating (80 C for 60 min; 120 C for 30 min; and 180 C for 30 min). Samples were dried at 260 C and redissolved in 5 mL of 1% HNO₃. Solution was then transferred to a 25-mL volumetric flask and filled with 1% HNO₃. Finally, target elements (Cu, Fe, Mn and Zn) in samples were determined on ICP-OES (Varian 720ES Agilent, Santa Clara, CA, USA) for confirmation with standard references.

Analysis of indole and skatole contents in chyme

Indole and skatole (3-methylindole) of luminal contents in cecal and colon were analysed as described previously on high-performance liquid chromatography (HPLC, Agilent 1260, Agilent Technologies Inc., USA) [15]. Briefly, luminal contents in cecal and colon were weighted (1.000 g) and extracted by 8 mL 0.4 mol/L perchloric acid overnight. Then the suspension was acquired by centrifuge 10 min at 8000 r/min at 4°C. 1 mL of supernatant was being homogenate with 1 mL sodium hydroxide (2 mol/L, pH 10.6)-saturated sodium bicarbonate buffer solution, then added 1 ml of dansulfonyl chloride (10 mg/ml acetone solution). The solution incubated at 40°C water in a dark place for 30 min, and then add 1 mL 5% of ammonia water. Finally, 3 mL of anhydrous ether was used to extract the solution. The anhydrous ether layer was dried and dissolved in 1 mL methanol, filtered by 0.22 µm syringe filter membrane and analyzed on the machine.

Statistics analysis

Gene expression data from replicate measurement within the same RNA extraction were averaged and analyzed using Livak's (2001) [16] method to measure the difference between the five DAR diets. All data were analyzed using the General Linear Model (GLM) procedure of SAS 8.0. Duncan differences were determined to compare differences among the groups. The variability in data was showed as standard error of mean (SEM), and the $P < 0.05$ was considered statistical significance.

Results

Serum biochemical variables and free amino acids at day 15

Serum TP, ALB, ALT, AST, BUN, TG, AMS at day 15 were not affected by DAR (Table 1). Serum CREA was higher in diet 0.80 than in other groups ($P < 0.05$). Serum GLU was higher in group 0.20 than in groups 0.00 and 0.40 ($P < 0.05$), with no significant difference compared with groups 0.60 and 0.80 ($P > 0.05$). Serum CHOL was higher in groups 0.00 and 0.60 than in group 0.40 ($P < 0.05$).

Serum essential amino acid at day 15 was not affected by DAR ($P > 0.05$) except that groups 0.00 had the lowest serum arginine compared with groups 0.40 and 0.60 ($P < 0.05$) (Table 2). Serum non-essential amino acid was not affected by DAR except alanine, aspartate, proline, sarcosine, citrulline, anserine, carnosine, α -aminoadipic acid, α -amino-n-butyric acid, γ -aminobutyric acid and ethanolamine ($P > 0.05$). Serum proline was lower but anserine was higher in diet 0.00 than in other groups ($P < 0.05$). Serum aspartate and ethanolamine were higher in diets 0.20 than in diets 0.60 and 0.80 ($P < 0.05$). Serum sarcosine and carnosine were higher in diet 0.40 than in groups 0.00, 0.20 and 0.80 ($P < 0.05$). Serum γ -aminobutyric acid was higher in diet 0.60 than in other groups ($P < 0.05$). Serum alanine, citrulline and α -amino-n-butyric acid was higher in diet 0.80 than in diets 0.00, 0.40 ($P < 0.05$). Serum α -aminoadipic acid was higher in diet 0.80 than in diets 0.20, 0.40, 0.60 ($P < 0.05$).

Absolute and relative organ weight

Absolute weight of organ was not affected by DAR (Table 3, $P > 0.05$). LPS stress caused increase in absolute weight of liver and decrease in large intestine ($P < 0.05$). Absolute weight of kidney tended to increase after LPS stress ($P = 0.051$). Relative organ weight such as stomach, liver, spleen, kidney and length of small intestine were not affected by DAR ($P > 0.05$). Relative weight of small intestine increased after LPS stress in diet 0.20 and 0.80 but decreased in other groups ($P < 0.05$). Relative weight of large intestine decreased after LPS stress ($P < 0.05$).

Serum biochemical variables and free amino acids at day 29

Serum TP, ALB, ALT, AST, BUN, CREA, GLU, CHOL and IGM was not affect by DAR (Table 4). Serum TG was lower in diet 0.40 than in groups 0.60 and 0.80 ($P < 0.05$). Serum AMS was lower in diets 0.00 than in diet 0.20, which lower than in diet 0.60 ($P < 0.05$). Serum IGG was lower in diet 0.00 than in diet 0.20, which was lower than in diet 0.40 and diet 0.80 had the highest IGG concentration ($P < 0.05$). Serum ALT, CREA, AMS and LIPC decreased but serum GLU, BUN, AST increased after LPS stress ($P < 0.05$). Serum IGG increased after LPS stress in diets 0.80 but decreased after LPS stress in other groups ($P < 0.05$).

Serum essential amino acid was not affected by DAR ($P > 0.05$) except that diet 0.80 had the highest serum arginine, higher than in groups 0.00, 0.20 and 0.40 (Table 5, $P < 0.05$). Serum non-essential amino acid was not affected by DAR ($P > 0.05$) except proline, sarcosine, citrulline, anserine, hydroxylysine, β -aminoisobutyric acid and hydroxy-L-proline ($P < 0.05$). Serum proline was higher in diet 0.60 than in 0.80, 0.40 and 0.00 ($P < 0.05$). Piglets consuming diet with DAR 0.80 had the highest concentration of serum sarcosine, citrulline, anserine, hydroxylysine, β -aminoisobutyric acid. Serum hydroxy-L-proline was higher in diets 0.20 and 0.60 than in diet 0.00 ($P < 0.05$). Serum essential amino acid decreased after LPS stress ($P < 0.05$) except histidine which was not affected by LPS stress ($P > 0.05$). Serum tyrosine, alanine, aspartate, glutamate, cysteine, proline, urea, citrulline, carnosine, ornithine, α -Amino-n-butyric acid, Hydroxy-L-proline were affected by LPS stress ($P < 0.05$). There is significant effect of interaction between AAR and LPS on serum methionine, phenylalanine, valine, arginine, tyrosine, serine, hydroxy-L-proline ($P < 0.05$). Serum phenylalanine increased after LPS stress in diet 0.40 but decreased in other groups ($P < 0.05$). Serum valine decreased after LPS stress but maintained stable in other groups ($P < 0.05$). Serum arginine and tyrosine maintain stable in diet 0.00 after LPS stress but decreased in other groups ($P < 0.05$). Serum serine decreased after LPS stress in diets 0.20 and 0.80 but increased in other groups ($P < 0.05$). Serum hydroxy-L-proline increased in diets 0.00 and 0.60 after LPS stress but maintained stable in other groups ($P < 0.05$).

Amino acid contents in liver and dorsal muscle

Amino acid concentration of *longissimus dorsi* muscle was not affected by DAR and LPS stress (Table 6, $P > 0.05$). Hepatic cysteine was lower in diet 0.80 than in diets 0.00 and 0.20 ($P < 0.05$). There was significant effect of interaction between DAR and LPS stress on hepatic isoleucine, leucine, phenylalanine, threonine, aspartate, serine, glutamate, glycine concentrations ($P < 0.05$). All these amino acids of liver decreased after LPS stress in diet 0.20 but increased in other groups ($P < 0.05$).

Antioxidant function of liver and muscle

Concentration of MDA in muscle was not affected by DAR or LPS stress (Table 7, $P > 0.05$). Activity of T-AOC in muscle was higher in diets 0.60 and 0.80 than in diets 0.00, 0.20 and 0.40 ($P < 0.05$), it decreased after LPS stress in diet 0.40 but increased in other groups ($P < 0.05$). Activity of T-AOC in liver was not affected by DAR or LPS stress ($P > 0.05$). Activities of GSH-PX was higher in diet 0.20 than in diets 0.40, 0.60 and 0.80 ($P < 0.05$), they increased after LPS stress ($P < 0.05$). Concentration of MDA was higher in diet 0.20 than in diets 0.00, 0.40 and 0.80 ($P < 0.05$). Hepatic T-SOD activity decreased after LPS stress in diet 0.40 but increased in diets 0.00 and 0.80 ($P < 0.05$). Activity of Cu-SOD was lower in diet 0.20 than in other groups ($P < 0.05$).

Gene related to antioxidant function such as SOD1, Nrf2, GPX1 mRNA expression was affected by DARA, which was higher in group 0.00 than in other groups ($P < 0.05$). LPS stress caused increased in abundance of gene SOD1 and GPX1 ($P < 0.05$). mRNA expression of SOD1 and Nrf2 decreased after LPS stress in

diets 0.40 and 0.60 but increased in other groups ($P < 0.05$). Gene of GCLC expression decreased after LPS stress in diets 0.40, 0.60, 0.80 but increased after LPS stress in diets 0.00 and 0.20 ($P < 0.05$).

Mineral element contents in liver and muscle

All of the tested mineral element in muscle was not affected by LPS stress (Table 8, $P > 0.05$). Mineral element concentrations such as Ca, Fe, Mg, S were not affected by DAR ($P > 0.05$). Muscle from piglets in diet 0.80 had the lowest Cu and Zn concentrations compared with other groups ($P < 0.05$). Diet 0.80 tended to have the lowest Mn in muscle ($P = 0.052$). Mn of muscle decreased after LPS stress in diets 0.20 and 0.60 but increased in other groups ($P < 0.05$).

Indole and skatole contents in chyme

Cecal indole concentration was higher in diet 0.00 than in diet 0.80 ($P < 0.05$), it was not affected by LPS stress ($P > 0.05$). Cecal skatole and colonic indole were not affected by DAR or LPS stress ($P > 0.05$). Diet 0.00 had the lowest colonic skatole than in diet 0.40, 0.60 and 0.80 ($P < 0.05$). Skatole in colon increased after LPS stress in diets 0.40 and 0.80 but decreased in other groups ($P < 0.05$).

Discussion

Oxidative stress is condition in which production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant system, it caused tissue damage and dysfunction. Generally, the ROS including MDA which is a metabolite produced after lipid peroxidation [17]. Increases in indices of lipid peroxidation in rat were accompanied by reduced activity levels of liver GPX and CuZn-SOD. Serum AST activity is a key biochemical markers of liver health and function. LPS stress caused elevation of serum AST level, which could be considered to be a sensitive indicator of liver injury [18]. In order to overcome LPS stress, antioxidant activity represented by GSH-PX activity and gene expression of GPX1 and SOD1 increased in liver of weaned piglets. The SOD enzymes keep the intracellular steady-state concentration of O_2^- low, while GPX remove the H_2O_2 generated from the dismutation [19]. Our results are inconsistent with [20], who summarized that the *SOD1* and *GPX1* activity of mice will down-regulate, and the expression levels of *GCLC* and *Nrf2* were significantly decreased 2 h after 8 mg/kg LPS challenge [21]. This has to do with our sampling time. We sample 12h after LPS challenge, 12-hour recovery time was more effective in fully restored piglet liver function than 2 hours. 15 day after LPS stress, proteins involved in inflammatory response, oxidation-redox processes and defense reactions were enriched in a phagosome pathway in liver of weaning piglet [4].

Weaned piglets supplemented diet with DAR 0.40 showed weak response to LPS stress, T-SOD of liver and T-AOC concentration in muscle decreased after LPS stress in diet with DAR 0.40 but increased in group 0.80. Addition of resistant starch (RS) in the high fat diet will significantly increase the glutamate-cysteine ligase catalytic subunit (*GCLC*) gene expression, which involved in cellular antioxidant response to ROS, and then perform the better efficiently in suppressing stress by elevating T-AOC and GSH-PX levels [22]. Copper, zinc-superoxide dismutase (SOD1) is a major intracellular antioxidant enzyme in mammals, its activity is important for the catalysis of LPS-induced hepatic protein nitration in mice [23]. Eukaryotic cells have two forms of SOD: one is founded in the mitochondrial matrix (Mn-SOD) and another is founded in the cytosol (CuZn-SOD) [24]. Relative organ weight of intestine increased after LPS stress in diet 0.80, which indicates an increase in digestion and absorption. As a result of the hypertrophy of the cecal wall and of the cecal acidification, raw potato starch (RS2) fermentation increases intestinal absorption of Ca, Mg, Fe, Zn and Cu [25]. Hepatic Cu concentration and muscular Mn increased after LPS stress in diet 0.80 but not in other groups. Accordingly, SOD1 mRNA expression decreased after LPS stress in diet 0.40 but increased in diet 0.80. Indoxyl sulfate and P-cresole sulfate are among the major microbial derived pro-inflammatory and pro-oxidant uremic toxins. Production of indoxyl sulfate and P-cresole sulfate increased at higher pH [26]. By increasing production of SCFA, production of indoles of cecum decreased as a result of lowering the luminal pH in diet 0.80. Serum α -Amino-n-butyric acid was the highest in diet 0.80. Serum IGG decreased after LPS stress in other groups but increased in diet 0.80. Increase of immunoglobulin increases the body's resistance to inflammation, which could be benefit to antioxidant ability as oxidative stress and inflammation are inseparable linked as each trigger and amplifies the other.

Studies assumed that an increase RS would decrease the abundance of protein fermentation products [27, 28]. After 24 h incubation of cecal and colonic digesta, production of ammonia-nitrogen and branched-chain fatty acids, which indicated protein fermentation, decreased when the corn resistant starch levels increased [27]. BCFA including isobutyrate and isovalerate concentrations significantly increased when the DAR was 0.20, suggesting that the DAR changed fermentation pattern and increased protein fermentation (data not shown). Protein fermentation products have been associated with toxic and proinflammatory impacts on the intestinal epithelium [29], which resulted in highest hepatic MDA concentration in diet 0.20. Normally, liver protein synthesis increased when animals undergoing immune challenge. Due to higher protein fermentation, hepatic isoleucine, leucine decreased after LPS stress in diet 0.20 because BCFA such as iso-butyrate, 2-methyl-butyrate, iso-valerate originate from deamination of valine, isoleucine and leucine. We should notice that serum serine decreased after LPS stress in diet 0.20 and 0.80 but increased in other groups. Serine participates in a metabolic network that interlinks the folate cycle and methionine cycle to support cell proliferation [30]. Serine is a precursor of glycine and cysteine that constitutes components of the cellular antioxidant glutathione (GSH). On the other side, hepatic amino acids such as phenylalanine, threonine, aspartate, serine, glutamate, glycine could be mobilized to build defences battle with MDA. Increase in the quantity of amino acid which enter the portal vein could be useful in improving tissue protein synthesis [31]. Higher hepatic cysteine in group 0.20 suggests that glutathione is in the process of synthesis in this group. Finally, it (diet 0.20) had highest GSH-PX activity in liver than in other groups. Gene expression of *Nrf2* and glutamate-cysteine ligase catalytic subunit (*GCLC*) of liver increased after LPS stress in diet 0.00 and 0.20 but decreased in other groups. *Nrf2* is the master regulator of close to 250 genes encoding antioxidant and cytoprotective enzymes and proteins [32].

Conclusions

In conclude, antioxidant ability of liver was affected by dietary amylose/amylopectin ratio when weaned piglets suffered LPS stress. More hepatic amino acids would be mobilized to synthesis glutathione peroxidase to defend LPS stress when weaned piglets supplemented diet with DAR 0.20. More Cu and Zn deposited in the liver and more Mn deposited in the muscle, and then antioxidant ability of liver and muscle increased after LPS stress when weaned piglets consuming diet with DAR0.80. Dietary amylose/amylopectin ratio (DAR) for most of the diet used in swine production is 0.40. Our study confirmed that diet with DAR 0.40 had disadvantageous when weaned piglets had experienced feed transition and then LPS stress. Supplemented amylose or amylopectin would be better for piglets under LPS stress through improving antioxidant function or amino acid metabolism.

Declarations

Ethics approval and consent to participate

Experimental procedure in this study was reviewed and approved by the Animal Care and Use Committee of the Hunan international joint laboratory of animal intestinal ecology and health, Hunan Normal University.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yin Yulong and Yang Huansheng organized the experiment and gave some advice on experiment idea. Yang Can conducted the experiment and was a major contributor in writing the manuscript. Yang Can, Wang Min, Tang Xiaowu and Wang Yancan conducted the experimental analysis. Li Fengna and Li Jianzhong reviewed the manuscript and gave some advice on experiment idea. All authors read and approved the final manuscript

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Authorship

Can Yang, post doctor in Hunan Normal University, tutor is Dr. Yulong Yin.

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Tables

Table 1
Effects of dietary amylose/amylopectin ratio on serum biochemical index on day 15 in weaned piglets

Item	0.00	0.20	0.40	0.60	0.80	SEM	<i>P</i> value
TP, g/L	47.22	49.51	45.60	45.97	45.12	0.68	0.260
ALB, g/L	25.59	26.73	24.53	26.29	25.70	0.52	0.725
ALT, U/L	34.48	36.59	42.79	44.38	39.13	1.58	0.264
AST, U/L	55.73	62.25	55.67	53.67	51.00	2.50	0.693
AST/ALT	4.54	5.13	4.83	4.48	4.73	0.22	0.895
BUN, mmol/L	60.00	63.92	66.92	63.75	63.00	1.60	0.762
CREA, umol/L	4.68 ^B	4.73 ^B	4.53 ^B	4.58 ^B	5.32 ^A	0.08	0.027
GLU, mmol/L	0.45 ^B	0.60 ^A	0.40 ^B	0.47 ^{AB}	0.53 ^{AB}	0.02	0.023
TG, mmol/L	1.87	2.07	1.87	2.02	2.05	0.05	0.405
CHOL, mmol/L	2642 ^A	2230 ^{AB}	2022 ^B	2493 ^A	2226 ^{AB}	63.79	0.031
AMS, U/L	4.22	4.56	3.93	4.55	4.13	0.12	0.421
TP: total protein; ALB: albumin; ALT: alanine transaminase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CREA: creatinine; GLU: glucose; TG: triglyceride; CHOL: total cholesterol; AMS: amylase; LIPC: hepatic lipase; SEM: standard error of the mean;							

Table 2
Effects of DAR on serum free AA concentration on day 15 in weaned piglets

Item	0.00	0.20	0.40	0.60	0.80	SEM	<i>P</i> value
Essential amino acid							
Lysine	418.65	488.86	441.79	413.45	432.75	19.48	0.752
Methionine	118.90	131.43	122.62	134.92	147.40	4.92	0.403
Histidine	137.54	154.34	126.72	140.73	144.51	3.44	0.154
Isoleucine	200.15	227.27	203.17	206.78	212.50	5.90	0.623
Leucine	272.47	305.75	255.92	261.08	252.83	7.08	0.126
Phenylalanine	206.62	219.99	197.31	202.84	203.49	4.55	0.590
Valine	368.39	402.46	347.66	370.04	365.57	8.80	0.400
Threonine	131.98	174.13	138.68	140.79	160.39	8.79	0.537
Arginine	407.26 ^B	471.80 ^{AB}	540.05 ^A	528.63 ^A	434.36 ^{AB}	15.44	0.037
Non-essential amino acid							
Tyrosine	156.28	177.26	152.29	166.32	187.46	5.92	0.314
Alanine	648.46 ^B	781.34 ^{AB}	710.91 ^B	710.52 ^B	860.09 ^A	21.02	0.029
Aspartate	100.80 ^A	102.90 ^A	91.02 ^{AB}	80.77 ^B	82.94 ^B	2.51	0.019
Glutamate	734.33	747.36	586.50	639.88	820.27	29.13	0.101
Glycine	810.43	773.85	811.29	758.24	875.34	30.42	0.773
Serine	173.97	188.12	183.17	158.89	195.41	6.19	0.390
Cysteine	93.28	119.84	101.66	105.77	109.84	2.94	0.082
Proline	290.28 ^B	370.19 ^A	412.60 ^A	400.86 ^A	453.46 ^A	12.43	0.003
Other AA							
Taurine	237.35	242.97	214.22	191.53	216.51	5.98	0.065
Urea	1971.87	2219.22	1948.99	1891.50	1967.06	82.46	0.750
Sarcosine	5.21 ^B	9.82 ^B	31.15 ^A	14.98 ^B	10.27 ^B	2.30	0.008
Citrulline	132.51 ^C	161.98 ^{AB}	132.65 ^C	141.87 ^{BC}	170.41 ^A	4.01	0.009
Anserine	20.60 ^A	1.61 ^B	2.51 ^B	3.19 ^B	1.80 ^B	1.83	0.008
Carnosine	40.20 ^B	48.23 ^B	94.83 ^A	110.27 ^A	52.58 ^B	5.76	0.001
Cystathionine	13.07	16.78	15.94	15.59	18.45	0.55	0.054
Hydroxylysine	14.74	17.63	16.17	14.23	17.52	0.67	0.383
Ornithine	118.98	136.44	118.74	117.85	140.36	5.55	0.537
α -Aminoadipic Acid	118.00 ^{AB}	91.46 ^B	33.50 ^C	49.56 ^C	133.74 ^A	5.34	0.0001
α -Amino-n-butyric Acid	12.61 ^C	20.14 ^{AB}	16.63 ^{BC}	17.72 ^{ABC}	23.40 ^A	0.94	0.012
β -Alanine	37.47	33.59	36.23	36.23	35.88	1.82	0.975
β -Aminoisobutyric Acid	3.49	6.82	6.70	8.78	7.22	0.57	0.085
γ -Aminobutyric Acid	0.97 ^B	1.133 ^B	1.11 ^B	1.88 ^A	0.96 ^B	0.11	0.043
Ethanolamine	10.25 ^B	12.49 ^A	9.16 ^B	9.32 ^B	9.18 ^B	0.23	0.0001
1-Methylhistidine	61.51	57.55	55.54	49.90	56.91	2.41	0.666
3-Methylhistidine	33.20	32.72	30.37	33.34	34.29	1.71	0.963
Hydroxy-L-proline	181.21	156.19	137.98	132.13	142.29	6.13	0.112
Total	167.40	181.49	161.25	157.77	168.82	4.86	0.586
DAR: dietary amylose/amylopectin ratio							

Table 3
Effects of dietary amylose/amylopectin ratio on organ weight in weaned piglets¹.

Items	0.00		0.20		0.40		0.60		0.80		SEM	P value		
	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL		P _{DAR}	P _{stress}	P _{D*S}
Absolute organ weight,g														
Stomach	81.4	74.8	91.4	77.2	77.6	77.3	84.1	74.3	81.0	79.1	1.70	0.737	0.061	0.692
Liver	407.7	367.3	413.8	328.2	388.8	374.3	412.1	363.1	397.7	405.5	8.58	0.859	0.041	0.507
Spleen	32.8	26.0	28.0	27.4	32.5	31.3	23.2	21.6	30.1	25.4	1.23	0.186	0.228	0.917
Kidney	69.4	63.5	70.5	60.3	75.7	62.6	73.0	63.4	63.6	69.7	1.62	0.951	0.051	0.385
Small intestine, kg	0.693	0.757	0.776	0.691	0.648	0.741	0.677	0.753	0.709	0.743	0.01	0.933	0.237	0.362
Large intestine, kg	0.432	0.550	0.419	0.540	0.430	0.546	0.414	0.567	0.422	0.560	0.01	0.999	0.0001	0.989
Relative organ weight,g/kg														
Stomach	0.61	0.66	0.72	0.65	0.64	0.65	0.64	0.70	0.70	0.66	0.01	0.469	0.874	0.162
Liver	3.06	3.26	3.23	2.77	3.16	3.21	3.11	3.45	3.48	3.29	0.05	0.174	0.890	0.098
Spleen	0.25	0.23	0.22	0.23	0.27	0.26	0.17	0.20	0.27	0.21	0.01	0.162	0.701	0.713
Kidney	0.52	0.57	0.55	0.51	0.62	0.52	0.54	0.61	0.57	0.55	0.01	0.557	0.639	0.121
Small intestine	5.22	6.81	6.05	5.89	5.35	6.47	5.17	7.19	6.34	5.97	0.14	0.969	0.005	0.045
Large intestine	3.29	4.83	3.26	4.59	3.53	4.73	3.10	5.32	3.50	4.54	0.09	0.894	0.0001	0.303
Length of small intestine, m	1.29	1.18	1.24	1.16	1.23	1.19	1.26	1.20	1.17	1.19	0.01	0.760	0.070	0.696

Table 4
Effects of dietary amylose/amylopectin ratio and stress on serum biochemical index on day 29 in weaned piglets

Items	0.00		0.20		0.40		0.60		0.80		SEM	P value	
	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL		P _{DAR}	P _{stress}
TP, g/L	43.63	55.16	44.58	52.50	43.13	43.27	44.82	48.22	45.82	43.40	0.94	0.192	0.030
ALB, g/L	23.72	21.46	21.92	25.22	21.68	21.35	25.65	23.98	23.33	22.60	0.60	0.483	0.774
ALT, U/L	33.98	41.86	38.83	39.78	42.48	44.20	39.88	46.48	31.42	41.98	1.28	0.324	0.031
AST, U/L	67.83	72.80	89.50	69.00	80.17	77.17	110.67	56.33	99.67	54.50	3.72	0.853	0.002
AST/ALT	6.17	5.38	6.57	5.58	9.03	5.65	5.77	5.02	6.63	5.18	0.36	0.460	0.039
BUN, mmol/L	91.33	57.20	96.33	63.83	87.50	64.83	107.00	60.33	100.67	61.83	2.26	0.670	0.000
CREA, umol/L	4.43	4.90	4.17	5.23	3.83	5.22	3.88	4.97	3.37	5.33	0.12	0.869	0.000
GLU, mmol/L	0.73	0.46	0.66	0.43	0.56	0.48	0.71	0.46	0.71	0.50	0.02	0.664	0.000
TG, mmol/L	2.14 ^{AB}	2.01	2.12 ^{AB}	2.21	1.78 ^B	2.03	2.11 ^A	2.30	2.28 ^A	2.37	0.04	0.014	0.238
CHOL, mmol/L	2487.50	2248.80	2153.33	2454.67	1950.00	2429.50	2585.00	2370.33	2406.83	2593.50	81.08	0.712	0.519
AMS, U/L	3.55 ^C	3.80	3.75 ^{BC}	4.63	4.30 ^{AB}	5.22	4.58 ^A	6.02	4.15 ^{ABC}	4.65	0.16	0.027	0.014
LIPC, U/L	3.55 ^C	3.80	3.75 ^{BC}	4.63	4.30 ^{AB}	5.22	4.58 ^A	6.02	4.15 ^{ABC}	4.65	0.16	0.027	0.014
IGG, g/L	0.37 ^E	0.87	1.09 ^D	6.99	5.57 ^C	7.52	6.74 ^B	17.19	16.41 ^A	12.85	0.26	0.0001	0.0001
IGM, g/L	0.66	0.85	0.51	0.51	0.54	0.55	0.47	0.77	0.68	0.54	0.03	0.146	0.259

TP: total protein; ALB: albumin; ALT: alanine transaminase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CREA: creatinine; GLU: glucose; TG: triglyceride; CHOL: total cholesterol; AMS: amylase; LIPC: hepatic lipase;

Table 5
Effects of dietary amylose/amylopectin ratio on serum free AA concentration on day 29 in weaned piglets

Items	0.00		0.20		0.40		0.60		0.80		SEM	P value
Essential amino acid	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL		P _{DAR}
Lysine	290.83	461.73	299.34	439.69	344.25	383.30	289.37	517.96	362.64	529.83	14.10	0.332
Methionine	88.53	109.98	71.44	145.46	80.73	101.33	67.04	168.53	63.65	148.75	3.99	0.290
Histidine	111.83	114.56	111.21	124.90	129.58	116.57	97.37	126.11	118.01	128.32	3.08	0.662
Isoleucine	189.06	241.42	148.19	298.79	172.41	234.18	148.91	281.43	130.05	280.38	6.91	0.883
Leucine	209.84	248.70	186.51	270.96	222.90	213.75	180.88	240.34	179.25	247.79	5.85	0.789
Phenylalanine	190.14	191.68	158.86	228.87	205.48	185.08	159.12	232.94	176.52	242.75	5.15	0.811
Valine	321.02	375.51	292.89	450.46	350.52	357.90	279.32	407.13	273.61	427.02	9.16	0.886
Threonine	88.26	93.72	85.80	126.89	96.80	96.43	86.83	121.27	83.86	122.10	3.80	0.715
Arginine	328.56 ^B	365.26	300.14 ^B	430.07	310.30 ^B	417.24	275.51 ^{AB}	537.74	347.81 ^A	606.33	12.69	0.015
Non-essential amino acid												
Tyrosine	175.17	181.98	149.26	224.12	157.65	170.40	131.81	229.11	133.36	256.84	5.27	0.437
Alanine	817.28	732.17	881.35	834.48	961.51	669.89	1219.58	817.30	903.89	703.50	27.71	0.060
Aspartate	64.50	78.11	59.85	92.65	73.18	78.19	54.64	83.10	60.92	80.89	2.61	0.872
Glutamate	616.87	639.59	526.48	793.71	584.36	642.12	495.57	735.61	553.77	661.64	23.35	0.955
Glycine	785.89	620.18	722.03	948.18	874.09	789.05	1016.83	795.10	668.99	775.45	27.50	0.128
Serine	169.54	137.75	150.71	207.84	179.11	156.57	206.91	180.88	150.52	165.47	4.97	0.089
Cysteine	104.91	81.56	99.86	76.33	113.40	94.96	119.98	93.51	131.90	94.06	3.12	0.092
Proline	287.69 ^C	325.81	350.11 ^{AB}	477.26	350.40 ^{BC}	355.05	411.45 ^A	503.95	333.15 ^{BC}	405.19	10.57	0.001
Other AA												
Taurine	170.19	207.61	169.97	192.20	181.97	162.60	156.89	163.98	172.31	186.49	4.70	0.405
Urea	2985.70	2251.38	2747.70	2300.43	2445.53	2480.56	2459.77	2121.04	2912.18	2307.53	65.11	0.502
Sarcosine	5.03 ^C	6.57	23.50 ^A	27.01	14.50 ^B	14.04	31.95 ^A	34.50	29.80 ^A	30.71	1.06	0.0001
Citrulline	166.38 ^B	136.89	186.49 ^{AB}	164.00	190.54 ^{AB}	154.22	182.22 ^{AB}	174.65	221.01 ^A	190.20	4.85	0.024
Anserine	2.94 ^{BC}	2.37	0.52 ^C	0.00	4.11 ^{ABC}	5.22	5.44 ^{AB}	7.60	11.54 ^A	7.16	0.73	0.003
Carnosine	47.58	30.79	37.03	38.89	44.61	39.59	53.22	26.42	43.69	32.99	1.57	0.926
Cystathionine	20.40	14.14	17.15	20.00	17.05	15.95	18.66	17.41	17.04	14.88	0.74	0.787
Hydroxylysine	12.04 ^B	13.48	9.06 ^B	11.64	11.63 ^B	13.00	11.80 ^B	13.03	18.29 ^A	18.69	0.67	0.005
Ornithine	155.05 ^B	232.42	173.61 ^{AB}	243.92	171.88 ^B	211.13	165.96 ^{AB}	256.64	197.85 ^A	276.09	5.67	0.100
α-Aminoadipic acid	149.35	109.72	131.54	164.00	151.77	137.55	164.84	157.76	170.94	158.45	5.77	0.357
α-Amino-n-butyric acid	16.66	21.25	17.80	27.98	17.62	17.92	12.13	24.97	14.23	26.31	1.06	0.582
β-Alanine	57.39	31.15	34.28	30.19	41.16	26.63	45.87	39.06	53.16	35.74	1.89	0.115
β-Aminoisobutyric acid	5.80 ^A	7.28	0.92 ^B	1.72	3.10 ^B	1.59	4.18 ^A	8.38	8.11 ^A	4.98	0.42	0.000

γ-Aminobutyric acid	2.09	1.69	1.50	1.66	2.78	1.25	1.45	3.92	3.11	3.25	0.26	0.304
Ethanolamine	9.86	10.81	10.18	9.17	10.02	7.62	10.76	9.66	13.45	9.24	0.40	0.368
1-Methylhistidine	71.09	42.86	47.79	62.23	65.33	55.25	59.19	64.74	56.45	58.50	2.37	0.891
3-Methylhistidine	13.65	33.01	12.85	17.08	24.80	19.76	13.94	16.75	20.60	18.02	1.32	0.167
Hydroxy-L-proline	161.69 ^B	85.02	155.94 ^A	161.40	153.41 ^{AB}	155.79	199.73 ^A	138.12	142.28 ^{AB}	146.45	4.20	0.019

Table 6
Effects of dietary amylose/amylopectin ratio on amino acid composition in liver and muscle of weaned piglets¹.

Items	0.00		0.20		0.40		0.60		0.80		SEM	P value		
<i>Longissimus dorsi</i> muscle	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL		P _{DAR}	P _{stress}	P _{D*S}
Lysine	5.691	5.461	6.649	4.825	4.629	4.800	6.380	5.967	4.667	5.483	0.26	0.487	0.591	0.637
Methionine	1.790	1.789	2.301	1.801	1.625	1.812	2.281	2.043	1.622	1.877	0.10	0.595	0.783	0.787
Histidine	2.636	2.455	3.205	2.170	2.062	2.280	2.902	2.702	2.039	2.562	0.12	0.468	0.587	0.355
Isoleucine	3.855	3.800	4.977	3.802	5.737	4.001	4.841	4.471	3.461	3.981	0.25	0.576	0.292	0.680
Leucine	6.530	6.438	8.152	6.110	8.120	6.273	7.901	7.425	5.847	6.758	0.38	0.803	0.368	0.733
Phenylalanine	1.885	1.853	2.221	1.727	2.437	1.701	2.253	2.056	1.587	1.852	0.11	0.776	0.301	0.676
Valine	3.684	3.600	4.429	3.170	3.376	3.254	4.249	3.925	3.050	3.619	0.18	0.644	0.510	0.633
Threonine	3.277	3.253	3.971	2.851	2.586	2.889	3.773	3.508	2.769	3.247	0.15	0.436	0.695	0.553
Arginine	4.597	4.429	5.482	3.840	3.468	3.901	5.157	4.802	3.775	4.327	0.22	0.393	0.600	0.553
Aspartate	6.728	6.574	8.131	5.771	5.233	5.889	7.707	7.200	5.646	6.616	0.32	0.421	0.673	0.536
Serine	2.865	2.831	3.473	2.482	2.264	2.543	3.315	3.105	2.448	2.832	0.14	0.442	0.689	0.571
Glutamate	11.438	11.209	13.592	9.858	8.955	9.982	13.060	12.207	9.613	11.213	0.54	0.442	0.696	0.596
Glycine	3.792	3.368	4.663	3.034	3.108	3.330	4.263	4.082	3.251	3.307	0.19	0.520	0.327	0.601
Alanine	4.797	4.566	5.633	3.985	3.821	4.092	5.419	5.057	3.972	4.568	0.23	0.484	0.564	0.621
Cysteine	-	-	-	-	-	-	-	-	-	-				
Tyrosine	0.963	0.852	1.050	1.055	1.173	0.737	1.597	1.204	1.024	1.233	0.10	0.559	0.470	0.827
NH ₃	1.161	1.140	1.363	0.946	0.792	0.967	1.371	1.236	0.992	1.140	0.06	0.259	0.675	0.515
Proline	3.337	2.978	4.139	2.698	2.568	2.877	3.762	3.677	2.853	3.006	0.16	0.369	0.402	0.500
Liver														
Lysine	3.205	2.605	2.791	2.972	3.110	2.528	3.183	2.327	2.879	2.425	0.05	0.526	0.0001	0.057
Methionine	0.919	0.705	0.838	0.891	0.902	0.731	0.900	0.673	0.829	0.658	0.01	0.181	0.0001	0.083
Histidine	1.074	0.901	0.975	1.036	1.046	0.877	1.031	0.822	0.951	0.839	0.01	0.133	0.0001	0.120
Isoleucine	1.847	1.548	1.727	1.804	1.828	1.518	1.866	1.385	1.703	1.487	0.02	0.242	0.0001	0.034
Leucine	3.865	3.238	3.575	3.790	3.878	3.229	3.917	3.004	3.640	3.174	0.05	0.562	0.0001	0.049
Phenylalanine	2.048	1.763	1.902	2.038	2.060	1.713	2.046	1.570	1.902	1.612	0.03	0.148	0.0001	0.042
Valine	2.366	2.018	2.213	2.310	2.326	1.912	2.360	1.784	2.157	1.871	0.03	0.129	0.0001	0.062
Threonine	1.982	1.661	1.819	1.934	1.991	1.614	1.999	1.513	1.855	1.550	0.03	0.317	0.0001	0.031
Arginine	2.777	2.299	2.574	2.678	2.784	2.223	2.807	2.055	2.596	2.149	0.04	0.320	0.0001	0.059
Aspartate	4.000	3.367	3.694	3.880	4.039	3.291	4.048	3.085	3.763	3.182	0.05	0.414	0.0001	0.046
Serine	2.110	1.790	1.927	2.058	2.103	1.729	2.120	1.611	1.963	1.660	0.03	0.311	0.0001	0.037
Glutamate	6.118	5.148	5.659	5.870	6.211	5.061	6.190	4.754	5.792	4.887	0.07	0.419	0.0001	0.041
Glycine	2.483	2.147	2.283	2.424	2.478	2.083	2.522	1.891	2.294	2.026	0.03	0.378		0.047

													0.0001	
Alanine	2.764	2.487	2.680	2.823	2.862	2.407	2.944	2.237	2.713	2.336	0.04	0.464	0.0001	0.056
Cysteine	0.32 ^A	0.287	0.33 ^A	0.321	0.33 ^{AB}	0.223	0.36 ^{AB}	0.197	0.29 ^B	0.205	0.01	0.034	0.0001	0.126
Tyrosine	1.391	1.106	1.346	1.467	1.527	1.253	1.513	1.159	1.451	1.172	0.02	0.265	0.0001	0.051
NH3	0.686	0.570	0.644	0.622	0.674	0.534	0.679	0.514	0.600	0.502	0.01	0.065	0.0001	0.406
Proline	2.019	1.716	1.855	1.995	2.031	1.684	2.082	1.584	1.930	1.644	0.03	0.675	0.0001	0.052

Table 7
Effects of dietary amylose/amylopectin ratio on antioxidant activity in liver and muscle of weaned piglets¹.

Items	0.00		0.20		0.40		0.60		0.80		SEM	P value	
	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL		P _{DAR}	P _{stress}
Enzymatic activity													
Longissimus dorsi muscle													
T-AOC,mmol/mg pro	19.80 ^C	17.97	31.0 ^{BC}	20.37	19.20 ^B	47.20	47.85 ^A	45.32	51.17 ^A	31.63	1.37	0.0001	0.651
MDA,nmol/mg pro	0.12	0.09	0.07	0.16	0.21	0.43	0.10	0.18	0.17	0.23	0.04	0.305	0.250
Liver													
T-AOC,mmol/mg pro	61.30	71.64	71.57	108.91	73.33	89.47	121.65	74.43	111.92	73.52	5.12	0.379	0.685
GSH-PX,U/mg pro	1385 ^{AB}	576.11	1700 ^A	805.25	585.06 ^C	187.56	468.24 ^C	172.90	748.88 ^{BC}	422.90	73.75	0.001	0.001
MDA,nmol/mg pro	0.83 ^C	1.00	4.57 ^A	3.60	2.03 ^{BC}	2.01	2.91 ^{AB}	3.39	1.69 ^{BC}	2.15	0.20	0.000	0.958
T-SOD,U/mg pro	1102.68 ^A	358.90	54.75 ^C	55.93	172.82 ^{BC}	347.69	527.17 ^{AB}	513.93	401.35 ^{BC}	278.50	46.51	0.000	0.133
Cu-SOD,U/mg pro	497.18 ^A	212.97	39.01 ^B	40.47	181.45 ^A	365.42	468.21 ^A	459.46	321.30 ^A	225.84	37.94	0.001	0.500
mRNA expression in liver													
SOD1	2.51 ^A	1.06	1.05 ^B	0.77	0.71 ^B	0.89	1.08 ^B	1.27	0.82 ^B	0.69	0.07	0.000	0.044
Nrf2	2.40 ^A	1.05	1.34 ^B	1.01	0.94 ^B	1.27	1.30 ^{AB}	1.37	0.83 ^B	1.04	0.07	0.016	0.148
GPX1	2.95 ^A	1.10	1.73 ^B	0.50	0.92 ^B	0.64	1.27 ^B	1.05	1.48 ^B	0.67	0.10	0.006	0.000
GCLC	1.80	1.05	1.11	0.82	1.04	1.45	1.03	2.02	0.90	1.23	0.08	0.167	0.396
MDA: malonaldehyde; T-SOD: total superoxide dismutase; CuZnSOD: cooper-zinc; Mn-SOD: manganese SOD; GSH-PX: glutathione peroxidase; T-AOC: total ar capacity; GCLC: glutamate-cysteine ligase catalytic subunit; Nrf2: nuclear factor, erythroid 2 like 2; SOD1: superoxide dismutase; GPX1: and glutathione perox													

Table 8
Effects of dietary amylose/amylopectin ratio on mineral element concentrations in liver and muscle of weaned piglets¹.

Items,mg/g	0.00		0.20		0.40		0.60		0.80		SEM	P value
TRET	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL		P _{DAR}
Longissimus dorsi muscle												
Ca	541.57	600.98	602.88	567.83	768.83	638.92	643.99	486.53	435.03	508.39	23.97	0.060
Cu	24.78 ^A	31.34	31.38 ^A	38.26	46.12 ^A	33.71	32.96 ^A	32.07	16.21 ^B	7.17	2.53	0.014
Fe	785.50	996.63	594.41	5365.35	1122.98	1291.09	1171.42	656.81	444.76	91.47	308.17	0.089
Mg	159.31	127.06	141.41	109.02	135.51	138.45	139.88	118.24	125.57	149.06	5.26	0.836
Mn	11.24	9.72	3.76	22.78	13.38	10.61	7.21	10.03	4.71	1.36	1.10	0.052
S	6486.19	6789.44	5393.02	5427.91	7813.21	5840.90	6038.85	4916.15	5888.60	6008.67	253.02	0.266
Zn	151.52 ^A	189.55	126.15 ^A	216.93	248.71 ^A	209.02	174.17 ^A	203.53	101.84 ^B	57.07	13.34	0.018
Liver												
Ca	886.14	548.05	757.36	444.94	501.06	808.66	676.50	433.16	523.10	438.81	38.16	0.379
Cu	18.11 ^C	7.47	9.10 ^C	11.66	7.41 ^C	8.13	6.86 ^B	44.77	58.99 ^A	47.34	1.67	0.0001
Fe	173.35 ^B	126.28	98.46 ^B	60.56	85.90 ^B	125.01	261.27 ^A	12102.46	8588.29 ^A	14655.24	863.02	0.000
Mg	169.61	128.46	176.96	108.27	145.03	165.15	151.72	95.68	118.25	99.10	6.99	0.249
Mn	6.59 ^B	1.29	1.66 ^B	1.22	1.35 ^B	1.95	5.98 ^B	43.89	31.97 ^A	86.69	5.09	0.003
S	7964.21	5326.40	7390.98	3577.73	5095.49	5229.55	5351.62	5235.06	7050.15	5380.98	368.17	0.684
Zn	147.72 ^{BC}	57.51	81.37 ^C	79.64	63.50 ^C	79.85	117.30 ^B	174.49	257.85 ^A	241.75	7.84	0.0001

Table 9
Effects of dietary amylose/amylopectin ratio on indole and skatole concentrations in cecal and colonic chyme of weaned piglets¹.

Items	0.00		0.20		0.40		0.60		0.80		SEM	P value		
Indol(e),µg/g	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL	LPS	SAL		P _{DAR}	P _{stress}	P _{D*S}
Cecal chyme	11.79 ^A	9.71	19.24 ^{AB}	3.62	9.84 ^{AB}	6.54	3.47 ^{AB}	2.86	0.00 ^B	4.07	1.01	0.031	0.123	0.091
Colonic chyme	2.73	3.21	5.47	14.37	10.05	7.43	4.00	8.97	5.28	4.90	0.94	0.246	0.271	0.402
Skatole,µg/g														
Cecal chyme	11.01	8.93	23.64	13.57	20.69	6.59	14.17	5.44	0.35	8.78	1.46	0.102	0.108	0.214
Colonic chyme	3.59 ^C	8.18	8.81 ^{BC}	28.18	40.14 ^{AB}	23.62	30.56 ^A	44.94	39.81 ^{AB}	15.88	2.20	0.002	0.930	0.019

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