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Effects of L-proline on the Growth Performance, and Blood Parameters in Weaned Lipopolysaccharide (LPS)-challenged Pigs

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ABSTRACT: This trail was conducted to study the effect of L-proline on the growth performance, and blood parameter in the weaned lipopolysaccharide (LPS)-challenged pigs. Thirty six pigs (9.13±0.85 kg) were assigned randomly to dietary treatments in a 2×3 factorial arrangement in a 20-d growth assay. Factors were intraperitoneal injection with saline or LPS, and three dietary L-proline supplement levels (0%, 0.5%, or 1.0%). On d 10, blood samples were collected at 3 h after LPS (100 μg LPS/kg body weight [BW]) or saline injection. On d 20 of the trial, all pigs were orally administrated D-xylose (0.1 g/kg BW) at 2 h, and blood samples were collected at 3 h after LPS or saline injection. As a result, dietary supplementation with 0.5% proline had a tendency to increase average daily gain (ADG) in piglets during d 10 to 20 ($p = 0.088$). Without LPS challenge, dietary supplementation with 1.0% proline had no effect on growth hormone (GH) concentrations on d 10 ($p>0.05$), but decreased it after LPS challenge ($p<0.05$). There was LPS challenge×proline interaction for GH concentrations on d 10 ($p<0.05$). Dietary supplementation with 1.0% proline decreased glucagon concentration on d 10 after LPS challenge ($p<0.05$). In addition, dietary supplementation with proline increased superoxide dismutase (SOD) activity significantly on d 10 and 20 ($p<0.05$), and 1.0% proline increased heat shock proteins-70 concentration on d 10 ($p<0.05$). Moreover, proline supplementation increased diamine oxidase (DAO) concentrations after LPS challenge ($p<0.05$). There was LPS challenge×proline interaction for DAO ($p<0.05$). Furthermore, dietary supplementation with 1.0% proline increased the D-xylose level when no LPS challenge ($p<0.05$). These results indicate that proline supplementation could improve growth performance, increase SOD activities, and has a positive effect on the gastrointestinal tract digestibility in early weaned pigs. (**Key Words:** L-proline, Growth Performance, Blood Parameters, Gastrointestinal Tract Digestibility, Early Weaned Pigs, Lipopolysaccharide)

INTRODUCTION

Proline is one of the most abundant amino acid in sow's colostrum and milk. Previous studies have shown that proline is an indispensable amino acid in the diets of 2.5-kg pigs (Ball et al., 1986) and young chicks (Baker, 1977). Young pigs have a limited ability to synthesize proline from arginine, glutamine or glutamate in the small intestine (Wu et al., 1996), thus, dietary supplementation with proline could play an important role for the weaned piglets.

There has been growing interest in proline metabolism and nutrition over the past decade (Phang and Liu, 2012). Growing evidence shows that proline plays an important role in differentiation and multiple biochemical and physiological processes in cells (Phang and Liu, 2012), as well as conceptus growth and development (Wu et al., 2008). Wu (1997) reported that proline was a major dietary precursor for *de novo* synthesis of arginine by the pig small intestine, suggesting that proline could spare a portion of the arginine requirement. Bertolo et al. (2003) concluded that the piglet could not synthesize sufficient proline to maintain its concentrations in plasma, and arginine synthesized from proline was also diminished as a result of gut atrophy during parenteral feeding. In addition, proline can serve as a major amino acid for the synthesis of

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polyamines in the small intestine (Wu et al., 2000). Therefore, proline could play an essential role for the weaned piglets. Previous study has shown that L-proline can improve small-intestinal morphology in the weanling piglet (Wu et al., 2011). However, the underlying mechanisms are unknown.

Newly weaned piglets are highly susceptible to various stressors. Because lipopolysaccharide (LPS) has been used as a tool to induce sickness behavior in the pigs (Lay et al., 2011), we hypothesized that proline could improve growth performance and relieve LPS challenge by reducing intestinal oxidative stress, stimulating some growth factors or hormones secretion, and improving the digestibility in piglets. In modern animal improvement, blood biochemical characteristic is an important indicator (El Darawany and Farghaly, 1999), accordingly, in this study, the growth performance and selected blood metabolites were measured to test our hypothesis.

MATERIAL AND METHODS

The animal use protocol for this research was approved by the Animal Care and Use Committee of Hubei Province. A total of thirty-six healthy crossbred (Duroc×Landrace×Yorkshire) piglets (9.13±0.85 kg) were weaned at 21±3 d of age and used in a 20-day feeding trail. During d 0 to10 of feeding trial, pigs were assigned randomly to three diet treatments, i) basal diet (0% proline [PRO]), ii) basal diet supplemented with 0.5% PRO, and iii) basal diet supplemented with 1.0% PRO. Each treatment had six replicates and each replicate comprised two pigs. On d 10, two pigs in each replicate were separated and housed individually, and one pig was injected intraperitoneally with saline (–LPS), and the other pig was injected intraperitoneally with LPS (+LPS). Therefore, pigs were assigned in a 2×3 factorial arrangement during d 10 to 20, each treatment had six replicates and each replicate comprised one pig. On d 10 and 20, pigs were administrated with intraperitoneal injections with either 100 μg LPS/kg body weight (BW) or an equivalent volume of sterile saline. On d 20, D-xylose was given via i.g. gavage to all pigs at the dosage of 0.1 g/kg BW (infused with 10% D-xylose at 1 mL/kg BW), 2 h after LPS challenge or saline injection. The basal diet was prepared to meet or exceed NRC (1998) nutrient requirement (Table 1), alanine was used to make the treatments isonitrogenous.

Table 1. The composition and nutrient contents of basal diet (air dry basis)

Item	Content
Ingredients (%)	
Corn	61.88
Soybean meal	21.98
Wheat middling	4.00
Fish meal	3.00
Dried whey	3.00
Soy protein concentrate	1.50
$CaHPO_4$	1.25
Premix[1]	1.00
Limestone	0.69
Soy oil	0.50
Acidifier	0.30
NaCl	0.30
L-lysine·HCl	0.25
Choline chloride	0.20
Mould inhibitor	0.10
DL-methionine	0.05
Total	100
Nutrient levels	
DE (MJ/kg)	14.22
CP (%)	20.9
Lys (%)	1.15
Met (%)	0.30
Met+cys (%)	0.65
Thr (%)	0.74
Trp (%)	0.21
Ca (%)	0.70
P (%)	0.60
Available phosphorus (%)	0.32
NaCl (%)	0.38
Proline (%)	1.44
Alanine (%)	0.47

DE, digestible energy; CP, crude protein.

[1] The premix provides for a kg of feed: Fe (as ferrous of sulfate), 100 mg; Cu (as copper sulfate), 150 mg; Mn (as manganese sulfate), 40 mg; Zn (as zinc sulfate), 100 mg; I, 0.5 mg; Se (as sodium selenite), 0.3 mg; vit A, 10,800 IU; vit D_3, 4,000 IU; vit E, 40 IU; vit K_3, 4 mg; vit B_1 (thiamine), 6 mg; vit B_2 (riboflavin), 12 mg; vit B_6 (pyridoxin), 6 mg; vit B_{12} (cobalamin), 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg.

Performance

Feed intake was measured every day during the entire experimental time, and pigs were weighed on d 0, 10, and 20 to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed:gain ration (F/G).

Blood collection

Blood samples were collected via the jugular vein into 10 mL heparinized vacuum tubes at 3 h after LPS or saline injection on d 10 and 20, and then centrifuged at 3,000×g for 10 min to collect plasma. Plasma was frozen at –80℃ until analysis. All blood samples were analyzed in duplicate.

Chemical analysis

Epidermal growth factor, insulin, nitric oxide, NO synthase, growth hormone and insulin-like growth factor 1 assay: Plasma concentrations of epidermal growth factor

(EGF), and insulin (INS) were measured with radioimmunoassay kits (Beijing SINO-UK Institute of Biological Technology, Beijing, China). The detection limits for EGF and INS assays were 0.1 ng/L and 2 μU/mL, respectively. The intra- and inter- assay coefficients of variation were 5% and 10% for EGF, 10% and 15% for INS, respectively. Plasma concentrations of nitrite plus nitrate nitric oxide (NO), total NO synthase (NOS), growth hormone (GH), and insulin-like growth factor (IGF)-I were measured, as we previously described (Kang et al., 2010).

Superoxide dismutase, malondialdehyde, and catalase assays: Plasma concentrations of catalase (CAT), malondialdehyde (MDA), and superoxide dismutase (SOD) were measured using commercial kits (Nanjing Jiancheng Biotechnology Co, Ltd, Nanjing, China). The SOD activity assay is based on nitroblue tetrazolium (NBT) which can be reduced to blue formazan by O^{2-}, and has a strong absorbance at 550 nm. One unit (U) of SOD is defined as the amount that inhibits the NBT reduction by 50% per minute in 1 mL plasma. The calculated SOD activity is expressed as U/mL plasma. Total CAT activity was determined as the consumption of H_2O_2 measured at 405 nm for 1 min at 37℃. One unit (U) of CAT is defined as 1 mL plasma that breaks down 1 μmol H_2O_2 per second at 37℃. The calculated CAT activity is expressed as U/mL plasma. The method for measuring MDA is based on the reaction with thiobarbituric acid (TBA), the absorbance of the supernatant fluid was recorded at 535 nm. Malondialdehyde results are expressed as nmol/mL plasma (Buege and Aust, 1978).

Plasma D-xylose content: The D-xylose absorption test was carried out according to the method described by Mansoori et al. (2009). Briefly, 50 μL of the collected plasma was added to 5 mL of phloroglucinol (Sigma Chemical Inc., St. Louis, MO, USA) colour reagent solution and heated at 100℃ for 4 min. The samples were allowed to cool to room temperature in a water bath. D-xylose standard solutions were prepared by dissolving D-xylose in saturated benzoic acid (prepared in deionized water) to obtain 0, 0.7, 1.3, 2.6 mmol/L. They were added to colour reagent solution as described for samples. The absorbance of all samples and standard solutions were measured, using a spectrophotometer (Model 6100, Jenway LTD., Felsted, Dunmow, CM6 3LB, Essex, England, UK), set at 554 nm. The standard solution of 0 mmol/L D-xylose was considered as blank.

Diamine oxidase assay: Diamine oxidase (DAO) activity in plasma was determined using spectrophotometry as described by Hosoda et al. (1989). The assay mixture (3.8 mL) contained 3 mL of phosphate buffer (0.2 M, pH 7.2), 0.1 mL (0.004%) of horseradish peroxidase solution (Sigma Chemicals), 0.1 mL of *o*-dianisidine–methanol solution (0.5% of *o*-dianisidine [Sigma Chemicals] in methanol), 0.5 mL of plasma, and 0.1 mL of substrate solution (0.175% of cadaverine dihydrochloride, Sigma Chemicals). This mixture was incubated for 30 min at 37℃, and absorbance at 436 nm was measured to indicate DAO activity.

Heat shock proteins-70 and α1-AGP assay: Heat shock proteins-70 (HSP-70) and α1-acid glycoprotein (α1-AGP) in plasma were determined by using commercially available enzyme-linked immunosorbent assays (ELISA) kits (Beijing SINO-UK Institute of Biological Technology, China). Optical density values were read at 450 nm. The intra- and inter-assay coefficients of variation were <10%. The detection limits for HSP-70 and α1-AGP were 0.1 ng/mL and 1.0 μg/mL, respectively.

Statistical analysis

Data were analyzed by analysis of variance using the general linear model procedures of SAS (SAS Inst. Inc., Cary, NC, USA) appropriate for a factorial arrangement of treatments with completely randomized design with 2×3 factorial arrangement. The statistical model included the effects of challenge (saline or LPS), proline (0, 0.5, and 1.0%), and their interaction. The experimental unit for the performance during d 0 to10 was the two pigs, and other statistical procedures was the individual pig. A repeated measures for the blood parameters was analyzed for the responses following LPS challenge. The normality and constant variance for experimental data were tested by the Levene's test (Wei et al., 2012). If data did not have homogenous variance, they underwent logarithm transformation to meet the necessary assumptions of analysis of variance (Wei et al., 2012). Differences among treatment means were determined by the Duncan's multiple range test. The statistical significance level for all analyses was set at $p<0.05$, and $0.05<p<0.10$ were discussed as trends.

RESULTS

Growth performance of weanling pigs

Data on growth performance of weanling pigs were shown in Table 2 and 3. Dietary supplementation with proline had no effect on ADG, ADFI, and F/G during d 0 to10 ($p>0.05$). LPS challenge could decrease ADG and ADFI ($p<0.05$), however, dietary supplementation with 0.5% proline had a tendency to increase ADG in both saline treated and LPS-challenged pigs during d 10 to 20 ($p<0.1$). There was no LPS challenge×proline interaction for ADG, ADFI, and gain/feed during d 10 to 20 ($p>0.05$).

Plasma growth factor and hormone concentrations

As shown in Table 4, LPS challenge could decrease

Table 2. Effects of L-proline supplementation on growth performance of piglets during 0-10 d[1]

Items	0% PRO	0.5% PRO	1.0% PRO	SEM	p value
Average daily gain (ADG, g)	439	457	412	20	0.15
Average daily feed intake (ADFI, g)	725	716	689	38	0.48
Feed:gain (F/G)	1.65	1.57	1.67	0.11	0.76

PRO, proline; SEM, standard error of the mean.
[1] Values are means for twelve pigs (two pigs per replicate).

Table 3. Effects of L-proline supplementation on growth performance of weanling piglets after LPS challenge or saline injection (10-20 d)[1]

Items	–LPS			+LPS			Pooled SEM	p value		
	0% PRO	0.5% PRO	1.0% PRO	0% PRO	0.5% PRO	1.0% PRO		LPS	PRO	LPS×PRO
ADG (g)	476	552	472	418	452	419	10	0.003	0.088	0.64
ADFI (g)	960	1020	926	825	850	795	52	0.004	0.547	0.71
F/G	2.02	1.85	1.96	1.99	1.88	1.90	0.19	0.92	0.88	0.44

LPS, lipopolysaccharide; PRO, proline; SEM, standard error of the mean; ADG, average daily gain; ADFI, average daily feed intake; F/G, feed:gain ration.
[1] Lipopolysaccharide was injected on d 10 and 20. Values are means for six pigs (one pig per replicate).

EGF and INS concentrations on d 20 ($p<0.05$). There was LPS challenge×proline interaction for GH concentrations on d 10 ($p<0.05$). When no LPS challenge, dietary supplementation with 1.0% proline had no effect on the GH concentrations on d 10 ($p>0.05$), but could decrease it after LPS challenge ($p<0.05$). Dietary supplementation with 1.0% proline could decrease glucagon concentration on d 10 after LPS challenge ($p<0.05$).

Plasma malondialdehyde content and activities of catalase and superoxide dismutase

As shown in Table 5, dietary supplementation with proline could increase SOD activity significantly on d 10 and 20 ($p<0.05$) in both saline-treated and LPS-challenged pigs. There was no LPS challenge×proline interaction for CAT, MDA, and SOD ($p<0.05$).

Plasma heat shock proteins-70 and α1-acid glycoprotein concentrations

Plasma HSP-70 and α1-AGP concentrations were shown in Table 6. Dietary supplementation with 1.0% proline could increase HSP-70 concentration on d 10 in both saline-treated and LPS-challenged pigs ($p<0.05$). There was no LPS challenge×proline interaction for α1-AGP and HSP-70 concentrations on d 10 and d 20 ($p>0.05$).

Plasma diamine oxidase, D-xylose, nitric oxide synthase, and NOx concentrations

Plasma DAO, D-xylose, NOS and NOx concentrations were summarized in Table 7. LPS challenge could decrease D-xylose concentrations on d 20, and NOS concentrations on d 10 ($p<0.05$). Proline supplementation had no effect on DAO concentrations, however, after LPS challenge, it could increase DAO concentrations ($p<0.05$). There was LPS

Table 4. Effects of L-proline supplementation on hormone concentrations in plasma of weanling piglets after LPS challenge (10-20 d)[1]

Items	Day	–LPS			+LPS			Pooled SEM	p value		
		0% PRO	0.5% PRO	1.0% PRO	0% PRO	0.5% PRO	1.0% PRO		LPS	PRO	LPS×PRO
EGF (U/mL)	10	1.29	1.21	1.27	1.02	1.50	1.14	0.058	0.73	0.28	0.087
	20	1.44	1.67	1.80	1.1	1.23	1.12	0.062	0.001	0.28	0.42
IGF-1 (ng/mL)	10	218.77	212	223	240	254	219	7.43	0.139	0.735	0.333
	20	224	193	239	237	225	202	9.66	0.875	0.609	0.307
GH (ng/mL)	10	4.87^{ab}	4.38^{ab}	5.38^{a}	4.60^{ab}	5.26^{ab}	4.27b	0.19	0.607	0.970	0.048
	20	5.19	5.17	5.11	5.59	5.24	4.32	0.11	0.731	0.151	0.211
Glucagon (pg/mL)	10	103	125	104	126	128	92	5.45	0.61	0.031	0.25
	20	99.9	92.2	101	75.8	110	103	6.21	0.93	0.40	0.20
INS (U/mL)	10	10.2	13.2	10.1	13.0	12.5	10.0	0.65	0.51	0.084	0.31
	20	8.55	11.7	8.60	6.82	8.48	8.03	0.38	0.046	0.08	0.47

LPS, lipopolysaccharide; PRO, proline; SEM, standard error of the mean; EGF, epidermal growth factor; IGF-I, insulin-like growth factor 1; GH, growth hormone; INS, insulin.
[1] Lipopolysaccharide was administered on d 10 and 20. Values are means for six pigs (one pig per replicate).
Means in a row with different letters differ significantly ($p<0.05$).

Table 5. Effects of L-proline supplementation on MDA content and activities of CAT and SOD of weanling piglets after LPS challenge (10-20 d)[1]

Items	Day	–LPS			+LPS			Pooled SEM	p value		
		0% PRO	0.5% PRO	1.0% PRO	0% PRO	0.5% PRO	1.0% PRO		LPS	PRO	LPS×PRO
CAT (U/mL)	10	6.25	7.16	9.09	7.31	10.5	9.63	0.67	0.19	0.20	0.63
	20	4.09	4.65	2.55	3.64	3.06	3.29	0.38	0.58	0.51	0.46
MDA (nmol/mL)	10	10.5	11.1	5.54	9.05	8.23	8.42	0.61	0.67	0.103	0.129
	20	7.33	12.0	8.62	9.24	9.27	10.61	0.89	0.78	0.44	0.34
SOD (U/mL)	10	138	156	174	139	155	149	4.06	0.13	0.004	0.11
	20	149	154	151	148	162	155	1.71	0.16	0.025	0.47

MDA, Malondialdehyde; CAT, catalase; SOD, superoxide dismutase; LPS, lipopolysaccharide; PRO, proline; SEM, standard error of the mean.
[1] Lipopolysaccharide was administered on d 10 and 20. Values are means for six pigs (one pig per pen).

Table 6. Effects of L-proline supplementation on plasma α1-AGP, and HSP-70 concentration of weanling piglets after LPS challenge (10-20 d)[1]

Items	Day	–LPS			+LPS			Pooled SEM	p value		
		0% PRO	0.5% PRO	1.0% PRO	0% PRO	0.5% PRO	1.0% PRO		LPS	PRO	LPS×PRO
α1-AGP (μg/mL)	10	309	470	283	383	375	383	47.62	0.76	0.64	0.58
	20	187	206	237	187	197	278	15.51	0.72	0.16	0.79
HSP-70 (ng/mL)	10	7.15	8.98	9.32	6.89	9.25	9.89	0.52	0.82	0.048	0.92
	20	10.08	9.34	11.48	8.80	10.24	8.83	0.44	0.22	0.67	0.20

α1-AGP, α1-acid glycoprotein; HSP-70, heat shock proteins-70; LPS, lipopolysaccharide; PRO, proline; SEM, standard error of the mean.
[1] Lipopolysaccharide was administered on d 10 and 20. Values are means for six pigs (one pig per pen).

challenge×proline interaction for DAO ($p<0.05$). Dietary supplementation with 1.0% proline could increase the D-xylose level when no LPS challenge ($p<0.05$).

DISCUSSION

Just like many previous studies, results of our study also demonstrated that both ADG and ADFI decreased after LPS challenge, which likely resulted from the impaired intestinal and immune functions (Wang et al., 2008). In this study, dietary supplementation with proline had a tendency to increase ADG, indicating a beneficial effect of supplemental appropriate proline on growth performance in piglet. However, Samuels (1989) reported that dietary supplementation with 1.0% to 1.5% proline had no effect on the performance in piglets from 1 to 10 d of age, suggesting that the requirement of proline in neonatal pig might be more than in young pigs.

Epidermal growth factor (EGF) is a 53-amino acid single-chain polypeptide. Its role in stimulating intestinal epithelium proliferation, differentiation, and intestinal maturation has been documented (Dignass and Sturm, 2001). Previous studies had reported that LPS induced the release of EGF (Ohyama et al., 2001). However, in the present study, LPS challenge had no effect on its concentration. Dietary supplementation with 0.5% proline had a tendency to increase EGF concentration after LPS challenge, which indicated that LPS challenge might provide a potential function to mobilize proline, which then stimulated EGF secretion to alleviate the LPS stress.

Table 7. Effects of L-proline supplementation on plasma D-xylose, DAO, NOx, and NOS concentration in weanling piglets after LPS challenge (10-20 d)[1]

Items	Day	–LPS			+LPS			Pooled SEM	p value		
		0% PRO	0.5% PRO	1.0% PRO	0% PRO	0.5% PRO	1.0% PRO		LPS	PRO	LPS×PRO
DAO (U/mL)	20	39.3^{ab}	28.9^{a}	46.0^{b}	29.67^{a}	46.4^{b}	47.6^{b}	2.02	0.40	0.027	0.024
D-xylose (mmol/L)	20	0.49	0.56	0.67	0.39	0.35	0.49	0.02	0.002	0.05	0.61
NOS (U/mL)	10	27.9	25.4	24.9	21.1	16.1	21.8	1.11	0.005	0.32	0.49
	20	23.7	21.7	20.8	19.7	21.9	24.5	0.89	0.97	0.86	0.14
NO (μmol/L)	10	224	232	172	162	240	172	15.09	0.53	0.20	0.55
	20	207	255	182	215	385	194	14.39	0.43	0.21	0.67

DAO, diamine oxidase; NOS, total NO synthase; NO, nitric oxide; LPS, lipopolysaccharide; PRO, proline; SEM, standard error of the mean.
[1] Lipopolysaccharide was administered on d 10 and 20. Values are means for six pigs (one pig per pen).
Means in a row with different letters differ significantly ($p<0.05$).

Growth hormone–IGF-I axis plays a major role in growth regulation. Soto et al. (1998) reported that the decrease in GH and IGF-I secretion was important mechanism in BW loss during chronic inflammation. In our study, we found that 1.0% proline could decrease GH level after LPS challenge, and there was a interaction between proline and LPS-challenge, which indicated that proline supplementation could affected GH concentration just under the LPS challenge conditions. The results were also consistent with the ADG results.

Oxidative stress is one of the major factors that impair the integrity of the gastrointestinal tract barrier and increase intestinal permeability (Kaplan et al., 2007). Antioxidant enzymes are an important part of the antioxidant system, and the antioxidant system in the body can be assessed by the determination of antioxidant enzyme activities (Buonocore and Groenendaal, 2007). Antioxidant enzymes, including CAT and SOD, require dietary supplies of the appropriated nutrients. Roecker et al. (2012) reported that acute administration of proline reduced CAT and increased SOD activities, while chronic treatment increased the activities of CAT and SOD in erythrocytes in the plasma of rats. In agreement with these findings, we found proline could increase SOD activity in piglets, with or without an LPS challenge. However, we found that proline had no effect on CAT activity.

The AGP level is an indicator of the immune or physiological status (Sorrells et al., 2006). Its serum concentration has often been used as a marker of disease. Activation of the immune system, such as inflammation, tissue injury, and infection, is associated with the release of acute phase proteins by the liver, known as the acute phase response (Suffredini et al., 1999). Heat shock proteins 70 can act as a biomarker of oxidative injury (EI Golli-Bennour and Bacha, 2011), plays essential roles in protein metabolism and stimulate cell-mediated immunity (Moroi et al., 2000). In our study, LPS challenge had no effect on α1-AGP and HSP70 concentration, however, dietary supplementation with 1.0% proline could increase HSP-70 concentration on d 10 in both saline-treated and LPS-challenged pigs, which indicated that proline could stimulate immunity response in early weaned pigs.

D-xylose, a pentose sugar, is absorbed from the upper small intestinal tract. It is poorly metabolized by the body and has been widely applied for the investigation of small intestinal absorption (Gyr et al., 1974). In the present study, proline supplementation could increase D-xylose concentration indicating that proline could have a positive effect on the gastrointestinal tract digestibility. The activity of the DAO is so high in the intestinal mucosa, that plasma DAO activity may be useful for monitoring and evaluating gastrointestinal tract injury (Namikawa et al., 2012). In the present study, 0.5% proline supplementation decreased DAO concentrations, however, it could increase DAO concentrations after LPS challenge, which suggested that proline has a positive effect on maintenance of the mucosal barrier after LPS challenge.

CONCLUSION

Proline supplementation could improve growth performance, increase SOD activities, and has a positive effect on the gastrointestinal tract digestibility in early weaned pigs. These results indicated that exogenous proline should be appropriate to add in the early weaned pigs' diets in order to achieve better growth performance.

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