

Effects of betaine on growth performance, intestinal health, and immune response of goslings challenged with lipopolysaccharide

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ABSTRACT The objective of this experiment was to investigate the effects of betaine on growth performance, serum parameters, intestinal health, and immune performance of goslings in response to lipopolysaccharide (LPS) challenge. A total of 168 healthy male 15-day-old Jiangnan White Goslings were randomly divided into 4 groups, with 6 replicates per treatment and seven goslings per replicate. A 2 × 2 factorial arrangement included 2 factors, that is, LPS challenge (injection of LPS or physiological saline) and betaine (added 0 or 0.06% betaine in diet). The results indicated that LPS challenge significantly reduced the average daily feed intake (ADFI), average daily gain (ADG), and body weight (BW) at 21 D of the goslings, while dietary betaine supplementation tended to increase the ADFI during the LPS stress period ($P = 0.08$) and BW at 21 D of the goslings ($P = 0.09$). The LPS-challenged goslings showed higher pro-inflammatory cytokines (interleukin-1 [IL-1 β], interleukin-6 [IL-6], tumor necrosis factor- α (TNF- α), and Interferon-gamma [IFN- γ]) and lower anti-inflammatory cytokine (Interleukin-10 [IL-10]) ($P < 0.05$) at 21 D of age. Dietary betaine supplementation

alleviated LPS-induced increase in pro-inflammatory cytokines. The LPS challenge significantly decreased duodenal and jejunal villus height (VH) and villus height and crypt depth ratio (VCR), while the addition of betaine significantly increased duodenal VH and VCR ($P < 0.05$). On the other hand, addition of betaine significantly alleviated decline of enzyme activity on lipase, amylase, trypsin, and chymotrypsin in the intestinal of goslings. The LPS challenge significantly increased the content of serum D-lactic acid (D-LA) and the activity of diamine oxidase (DAO) at 21 D of the goslings. The LPS challenge and betaine addition significantly increased the mRNA expression of Occludin (OCLN) in jejunal mucosa at 28 D of the goslings ($P < 0.05$). In conclusion, our research demonstrated that betaine can alleviate the decline of growth performance and immune performance in goslings caused by LPS. The results also indicate betaine possesses anti-inflammation properties and improves intestinal barrier functions. We recommend that 0.06% betaine be added into the diet to improve the intestinal health and immune performance of goslings.

Key words: betaine, goslings, lipopolysaccharide, immune performance, tight junction-related gene expression

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INTRODUCTION

Infection, pathogenic microorganisms, and environmental pollution can lead to immune stress and oxidative stress in poultry (Li et al., 2015). Immune stress also occurs in the reproduction of birds, which often results in inflammatory reactions and affects the growth of meat goslings (Liu et al., 2011). Lipopolysaccharide

(LPS) is an inflammatory stimulator that can destroy the intestinal barrier (Ma et al., 2004), which leads to an increase in intestinal permeability. Therefore, the poultry industry has used injections of LPS as a reliable model to study systemic inflammation and oxidative stress (Zheng et al., 2020). Previous studies showed that negative reactivity of birds induced by LPS can be modulated by adding feed additives, such as vitamins, synthetic antioxidants, and natural plant extracts (Fylaktakidou et al., 2004; De Boever et al., 2009).

Betaine (Bet) (N, N, N-trimethylglycine), a nontoxic amino acid derivative, has been found in several plants and organisms and provides the methyl groups for the synthesis of many substances such as methionine,

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carnitine, and creatine (Chen et al., 2018; Wu et al., 2020). Our previous studies have shown that betaine supplementation can improve the amino acid digestibility and increased lipolysis in the finishing period of goslings via reducing feed intake (Yang et al., 2016; Yang et al., 2021). In addition, betaine is anti-inflammatory and can improve intestinal function in goslings. Some researchers reported that betaine has osmotic protective properties and helps protect proteins and enzymes in intestinal cells from environmental stress (Lan and Kim, 2018; Zhao et al., 2018). Ratriyanto and Mosenthin, (2018) reported that dietary betaine supplementation could have beneficial effects on relieving physical reactions to heat stress in poultry. However, research of betaine in poultry primarily focuses on chickens and less on goslings. Furthermore, the specific mechanism regarding how betaine improves the intestinal function remains elusive. Thus, in the present study, we challenged to clarify the effect of betaine on growth performance, serum inflammatory cytokines, intestinal barrier functioning, and tight junction-related gene expression with LPS challenge.

MATERIALS AND METHODS

Ethics Statement

This animal study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Yangzhou University Animal Experiments Ethics Committee under permit number SYXK (Su) 2021-0028. All experimental procedures involving goslings were performed in accordance with the regulations for the administration of affairs concerning experimental animals approved by the State Council of the People's Republic of China.

Animals, Experimental Design, and Diets

The study was undertaken using Jiangnan White Goslings, a 3-line-cross commercial white goose with the characteristics of rapid early growth, intermediate size, superior meat quality, and a strong tolerance and adaptability to coarse feed.

The experiment was conducted using a total of 168 healthy male 15-day-old Jiangnan White Goslings, which were obtained from a commercial hatchery (Changzhou Four Seasons Poultry Industry Co. Ltd., Jintan, China). All the birds were randomly divided into four groups containing 6 replicates per treatment and 7 goslings per replicate. A 2×2 factorial arrangement included 2 factors, that is, LPS challenge (injection of LPS or physiological saline) and Bet (added 0 or 0.06% Bet in diet). This resulted in 4 treatments: control group (Ctrl) (add 0 Bet in diet + intraperitoneal injection physiological saline), Bet group (add 0.06% Bet in diet + intraperitoneal injection physiological saline), LPS group (add 0 Bet in diet + intraperitoneal injection LPS), Bet + LPS group (add 0.06% Bet in diet + intraperitoneal injection of LPS). In the morning at 16, 18, 20 D of age goslings were intraperitoneally injected with LPS or physiological saline at 1.5 mg/kg BW. The dosage of LPS in the

goslings was based on broilers (Yang et al., 2019). Betaine was added in the gosling diet after the first LPS challenge (16–28 D). SMILYCINE betaine anhydrous 75% was added on top of the basal diet, provided by Beijing Xin Dayang Co. Ltd., (Beijing, China). The composition of betaine is as follows: 75% betaine and the remaining 25% sodium chloride. The basal corn-soybean meal diet was formulated mainly according to NRC (1994) for goslings and prior research from our laboratory (Shi et al., 2007; Wang et al., 2010) (Table 1). The experiment was divided into a stress period (16–21 D) and a recovery period (22–28 D). At 21 and 28 D of age, goslings were weighed and samples taken.

All goslings had access to feed and water ad libitum throughout the trial. Water was provided in a half-open plastic cylindrical water tank, and feed was provided in feeders on one side of each pen. The feed was given in separate plastic-floored pens with 2 cm² square holes that were laid 70 cm above ground. Feces under the net bed were cleaned daily with an automatic fecal belt. The goose house was cleaned at the end of the trial. The goslings were reared indoors under similar environmental conditions (temperature: 26.0°C $\pm$ 3.0°C; relative humidity [RH]: 65.5 $\pm$ 5.0%; lighting period: 16 h; space allocation: 0.5 m²/gander).

Sample Collection and Preparation

On d 21 and 28, all birds were weighed by pen to calculate body weight (BW) and average daily gain (ADG). Feed intake (FI) by pen was utilized daily to determine the average daily feed intake (ADFI). Feed conversion

Table 1. Ingredients and nutrient composition of the experimental diets.

Ingredients	15–28 d
Maize	63.1
Soybean meal	27.8
Wheat bran	5.6
Limestone	1.0
Calcium hydrogen phosphate	1.1
DL-methionine	0.1
Salt	0.3
Premix ¹⁾	1.0
Total	100.0
Calculated values (as-fed basis)	
ME (MJ/kg) ²⁾	11.60
Non-phytase phosphorus (%)	0.40
Analyzed values (as-fed basis)	
Crude protein (%)	18.01
Crude fiber (%)	3.03
Calcium (%)	0.79
Total phosphorus (%)	0.65
Methionine (%)	0.36
Lysine (%)	1.03

¹⁾Premix was provided by the Yangzhou University Feed Company (Yangzhou, China). One kilogram of premix contained the following: retinol, 1,200,000 IU; cholecalciferol, 400,000 IU; α -tocopherol, 1,800 IU; 2-methyl-1,4-naphthoquinone, 150 mg; thiamin, 90 mg; riboflavin, 800 mg; pyridoxine, 320 mg; cobalamin, 1 mg; nicotinic acid, 4.5 g; pantothenic acid, 1,100 mg; folic acid, 65 mg; biotin, 5 mg; choline, 45 mg; Fe (as ferrous sulfate), 6 g; Cu (as copper sulfate), 1 g; Mn (as manganese sulfate), 9.5 g; Zn (as zinc sulfate), 9 g; I (as potassium iodide), 50 mg; Se (as sodium selenite), 30 mg.

²⁾The values were calculated from the ingredients' apparent metabolizable energy (AME) values for chickens.

ratios (**FCR**) were calculated from 16 to 21 d and 22 to 28 d, and mortality was recorded as it occurred.

On d 21 and 28, two goslings from each pen were randomly selected for blood sample collection. A butterfly needle with a luer adapter was inserted in wing veins of the goslings, and 3 mL blood was collected into a disposable negative pressure blood collection vessel. Blood samples were centrifuged for 10 min at 4,500 rpm to obtain serum for the measurement of biochemical indices. The serum was stored at -20°C for the analysis of clinical blood parameters.

After taking blood, the goslings were killed by cervical dislocation to obtain intestinal samples. Two-centimeter-segments from the median sections of the duodenum and jejunum were collected and placed in 10% neutral buffered formalin for further morphological measurements. Duodenum spanned the gizzard outlet to the end of the pancreatic loop and jejunum spanned the pancreatic loop to Meckel's diverticulum. The digesta was gently squeezed from the duodenum and jejunum with tweezers and stored in deactivation centrifuge tubes to detect digestive enzyme activity. The digesta was stored at -20°C for intestinal digestive enzyme activity. After taking the digesta, duodenum and jejunum were opened gently, rinsed with distilled water, and the mucosa was gently scraped off the underlying musculature from the middle portion by using a glass slide and stored in a deactivation centrifuge tube. The mucosa samples were quickly frozen in liquid nitrogen and stored at -80°C for RT-PCR and western blot analysis.

Serum Parameters

The protein levels of interleukin-1 (**IL-1 β**), interleukin-6 (**IL-6**), tumor necrosis factor- α (**TNF- α**), interferon-gamma (**IFN- γ**), and interleukin-10 (**IL-10**) in the serum of goslings were tested using ELISA kits, which were purchased from Shanghai Yubo Biotechnology Co. Ltd., (Shanghai, China). Both in-batch and inter-batch coefficients of variation were less than 10%.

D-lactic acid (**D-LA**) and diamine oxidase (**DAP**) content in the gosling serums were tested using ELISA kits, which were purchased from Shanghai Yubo Biotechnology Co. Ltd., (Shanghai, China).

Intestinal Morphology

The duodenal and jejunal segments were embedded in wax, sectioned at a thickness of 5 μm , and stained with

hematoxylineosin. For histological morphometric observations, the slides were analyzed by light microscopy, and digital images were captured (LY-WN-HP SUPER CCD, Chengdu, China). The villus height (**VH**) and crypt depth (**CD**) were measured for 10 villi at $\times 40$ magnification as described by (Sarker et al., 2021), with minor modifications. Villus height and crypt depth ratio (**VCR**) were calculated by VH:CD .

Intestinal Digestive Enzyme Activity

The duodenal and jejunal digestive enzyme activity of lipase, amylase, trypsin, and chymotrypsin were measured using kits purchased from Nanjing Jiancheng Biotechnology Co., Ltd (Nanjing, China).

RT-PCR

Reverse transcriptase-polymerase chain reaction was used to detect the expression of relative mRNA of Occludin (**OCLN**), tight junction protein 1 (**TJP1**), and β -actin. Total RNA was extracted from livers using Trizol reagent (TIANGEN, Beijing, China). Thermal cycling was carried out with an ABI Prism 7500 sequence detection system (Applied Biosystems, Shanghai, China) using factory default conditions (95°C , 15 min) and 40 cycles at 95°C , 10 s; 55°C , 30 s; 72°C , 32 s). Results were presented as the ratio of each gene β -actin to correct for differences in the amounts of template DNA used. Primer sequences for amplification of OCLN, TJP1, and β -actin were synthesized by Shanghai Sunny Biotechnology Co., Ltd. (Shanghai, China) and are shown in Table 2. Fold changes from the reference were calculated as $2^{-\Delta\Delta\text{CT}}$, where Ct is the threshold cycle.

Western Blot

Total protein lysates were collected for standard immunoblot analysis. The protein concentrations were determined by bicinchoninic acid (**BCA**) protein assay (Thermo Fisher Technology (China) Co., Ltd, Shanghai, China). Aliquots of protein lysates (30 mg/lane) were loaded into gels for sodium dodecyl sulfide-polyacrylamide gel electrophoresis (**SDS-PAGE**), and the separated proteins were transferred to a polyvinylidene fluoride (**PVDF**) membrane (Thermo Fisher Technology (China) Co., Ltd). The membrane was blocked and incubated with OCLN and GAPDH antibodies

Table 2. Primer sequences used for qRT-PCR in this study.

Name	Primer sequence (5'→3')	Product size	Reference
Occludin-F	5'-TGCTTCCAGCTCCATCCAAG-3'	278 bp	XM_013199669.1
Occludin-R	5'-CTTGTCGTAGTCGCTCACCA-3'		
TJP1-F	5'-ACGCTGTTGAATGTCCC-3'	216 bp	XM_013177404.1
TJP1-R	5'-TCGAAGACTGCCGTTGC-3'		
β -actin-F	5'-GCACCCAGCACGATGAAAAT-3'	150 bp	XM_013174886.1
β -actin-R	5'-GACAAATGGAGGTTCCCGATT-3'		

Abbreviations: F, forward; qRT-PCR, quantitative reverse transcription polymerase chain reaction; R, reverse; TJP1, tight junction protein 1.

overnight at 4°C. The OCLN and GAPDH antibodies were purchased from Abcam Trading Co., Ltd (Shanghai, China). The blots were washed with TBST (Sigma-Aldrich, Shanghai, China) and incubated with corresponding horseradish peroxidase-conjugated secondary antibodies (Jackson Laboratory, Bar Harbor, ME). Finally, the blots were visualized with enhanced chemiluminescence and quantified by densitometry.

Statistical Analysis

The observation unit was the floor pen for growth performance, while for the other variables the observation unit was the individual gosling. The experimental data was preliminarily sorted using Excel 2020, and then analyzed with SPSS 20.0 software (Ver. 20.0 for Windows, SPSS, Inc., Chicago, IL). A randomized block ANOVA analysis of repeated measures was performed, and 2×2 factorial structure was used to investigate the 2 treatment factors (challenge of LPS and the presence of betaine) and their interactions. The significant differences among groups were determined using Tukey's multiple-range tests. Differences were reported as significant at $P < 0.05$. The data analysis results were expressed as mean values and standard errors.

RESULTS

Growth Performance

Mortality data was transformed before analysis. Effects of betaine on growth performance of goslings challenged by LPS are shown in Table 3. During the stress period of LPS injection (16–21 D), LPS challenge significantly reduced the ADFI, ADG, and BW at 21 D of the goslings, and significantly increased the FCR ($P < 0.05$). Dietary betaine tends to increase the ADFI during the LPS stress period ($P = 0.08$) and BW at 21 D of the goslings ($P = 0.09$). Significant interaction between LPS

challenge and dietary betaine was detected in terms of ADFI of the goslings ($P = 0.05$). In the recovery period (22–28 D), LPS challenge significantly reduced the ADFI, FCR, and BW at 28 D of the goslings ($P < 0.05$). Dietary betaine had no significant effect on the growth performance of the goslings during the recovery period.

Inflammatory Cytokines in Serum

Effects of betaine on inflammatory cytokines in the gosling serums challenged by LPS are shown in Table 4. During the stress period of LPS injection, LPS challenge significantly increased the serum pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α and IFN- γ) at 21 D of the goslings ($P < 0.05$) and decreased anti-inflammatory cytokine (IL-10). The addition of betaine significantly decreased serum pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α , and IFN- γ) at 21 D of the goslings ($P < 0.05$). Significant interaction between LPS challenge and dietary betaine was detected for pro-inflammatory cytokines during the LPS stress period.

In the recovery period, LPS challenge still significantly increased the serum pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α and IFN- γ) at 28 D of the goslings ($P < 0.05$), while decreasing anti-inflammatory cytokine (IL-10).

Intestinal Morphology

Effects of betaine on duodenal morphology of goslings challenged by LPS are shown in Table 5. During the LPS injection stress period, LPS challenge significantly decreased duodenal VH and VCR, but significantly increased duodenal CD ($P < 0.05$). The addition of betaine significantly increased duodenal VH and VCR ($P < 0.05$). LPS challenge and dietary betaine had significant interaction effects on duodenal VH ($P < 0.05$).

Effects of betaine on gosling jejunal morphology challenged by LPS are shown in Table 6. During the

Table 3. Effects of betaine on growth performance of goslings challenged by LPS.

		16–21 d					22–28 d			
Items		15 dBW/g	21 dBW/g	ADFI/g	ADG/g	FCR	28 dBW/g	ADFI/g	ADG	FCR
Groups	Ctrl	804	1,180	141.5	62.7	2.26	1,684	361.3	71.9	5.04
	Bet	801	1,179	140.7	63.1	2.24	1,693	358.8	73.3	4.91
	LPS	802	955	87.5	25.5	3.53	1,426	294.7	67.3	4.40
	Bet + LPS	807	995	101.1	31.4	3.22	1,498	314.2	71.8	4.38
SEM		3.39	10.90	3.19	1.93	0.11	23.97	7.79	2.41	0.13
Primary effects										
LPS	-	802	1,180	141.1	62.9	2.25	1,688	360.1	72.6	4.98
	+	805	977	94.9	28.7	3.36	1,465	305.2	69.7	4.39
Bet	-	803	1,078	117.0	45.8	2.84	1,567	331.0	69.8	4.75
	+	804	1,187	120.9	47.3	2.73	1,595	336.5	72.6	4.65
P-value	LPS	0.497	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.215	<0.001
	Bet	0.878	0.090	0.080	0.119	0.223	0.111	0.298	0.239	0.593
	Interaction	0.252	0.075	0.050	0.170	0.292	0.205	0.179	0.528	0.685

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; Bet, betaine; BW, body weight; FCR, Feed conversion ratio.

Control group (Ctrl): add 0 Bet in diet + intraperitoneal injection physiological saline; Bet group (Bet): add 0.06% Bet in diet + intraperitoneal injection physiological saline; LPS group (LPS): add 0 Bet in diet + intraperitoneal injection LPS; Bet + LPS group (Bet + LPS): add 0.06% Bet in diet + intraperitoneal injection of LPS.

In the same column, values with different small letter superscripts indicate a significant difference ($P \leq 0.05$), while with the same or no letter superscripts indicate no significant difference ($P > 0.05$).

Table 4. Effects of betaine on the contents of inflammatory cytokines in serum of goslings challenged by LPS pg/mL.

Items		21 d					28 d				
		IL-1 β	IL-6	TNF- α	IFN- γ	IL-10	IL-1 β	IL-6	TNF- α	IFN- γ	IL-10
Groups	Ctrl	23.43	17.31	107.00	31.88	62.17	24.46	17.83	109.94	29.08	59.62
	Bet	23.92	18.61	109.49	29.38	57.96	25.47	19.38	111.05	29.06	56.00
	LPS	71.12	33.18	221.43	62.84	28.09	50.22	22.19	164.33	58.55	32.64
	Bet + LPS	41.58	24.86	154.37	46.34	40.07	46.97	22.90	161.39	51.53	37.07
SEM		2.10	0.91	5.46	3.02	2.65	2.21	0.85	4.06	2.44	2.28
Primary effects											
LPS	-	23.68	17.96	108.24	30.63	60.06	24.96	18.60	110.49	29.07	57.81
	+	57.69	29.40	190.95	55.34	33.54	48.45	22.58	162.72	54.72	35.05
Bet	-	47.27	25.24	164.21	47.36	45.13	36.17	19.81	134.66	42.48	47.36
	+	31.95	21.45	129.89	37.09	49.83	36.22	21.14	136.22	40.30	46.53
<i>P</i> -value	LPS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Bet	<0.001	0.003	<0.001	0.006	0.189	0.638	0.216	0.826	0.186	0.866
	Interaction	<0.001	<0.001	<0.001	0.033	0.010	0.375	0.641	0.625	0.188	0.104

Abbreviations: Bet, betaine; IL-1 β , interleukin-1; IL-6, Interleukin-6; TNF- α , tumor necrosis factor- α ; IFN- γ , Interferon-gamma; IL-10, Interleukin-10.

Control group (Ctrl): add 0 Bet in diet + intraperitoneal injection physiological saline; Bet group (Bet): add 0.06% Bet in diet + intraperitoneal injection physiological saline; LPS group (LPS): add 0 Bet in diet + intraperitoneal injection LPS; Bet + LPS group (Bet + LPS): add 0.06% Bet in diet + intraperitoneal injection of LPS.

Table 5. Effects of betaine on duodenal morphology of goslings after challenged by LPS.

Items		21 d			28 d		
		VH (μ m)	CD (μ m)	VCR	VH (μ m)	CD (μ m)	VCR
Groups	Ctrl	806	248.4	3.25	924	249.1	3.72
	Bet	822	243.0	3.38	960	246.4	3.90
	LPS	686	266.9	2.57	964	257.4	3.75
	Bet + LPS	753	257.6	2.93	955	252.9	3.78
SEM		10.11	3.32	0.05	18.51	3.05	0.11
Primary effects							
LPS	-	813	246.0	3.31	940	247.9	3.80
	+	716	262.7	2.73	960	255.2	3.77
Bet	-	746	257.6	2.91	944	253.2	3.74
	+	787	250.3	3.16	957	250.0	3.83
<i>P</i> -value	LPS	<0.001	0.001	<0.001	0.416	0.028	0.724
	Bet	0.002	0.076	0.001	0.538	0.262	0.371
	Interaction	0.036	0.623	0.085	0.314	0.774	0.498

Bet, betaine; CD, Crypt depth; VCR, villus height and crypt depth ratio; VH, Villus height.

Control group (Ctrl): add 0 Bet in diet + intraperitoneal injection physiological saline; Bet group (Bet): add 0.06% Bet in diet + intraperitoneal injection physiological saline; LPS group (LPS): add 0 Bet in diet + intraperitoneal injection LPS; Bet + LPS group (Bet + LPS): add 0.06% Bet in diet + intraperitoneal injection of LPS.

stress period of LPS injection, LPS challenge significantly decreased jejunal VH and VCR, but significantly increased jejunal CD ($P < 0.05$). The addition of betaine significantly increased the jejunal VH and

VCR, but significantly decreased jejunal CD ($P < 0.05$). The LPS challenge and dietary betaine had significant interaction effects on jejunal CD and VCR ($P < 0.05$). In the recovery period, LPS challenge

Table 6. Effects of betaine on jejunal morphology of goslings challenged by LPS.

Items		21 d			28 d		
		VH (μ m)	CD (μ m)	VCR	VH (μ m)	CD (μ m)	VCR
Groups	Ctrl	862.3	264.3	3.26	1,046	251.9	4.17
	Bet	880.3	265.5	3.32	1,017	260.5	3.91
	LPS	777.1	302.6	2.57	1,062	243.0	4.40
	Bet + LPS	856.6	276.6	3.10	1,037	255.6	4.07
SEM		19.89	3.09	0.06	27.87	6.41	0.16
Primary effects							
LPS	-	870.3	264.9	3.28	1,031	256.2	4.04
	+	816.8	289.6	2.84	1,050	249.3	4.23
Bet	-	819.7	283.4	2.91	1,054	247.5	4.28
	+	867.1	271.7	3.20	1,027	258.1	3.99
<i>P</i> -value	LPS	0.031	<0.001	<0.001	0.540	0.315	0.255
	Bet	0.050	0.002	<0.001	0.364	0.130	0.094
	Interaction	0.198	0.001	0.003	0.931	0.761	0.843

Abbreviations: Bet, betaine; VH, Villus height; CD, Crypt depth; VCR, villus height and crypt depth ratio.

Control group (Ctrl): add 0 Bet in diet + intraperitoneal injection physiological saline; Bet group (Bet): add 0.06% Bet in diet + intraperitoneal injection physiological saline; LPS group (LPS): add 0 Bet in diet + intraperitoneal injection LPS; Bet + LPS group (Bet + LPS): add 0.06% Bet in diet + intraperitoneal injection of LPS.

Table 7. Effects of betaine on duodenal digestive enzyme activity of goslings challenged by LPS.

Items		21 d				28 d			
		Lipase (U/g)	Amylase (U/g)	Trypsin (U/g)	Chymotrypsin (IU/g)	Lipase (U/g)	Amylase (IU/g)	Trypsin (U/g)	Chymotrypsin (IU/g)
Groups	Ctrl	3.57	2.66	2.65	1.80	4.67	2.84	2.74	1.92
	Bet	4.94	2.92	2.85	2.03	5.23	3.45	3.01	2.14
	LPS	2.99	1.94	2.16	1.41	3.75	2.30	2.31	1.55
	Bet + LPS	4.16	2.42	2.40	1.72	4.59	2.74	2.65	1.75
SEM		0.30	0.10	0.07	0.07	0.23	0.12	0.09	0.06
Primary effects									
LPS	-	4.25	2.79	2.75	1.92	4.95	3.15	2.88	2.03
	+	3.58	2.18	2.28	1.56	4.20	2.52	2.48	1.65
Bet	-	3.28	2.30	2.40	1.60	4.25	2.57	2.53	1.74
	+	4.55	2.67	2.63	1.87	4.91	3.09	2.83	1.95
<i>P</i> -value	LPS	0.001	<0.001	<0.001	<0.001	0.005	<0.001	0.001	<0.001
	Bet	0.039	0.030	0.005	0.001	0.010	<0.001	0.005	0.002
	Interaction	0.743	0.240	0.746	0.584	0.592	0.484	0.711	0.791

Abbreviation: Bet, betaine.

Control group (Ctrl): add 0 Bet in diet + intraperitoneal injection physiological saline; Bet group (Bet): add 0.06% Bet in diet + intraperitoneal injection physiological saline; LPS group (LPS): add 0 Bet in diet + intraperitoneal injection LPS; Bet + LPS group (Bet + LPS): add 0.06% Bet in diet + intraperitoneal injection of LPS.

and betaine addition had no significant effects on VH, CD, and VCR ($P > 0.05$).

Intestinal Digestive Enzyme Activity

Effects of betaine on duodenal digestive enzyme activity of goslings challenged by LPS are shown in Table 7. During stress and recovery periods, LPS challenge significantly decreased the activity of lipase, amylase, trypsin, and chymotrypsin in the duodenum ($P < 0.05$). The addition of betaine significantly increased the activity of lipase, amylase, trypsin, and chymotrypsin in the duodenum ($P < 0.05$). The addition of betaine effectively alleviated the effect of LPS stimulation on gosling duodenal digestive enzyme activity.

Effects of betaine on jejunal digestive enzyme activity of goslings challenged by LPS are shown in Table 8. During the stress and recovery period, LPS challenge significantly decreased the activity of lipase, amylase, trypsin, and chymotrypsin in the jejunum ($P < 0.05$). The

addition of betaine significantly increased activity of lipase, amylase, and trypsin in the jejunum ($P < 0.05$). There was no significant interaction between LPS challenge and betaine addition on jejunal digestive enzyme activity ($P > 0.05$). The addition of betaine effectively alleviated the effect of LPS stimulation on gosling jejunal digestive enzyme activity.

Intestinal Permeability Parameters

Effects of betaine on gosling intestinal permeability challenged by LPS are shown in Table 9. During the stress and recovery periods, LPS challenge significantly increased the content of serum D-lactic acid (D-LA) and the activity of diamine oxidase (DAO) at 21 D of the goslings. In the stress period, LPS challenge and dietary betaine had significant interaction effects on the activity of serum DAO at 21 D of the goslings ($P < 0.05$).

Table 8. Effects of betaine on jejunal digestive enzyme activity of goslings challenged by LPS.

Items		21 d				28 d			
		Lipase (U/g)	Amylase (U/g)	Trypsin (U/g)	Chymotrypsin (IU/g)	Lipase (U/g)	Amylase (IU/g)	Trypsin (U/g)	Chymotrypsin (IU/g)
Groups	Ctrl	5.12	2.84	2.52	1.88	6.34	2.93	2.72	1.92
	Bet	6.04	3.42	2.74	1.94	6.81	3.50	3.04	2.14
	LPS	4.02	2.18	2.29	1.42	4.61	2.47	2.49	1.67
	Bet + LPS	4.87	2.51	2.53	1.64	5.14	2.83	2.66	1.88
SEM		0.25	0.13	0.09	0.07	0.22	0.11	0.08	0.07
Primary effects									
LPS	-	5.58	3.13	2.63	1.91	6.57	3.21	2.88	2.03
	+	4.45	2.34	2.41	1.53	4.88	2.65	2.57	1.78
Bet	-	4.57	2.51	2.41	1.65	5.47	2.70	2.61	1.80
	+	5.45	2.96	2.64	1.79	5.97	3.16	2.85	2.01
<i>P</i> -value	LPS	<0.001	<0.001	0.028	<0.001	<0.001	<0.001	0.001	0.003
	Bet	0.003	0.005	0.022	0.065	0.044	0.001	0.009	0.008
	Interaction	0.899	0.407	0.888	0.297	0.887	0.382	0.410	0.924

Abbreviations: Bet, betaine.

Control group (Ctrl): add 0 Bet in diet + intraperitoneal injection physiological saline; Bet group (Bet): add 0.06% Bet in diet + intraperitoneal injection physiological saline; LPS group (LPS): add 0 Bet in diet + intraperitoneal injection LPS; Bet + LPS group (Bet + LPS): add 0.06% Bet in diet + intraperitoneal injection of LPS.

Table 9. Effects of betaine on intestinal permeability of goslings challenged by LPS.

		21 d		28 d	
Items		D-LA(ng/mL)	DAO(U/mL)	D-LA(ng/mL)	DAO(U/mL)
Groups	Ctrl	17.08	0.87	17.30	0.76
	Bet	18.76	0.92	17.75	0.80
	LPS	31.80	1.64	32.53	1.41
	Bet + LPS	33.21	1.43	27.86	1.31
SEM		1.06	0.05	1.33	0.05
Primary effects					
	LPS				
LPS	-	17.92	0.90	17.53	0.78
	+	32.44	1.54	29.99	1.35
Bet	-	24.44	1.26	24.23	1.05
	+	35.33	1.15	22.81	1.05
<i>P</i> -value	LPS	<0.001	<0.001	<0.001	<0.001
	Bet	0.192	0.109	0.143	0.615
	Interaction	0.906	0.017	0.079	0.216

Abbreviations: D-LA, D-lactic acid; DAP, diamine oxidase.

Control group (Ctrl): add 0 Bet in diet + intraperitoneal injection physiological saline; Bet group (Bet): add 0.06% Bet in diet + intraperitoneal injection physiological saline; LPS group (LPS): add 0 Bet in diet + intraperitoneal injection LPS; Bet + LPS group (Bet + LPS): add 0.06% Bet in diet + intraperitoneal injection of LPS.

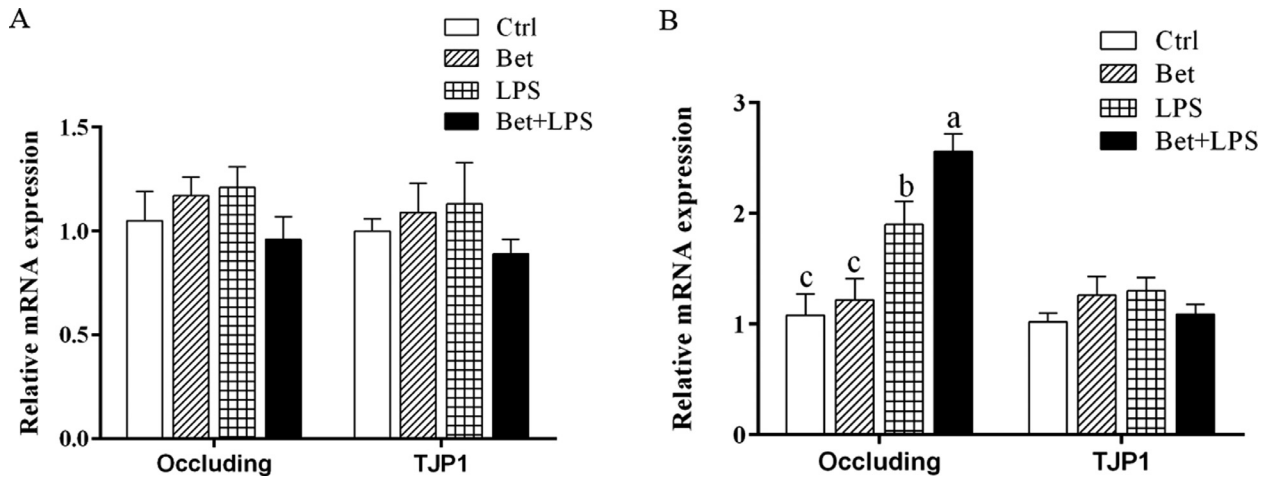


Figure 1. Effect of betaine on mRNA expression of Occludin and TJP1 in jejunal mucosa of goslings challenged by LPS (A, 21 days of age; B, 28 days of age).

Expression of OCLN and TJP1

Effect of betaine on mRNA expression of OCLN and TJP1 in jejunal mucosa of goslings challenged by LPS is shown in Figure 1. During the stress period of LPS injection (16–21 D), LPS stimulation and betaine supplementation had no significant effect on the mRNA expression of Occludin and TJP1 in jejunal mucosa at 21 D of the goslings ($P > 0.05$). In the recovery period (22–28 D), LPS challenge and betaine addition significantly increased the mRNA expression of Occludin in jejunal mucosa at 28 D of the goslings ($P < 0.05$).

Effect of betaine on protein expression of OCLN in jejunal mucosa of goslings challenged by LPS is shown in Figure 2. During the stress period of LPS injection (16–21 D), LPS stimulation tended to decrease the expression of OCLN proteins in jejunum of goslings.

DISCUSSION

A large number of studies on the application effect of betaine on animals have accelerated the development

and promotion of betaine as a feed additive. Betaine also has anti-inflammation properties and improve intestinal barrier. However, how betaine improves intestinal function is not clear and needs to be explored. In the present study, for the first time LPS challenge was administered to goslings to induce oxidative stress and inflammation in the starter stage. We challenged to clarify the effect of betaine on growth performance, serum inflammatory cytokines, intestinal barrier functioning, and tight junction-related gene expression with LPS challenge. Our results showed that LPS significantly reduced the ADFI, ADG, and BW at 21 D of the goslings, which was similar to previous research results in broilers (Hu et al., 2020), indicating an immune stress model of LPS in goslings was successfully constructed.

In the present study, dietary betaine supplementation induced a numerical increase in the ADG of goslings during 1 to 21 d of age, without reaching a statistically significant level. Some previous experiments reported that dietary supplementation of betaine improved laying performance of hens raised under heat stress conditions. Liu et al. (2019) demonstrated that, along with a dose effect,

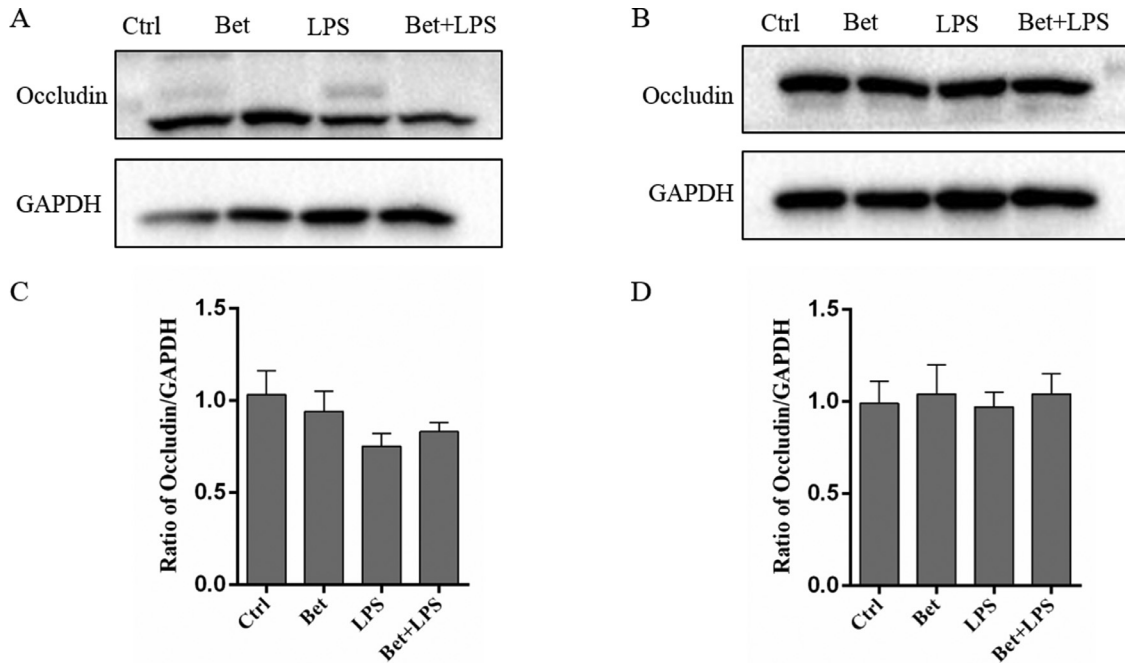


Figure 2. Effect of betaine on protein expression of Occludin in jejunal mucosa of goslings challenged by LPS. (A, Western blots diagram of 21 days of age; B, Western blots diagram of 28 days of age; C, densitometric analyses of Occludin/GAPDH ratio of 21 days of age, densitometric analyses of Occludin/GAPDH ratio of 28 days of age).

the addition of 0.05, 0.1, and 0.2% betaine to the diet could alleviate the adverse effects of heat stress on ADFI and BW in yellow feather broilers. Hamidi et al. (2010) reported that adding 0.12% betaine to the diet can significantly improve the daily weight gain of broilers infected with mixed coccidia, which may be related to the protective effect of betaine on intestinal epithelial cells and mucosa. The beneficial effect of betaine has been associated with requirements for methyl donors and an increase in nutrient digestibility and intestinal health (Yang et al., 2017). Thus, it is suggested that dietary supplementation of betaine has a positive effect on growth performance under LPS challenge.

Cytokines are low molecular, weight-soluble proteins induced by immunogens or other exogenous stimulants, which are key modulators of immunity (Yu et al., 2021). The LPS itself has no toxic effect, but as a nonspecific immunogen, it interacts with host effector cells when entering the microcirculation to produce inflammatory cytokines such as TNF- α and IL-1 β (Murray and Smale, 2012). The cytokines in serum could reflect the inflammatory reaction of the body. The results showed that LPS stimulation significantly increased the serum pro-inflammatory cytokine IL-1 β , IL-6, TNF- α , and IFN- γ in goslings. However, the content of anti-inflammatory cytokine IL-10 in serum was significantly decreased, indicating that the body experienced a systemic inflammatory response. The addition of betaine in the diet could reduce the serum pro-inflammatory cytokine IL-1 β , IL-6, TNF- α , and IFN- γ content in goslings. It was reported that IL-6 and TNF- α can effectively cause satiety and loss of appetite by stimulating the hypothalamus (Paulsen et al., 2017). Therefore, the addition of betaine in this experiment can significantly increase the feed intake of goslings, which may be related to the

inhibition of the release of inflammatory cytokines. At 28 d of age, LPS stimulation can significantly increase the content of serum pro-inflammatory cytokines IL-1 β , IL-6, TNF- α , and IFN- γ as well as reduce the content of serum anti-inflammatory cytokine IL-10. This may be due to the chronic immune stress effect caused by LPS and the need for a longer recovery for goslings.

The intestines act in feed digestion and absorption and serve as an important barrier against all pathogenic bacteria and toxic substances present in the intestinal lumen (Kheradmand et al., 2013). When the morphology of the villus is destroyed, the crypt depth increases, VH recovery slows, and digestive tract absorption functioning declines, adversely affecting the growth and performance of goslings. The present study showed that LPS stimulation significantly reduced the VH and VCR of duodenum and jejunum of goslings, while the addition of betaine significantly increased the VH and VCR of duodenum and jejunum, effectively alleviating the damage of LPS stress to the small intestine. Our result was similar to the previous research on the changes of intestinal morphology and structure under the stress of livestock and poultry (Amerah and Ravindran, 2015; Attia et al., 2019).

Intestinal enzyme activities play a crucial role in the physiological process and life activities of animals (Cho et al., 2012). It was widely reported that LPS stress has malabsorption syndrome regarding macronutrients and reduced activity of digestive enzymes (Cao et al., 2018). The present study showed that LPS stimulation significantly decreased gosling duodenal and jejunal digestive enzymes, including lipase, amylase, trypsin, and chymotrypsin. The possible reason was ascribed to the damage of LPS challenge, which lead to intestinal disorders, resulting in the alteration of nutrient digestion,

absorption function, and reduced growth performance of goslings. The addition of betaine in the gosling diet significantly improved intestinal digestive enzymes, including lipase, amylase, trypsin, and chymotrypsin. However, due to the limited data available regarding the effect of betaine on digestive enzymes activity in LPS challenged goslings, no more comparisons could be made. On the other hand, Wang et al. (2018) reported that the addition of betaine can increase the activities of amylase, lipase, trypsin, and chymotrypsin in the small intestine of stressed rats. Liu et al. (2019) also confirmed this in yellow-feathered broilers. A possible explanation for the increased enzyme activities of betaine was ascribed to improved intestinal morphology and nutrient absorption.

DAO is an enzyme in intestinal epithelial cells that suppresses cell proliferation by reducing polyamine concentrations, whereas D-LA is a bacterial metabolite produced by intestinal flora (Meng et al., 2016). In the process of intestinal infection and inflammation, the permeability of the intestinal wall is increased, and the translocation of many microorganisms from the intestinal tract to the circulation is increased. Intraluminal DAO and D-LA can easily enter the peripheral blood through the intestinal mucosa (Shi et al., 2022). The present study showed that LPS stimulation significantly reduced D-LA content and DAO activity, indicating that the intestinal permeability of the goslings was increased. Moreover, LPS stimulation and betaine addition had a significant interaction effect on DAO activity, suggesting that betaine addition could alleviate the LPS oxidative stress response by re-establishing intestinal barrier function. Dietary betaine can assemble in intestinal tissues and reinforce the structure of intestinal epithelium. The results of our study were consistent with the known effects of betaine in most studies. It has been suggested that the addition of betaine can increase the osmotic stability of intestinal cells and reduce the protein denaturation of intestinal cells, resulting in the decrease of intestinal permeability (Shin et al., 2018).

The current experiment measured tight junction-related gene expressions in the jejunal mucosa. The OCLN and TJP1 are transmembrane proteins of tight junction, which play a key role in maintaining the barrier function of tight connections (Ulluwishewa et al., 2011). The results of our study showed that mRNA expression of OCLN significantly increased in jejunal mucosa in the recovery period with the addition of betaine in diets. The current results showed that improvements in growth performance in goslings with betaine are likely associated with the selective modification of tight junction-related genes, especially the significant increase of OCLN expression (Shin et al., 2018). There was no significant difference in protein expression level, which may be due to apparent modification. However, it was unexpected that OCLN mRNA was also increased under LPS challenge as previous experiments reported OCLN were downregulated when tight junction integrity was decreased (Song et al., 2014). More research is

needed to observe the interaction among those selective gene expressions for tight junction integrity.

In conclusion, LPS stimulation significantly reduced growth performance and immune function, while adding betaine alleviated the increase of serum proinflammatory cytokines in goslings. The beneficial effects of betaine may be associated with the improvement of morphological structure and digestive enzyme activity of the small intestine and the change of intestinal permeability with selective modification of tight junction-related gene expressions in the gosling jejunal mucosa.

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DISCLOSURES

The authors have no conflicts of interest to declare.

REFERENCES

- Amerah, A. M., and V. Ravindran. 2015. Effect of coccidia challenge and natural betaine supplementation on performance, nutrient utilization, and intestinal lesion scores of broiler chickens fed suboptimal level of dietary methionine. *Poult. Sci.* 94:673–680.
- Attia, Y. A., A. S. El-Naggar, B. M. Abou-Shehema, and A. A. Abdella. 2019. Effect of supplementation with trimethylglycine (betaine) and/or vitamins on semen quality, fertility, antioxidant status, DNA repair and welfare of roosters exposed to chronic heat stress. *Animals* 9:547.
- Cao, S., Q. Zhang, C. Wang, H. Wu, L. Jiao, Q. Hong, and C. Hu. 2018. LPS challenge increased intestinal permeability, disrupted mitochondrial function and triggered mitophagy of piglets. *Innate. Immun.* 24:221–230.
- Chen, R., S. Zhuang, Y. P. Chen, Y. F. Cheng, C. Wen, and Y. M. Zhou. 2018. Betaine improves the growth performance and muscle growth of partridge shank broiler chickens via altering myogenic gene expression and insulin-like growth factor-1 signaling pathway. *Poult. Sci.* 97:4297–4305.
- Cho, I., S. Yamanishi, L. Cox, B. A. Methé, J. Zavadi, K. Li, Z. Gao, D. Mahana, K. Raju, I. Teitler, H. Li, A. V. Alekseyenko, and M. J. Blaser. 2012. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* 488:621–626.
- De, Boever, S., S. Croubels, E. Meyer, S. Sys, R. Beyaert, R. Ducatelle, and P. De Backer. 2009. Characterization of an intravenous lipopolysaccharide inflammation model in broiler chickens. *Avian. Pathol.* 38:403–411.
- Fylaktakidou, K. C., D. J. Hadjipavlou-Litina, K. E. Litinas, and D. N. Nicolaidis. 2004. Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities. *Curr. Pharm. Des.* 10:3813–3833.
- Hamidi, H., R. J. Jahanian, and J. Pourreza. 2010. Effect of dietary betaine on performance, immunocompetence and gut contents osmolarity of broilers challenged with a mixed coccidial infection. *Asian. J. Anim. Vet. Adv.* 5:193–201.
- Hu, R., Z. He, M. Liu, J. Tan, H. Zhang, D. X. Hou, J. He, and S. Wu. 2020. Dietary protocatechuic acid ameliorates inflammation and up-regulates intestinal tight junction proteins by

- modulating gut microbiota in LPS-challenged piglets. *J. Anim. Sci. Biotechnol.* 9:92.
- Kheradmand, A., M. Alirezai, and O. Dezfoulian. 2013. Cadmium-induced oxidative stress in the rat testes: protective effects of betaine. *Int. J. Pept. Res. Ther.* 19:337–344.
- Lan, R., and I. Kim. 2018. Effects of feeding diets containing essential oils and betaine to heat-stressed growing-finishing pigs. *Arch. Anim. Nutr.* 72:368–378.
- Li, Y., H. Zhang, Y. P. Chen, M. X. Yang, L. L. Zhang, Z. X. Lu, Y. M. Zhou, and T. Wang. 2015. *Bacillus amyloliquefaciens* supplementation alleviates immunological stress in lipopolysaccharide challenged broilers at early age. *Poult. Sci.* 94:1504–1511.
- Liu, B. Y., Z. Y. Wang, H. M. Yang, J. M. Wang, D. Xu, R. Zhang, and Q. Wang. 2011. Influence of rearing system on growth performance, carcass traits, and meat quality of Yangzhou geese. *Poult. Sci.* 90:653–659.
- Liu, W., Y. Yuan, C. Sun, B. Balasubramanian, Z. Zhao, and L. An. 2019. Effects of dietary betaine on growth performance, digestive function, carcass traits, and meat quality in indigenous yellow-feathered broilers under long-term heat stress. *Animals* 9:506.
- Ma, T. Y., G. K. Iwamoto, N. T. Hoa, V. Akotia, A. Pedram, M. A. Boivin, and H. M. Said. 2004. TNF α -induced increase in intestinal epithelial tight junction permeability requires NF- κ B activation. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 286:G367–G376.
- Meng, Y., Y. Zhang, M. Liu, Y. K. Huang, J. Zhang, Q. Yao, Y. L. Zhao, and J. J. Xiong. 2016. Evaluating intestinal permeability by measuring plasma endotoxin and diamine oxidase in children with acute lymphoblastic leukemia treated with high-dose methotrexate. *Anticancer. Agents. Med. Chem.* 16:387–392.
- Murray, P. J., and S. T. Smale. 2012. Restraint of inflammatory signaling by interdependent strata of negative regulatory pathways. *Nat. Immunol.* 13:916–924.
- Paulsen, Ø., B. Laird, N. Aass, T. Lea, P. Fayers, S. Kaasa, and P. Klestad. 2017. The relationship between pro-inflammatory cytokines and pain, appetite and fatigue in patients with advanced cancer. *PLoS One* 12:e0177620.
- Ratriyanto, A., and R. Mosenthin. 2018. Osmoregulatory function of betaine in alleviating heat stress in poultry. *J. Anim. Physiol. Anim. Nutr.* 102:1634–1650.
- Ulluwishewa, D., R. C. Anderson, W. C. McNabb, P. J. Moughan, J. M. Wells, and N. C. Roy. 2011. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J. Nutr.* 141:769–776.
- Sarker, M. T., Z. Y. Wang, H. Yang, X. Wan, and A. Emmanuel. 2021. Evaluation of the protective effect of lycopene on growth performance, intestinal morphology, and digestive enzyme activities of aflatoxinB1 challenged broilers. *Anim. Sci. J.* 92:e13540.
- Shi, P., T. Zhao, W. Wang, F. Peng, T. Wang, Y. Jia, L. Zou, P. Wang, S. Yang, Y. Fan, J. Zong, X. Qu, and S. Wang. 2022. Protective effect of homogeneous polysaccharides of Wuguchong (HPW) on intestinal mucositis induced by 5-fluorouracil in mice. *Nutr. Metab.* 18:36 19.
- Shi, S. R., Z. Y. Wang, H. M. Yang, and Y. Y. Zhang. 2007. Nitrogen requirement for maintenance in Yangzhou goslings. *Br. Poult. Sci.* 48:205–209.
- Shin, J. E., J. H. Kim, D. Goo, G. P. Han, F. M. Pitargue, H. K. Kang, and D. Y. Kil. 2018. Effect of dietary supplementation of betaine on productive performance, egg quality and jejunal tight junction-related gene expression in laying hens raised under hot environmental conditions. *Livest. Sci.* 214:79–82.
- Song, J., K. Xiao, Y. L. Ke, L. F. Jiao, C. H. Hu, Q. Y. Diao, B. Shi, and X. T. Zou. 2014. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. *Poult. Sci.* 93:581–588.
- Wang, H. C., S. S. Li, S. L. Fang, X. J. Yang, and J. Feng. 2018. Betaine improves intestinal functions by enhancing digestive enzymes, ameliorating intestinal morphology, and enriching intestinal microbiota in high-salt stressed rats. *Nutrients* 10:907.
- Wang, Z. Y., S. R. Shi, Q. Y. Zhou, H. M. Yang, J. M. Zou, K. N. Zhang, and H. M. Han. 2010. Response of growing goslings to dietary methionine from 28 to 70 days of age. *Br. Poult. Sci.* 51:118–121.
- Wu, J., C. He, J. Bu, Y. Luo, S. Yang, C. Ye, S. Yu, B. He, Y. Yin, and X. Yang. 2020. Betaine attenuates LPS-induced downregulation of Occludin and Claudin-1 and restores intestinal barrier function. *BMC. Vet. Res.* 4:75 16.
- Yang, L., G. Liu, X. Zhu, Y. Luo, Y. Shang, and X. L. Gu. 2019. The anti-inflammatory and antioxidant effects of leonurine hydrochloride after lipopolysaccharide challenge in broiler chicks. *Poult. Sci.* 98:1648–1657.
- Yang, Z., Z. Y. Wang, H. M. Yang, F. Z. Zhao, and L. L. Kong. 2016. Response of growing goslings to dietary supplementation with methionine and betaine. *Br. Poult. Sci.* 57:833–841.
- Yang, Z., Z. Y. Wang, H. M. Yang, L. Xu, and D. Q. Gong. 2017. Effects of dietary methionine and betaine on slaughter performance, biochemical and enzymatic parameters in goose liver and hepatic composition. *Anim. Feed Sci. Tech.* 228:48–58.
- Yang, Z., E. Asare, Y. Yang, J. J. Yang, H. M. Yang, and Z. Y. Wang. 2021. Dietary supplementation of betaine promotes lipolysis by regulating fatty acid metabolism in geese. *Poult. Sci.* 100:101460.
- Yu, H., W. Zou, C. Mi, Q. Wang, G. Dai, T. Zhang, G. Zhang, K. Xie, J. Wang, and H. Shi. 2021. Research note: expression of T cell-related cytokines in chicken cecal and spleen tissues following *Eimeria tenella* infection in vivo. *Poult. Sci.* 100:101161.
- Zhao, G., F. He, C. Wu, P. Li, N. Li, J. Deng, G. Zhu, W. Ren, and Y. Peng. 2018. Betaine in inflammation: mechanistic aspects and applications. *Front. Immunol* 24:1070.
- Zheng, Y. W., J. Y. Zhang, H. B. Zhou, Y. P. Guo, Q. G. Ma, C. Ji, and L. H. Zhao. 2020. Effects of dietary pyrroloquinoline quinone disodium supplementation on inflammatory responses, oxidative stress, and intestinal morphology in broiler chickens challenged with lipopolysaccharide. *Poult. Sci.* 99:5389–5398.