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Effects of yeast on inflammatory responses in yellow-feathered broilers induced by single and multiple lipopolysaccharide stimulation

Lijiao Zhang[1], Huixin Sun[1], Hainan Tao[1], Yinan Ding[1], Siming Xue[1], Yan Fang[1,2]*

[1] Changchun Normal University, Changchun, Jilin Province, P. R. China.
[2] Sanming University, Sanming, Fujian Province, P. R. China.

ABSTRACT - This study investigated the effect of yeast on the growth performance and inflammation of yellow-feathered broilers challenged with lipopolysaccharide (LPS). Four hundred and eighty-one-day-old broilers (BW 38.5±1.01 g) were randomly divided into six treatments with eight replicates of 10 birds each and fed a basal diet with or without 0.025% antibiotics and 0.05 and 0.5% yeast (*Saccharomyces cerevisiae,* 1.0×10^{10} cfu/g), respectively. Broilers from each replication were intra-abdominally injected with LPS (1.0 mg/kg body weight) or saline at 21, 23, 25, and 27 d of age. Samples were obtained after 3 or 12 h of the first injection (d 21) and the last injection (d 27), respectively. The results showed that LPS stimulation resulted in a significant decrease in productive performance (P<0.01) and a significant decrease in splenic index (P<0.05). Lipopolysaccharide injection caused significant (P<0.01) increase in serum α-AGP in the first hour and haptoglobin in the last 3 h (P<0.01), while other acute phase proteins were not significantly affected. All chickens fed diets supplemented with 0.5% yeast had elevated serum levels of IgA (P = 0.094) and lysozyme (P = 0.067) at 12 h of the first injection compared with those fed antibiotic diets. Lipopolysaccharide injection substantially increased the levels of cytokines in serum. A trend toward decreased levels of IL-10 (P = 0.078), transforming growth factor-beta 1 (P = 0.075), and interferon-gamma (P = 0.061) was observed in the LPS-stimulation × diet interaction. There was an LPS-stimulated × diet interaction (P<0.05) for interleukin-1beta transcript levels in the liver 12 h after repeated LPS injections. Chickens fed diets with 0.05 or 0.5% yeast tended to have higher levels of toll-like receptor 4 (P = 0.074) transcripts in the spleen at 3 h and β-defensin 1 (P = 0.065) transcripts at 12 h compared with those fed diets with antibiotics. The addition of 0.05 or 0.5% yeast alleviated the adverse effects caused by LPS injection, and this study provided a reference for the application of yeast in the broiler industry.

Keywords: growth performance, inflammation, live yeast, poultry

***Corresponding author:**
xiaohuawu19@126.com

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1. Introduction

In the process of livestock and poultry farming, poor feeding environments, such as high-density feeding, immunization, and heat stress, can cause stress reactions in livestock and poultry, which can lead to reduced feed intake (FI) and growth retardation. Of these, immunization stress is more common. Harmful microorganisms, endotoxins in the environment, and antigenic molecules in the feed tend to induce immune stress, which affects the behavior and growth of livestock, reduces growth performance, and causes economic losses (Liu et al., 2015). It has been reported that poultry injected with lipopolysaccharide (LPS) have elevated body temperature, reduced FI, and decreased body weight gain (BWG; Klasing, 2007; Al-Ogaili et al., 2022; Liu et al., 2023b). It was also found that the growth performance of livestock and poultry was related to the dose and time of LPS injection (Takahashi et al., 2000; Wright et al., 2000; Shen et al., 2010).

Antibiotics in feed can relieve immune stress in livestock, but many of the harmful consequences of antibiotics are increasingly being recognized. For example, antibiotics can lead to the development of drug resistance in livestock, residues in animal products, imbalance of intestinal bacterial flora, and environmental pollution, which ultimately threaten the sustainable development of animal husbandry and human health. With the total ban of feed antibiotics in the European Union, China is also gradually restricting the use of antibiotics. Seeking pollution-free and environmentally friendly alternatives to antibiotics, controlling livestock stress through nutritional regulation, and reducing economic losses caused by stress have become the main direction of current research.

Yeast is a facultative anaerobic microorganism, which can provide nutrients for livestock, promote intestinal digestion and absorption, regulate micro-ecological balance, enhance immunity, and ultimately improve animal growth performance (Trckova et al., 2014; Trevisi et al., 2015; Liu et al., 2023a). Lipopolysaccharide, a major component of the endotoxin cell wall of Gram-negative bacteria, induces a series of inflammatory responses and is a typical stress model. Numerous reports have shown that after LPS injection, FI and BWG were reduced, and the expression of inflammatory factors *in vivo* was also changed (Munyaka et al., 2013; Li et al., 2017). There are many reports on the application of active yeast in animals, but there are few applications in yellow-feathered broilers. Therefore, this study investigated the effect of active yeast on immune stress (LPS injection) and its possible mechanisms with a view to informing its application in the broiler industry.

2. Material and Methods

2.1. Birds, diets, and experimental design

This study was conducted in Changchun, Jilin, China (125°40' N, 43°91' W). Research on animals was conducted according to the institutional committee on animal use (protocol number 2019006).

A total of 480 yellow-feathered broilers (one-day-old, 38.5±1.01 g) were purchased from Changchun poultry company, and randomly divided into six groups with eight replicates per group and 10 broilers in each replicate. The experimental design was a 3 × 2 analytic factorial arrangement. Primary factors included dietary treatments (broiler basal diets supplemented with antibiotics and 0.05 or 0.5% yeast) and LPS stimulation (broilers injected intraperitoneally with LPS or sterile saline). The basal diets were formulated to meet or exceed the National Research Council (NRC, 1994) nutritional requirements for broilers (Table 1). On days 21, 23, 25, and 27, the LPS stimulation groups were injected with LPS (serotype O55:B5, Sigma Chemical Co., St. Louis, MO) at 1 mg/kg body weight (BW), and the unstimulated group was injected with equal amounts of sterile saline.

Chicks were raised in three-level battery cages with raised wire floors and housed in an environmentally controlled room maintain at 34 °C in the beginning of the experiment and was decreased by 2 °C each consecutive week until 24 °C through to slaughter. The light program was a 12-h light-dark cycle (light from 06:00 to 18:00 h). All broilers had *ad libitum* access to feed and water. The feeding trial lasted for 28 days. Mortality was recorded on a daily basis, and BWG, FI, and feed conversion ratio (FCR) were calculated.

2.2. Sample collection and procedure

On days 21 and 27, one broiler was randomly selected from each replicate 3 and 12 h after LPS injection, and wing venous blood samples were collected. Blood samples were kept at room temperature for 2 h, and then centrifuged at 3000 rpm for 15 min to separate the serum. Serum samples were frozen at −80 °C until analysis. One broiler was randomly selected in each replicate 3 and 12 h after injection on day 27, and the liver and spleen were removed and weighed at 10 g after slaughtering, rapidly frozen with liquid nitrogen, and stored at −80 °C for mRNA determination.

Table 1 - Composition and nutrient levers of diets (air-dry basis)

Ingredient (%)	
Corn	66.00
Soybean meal	26.00
Fish meal	2.00
Soybean oil	1.70
Limestone	1.30
Dicalcium phosphate	1.10
Wheat middling and red dog	0.60
NaCl	0.30
1% Premix[1]	1.00
Total	100.00
Chemical composition (%)[2]	
Metabolizable energy (MJ/kg)	12.13
Crude protein	21.0
Available P	0.45
Calcium	1.00
Lysine	1.15
Methionine	0.50

[1] Provided per kilogram of complete feed: vitamin A, 9,500 IU; vitamin D_3, 62.5 μg; vitamin K_3, 2.65 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_{12}, 0.025 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic, 12 mg; niacin, 50 mg; Cu ($CuSO_4.5H_2O$), 8 mg; Zn (ZnO), 75 mg; Fe ($FeSO_4.7H_2O$), 80 mg; Mn ($MnSO_4.H_2O$), 100 mg; Se ($NaSeO_3$), 0.15 mg; I (KI), 0.35 mg.
[2] Nutrient levels were calculated values.

2.3. Serum parameters measurement

The level of inflammatory cytokines interleukin-1beta (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon-gamma (IFN-γ), tumor necrosis factor-alfa (TFN-α), and transforming growth factor-beta 1 (TGF-β1), C-reactive protein (CRP), haptoglobin (HP), alfa-acid glycoprotein (α-AGP), serum amyloid A protein (SAA), and alfa1-antitrypsin (AAT) levels in serum were measured using ELISA commercial kits (Cusabio Biotech co., Ltd, Wuhan, China) according to the manufacturer's instructions. Levels of immunoglobulin G, A, and M (IgG, IgA, and IgM, respectively) and lysozyme activity in serum were determined by ELISA commercial kits (Jiancheng Biotechnology Institute, Nanjing, China) according to the manufacturer's instructions.

2.4. Quantification of mRNA expression by real-time PCR

Total RNA from the spleen and liver sample was extracted using Trizol Reagent (Invitrogen Life Technologies Co., Carlsbad, CA, USA) according to the manufacturer's protocol. Total RNA concentration was quantified by the NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and the integrity was assessed by formaldehyde-agarose gel electrophoresis. After determining the RNA concentration, total RNA (1 μg) was reverse-transcribed into cDNA by a Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, Wilmington, DE, USA) according to the manufacturer's instructions. The cDNA samples were stored at −20 °C until analyzed. Quantitative real-time PCR was performed on an Applied Biosystems 7500 Real-Time PCR System (Foster City, CA, USA) using a Roche FastStart Universal SYBR Green Master (Rox) (Roche Co., America). A reaction system of 20 μL included 2.5 μL of cDNA, 1 μL of each primer (10 uM) (Table 2), 12.5 μL of SYBR Green Master (Rox) (2X), and 8 μL of double distilled water. The PCR cycle conditions were set as follows: 95 °C for 5 min; 40 cycles of 95 °C for 5 s, 60 °C for 34 s, 72 °C for 30 s, and 72 °C for 5 min. All the samples were analyzed in triplicate, and negative controls were included to check for the nonspecific amplification of primers. The melting profile of each sample was analyzed after every PCR run to confirm PCR product specificity. The relative expression levels of targeted genes were calculated according to $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Table 2 - Sequence of real-time PCR primer

Gene	Primer sequence (5'-3')	Accession number	Product size
β-actin	F: GAGAAATTGTGCGTGACATCA R: CCTGAACCTCTCATTGCCA	NM_205518.1	112
IL-1β	F: AAGCCTCGCCTGGATTCTGA R: AAGGACTGTGAGCGGGTGTAG	NM_204524.1	260
IL-2	F: ATGGAGCATCTCTATCATCAGCA R: GTAACCACTTCTCCCAGGTAACAC	AF000631.1	169
IL-4	F: GTGCCCACGCTGTGCTTACA R: GTTCATCTTGACGCAGGAAACCT	GU119892.1	96
IL-6	F: AAATCCCTCCTCGCCAATCT R: CTCACGGTCTTCTCCATAAACG	NM_204628.1	104
IL-10	F: TCAATCCAGGGACGATGAACTTA R: AGGTGAAGAAGCGGTGACAGC	NM_001004414.2	233
TNF-α	F: GGAATGAACCCTCCGCAGTA R: CAGAGCATCAACGCAAAAGG	AY765397.1	258
IFN-γ	F: ATCATACTGAGCCAGATTGTTTCG R: ACGCCATCAGGAAGGTTGTT	AJ634956.1	125
TGF-β1	F: CGGGACGGATGAGAAGAACTG R: CAGGCACGGACCACCATATTG	JQ423909.1	303
TLR-2	F: AAGGAAACTTTGAGGGCATTGT R: CGATTTCAGACTTCCAGGCTC	NM_001161650.1	89
TLR-4	F: TTTGAAAATGTAAGGCGGCTC R: ATTTCCAGGGCTGAGTCCGA	NM_001030693.1	224
HSP-70	F: TGCAAGCAGCTATCCTCATGG R: GGGAATGGTGGTGTTACGCT	NM_001006685.1, FJ217667.1	140
β-defensin 1	F: GCTCATCCCCTTCTTCCTCTTGT R: CCCAACACGACAGAATCCTCCT	NM_204650.2	88

IL - interleukin; TNF-α - tumor necrosis factor-alfa; IFN-γ - interferon-gamma; TGF-β1- transforming growth factor-beta 1; TLR - toll-like receptor; HSP-70 - heat-shock protein 70; F - forward primer; R - reverse primer.

2.5. Statistical analysis

Data were analyzed by two-way ANOVA using the GKM procedure of SPSS 17.0 (SPSS Inc., Chicago, IL) as a 3 × 2 factorial arrangement with diet and LPS as main effects. When the P-values of the interaction of main effects was less than 0.10, one-way ANOVA was performed using Duncan multiple comparisons. The statistical model was as follows:

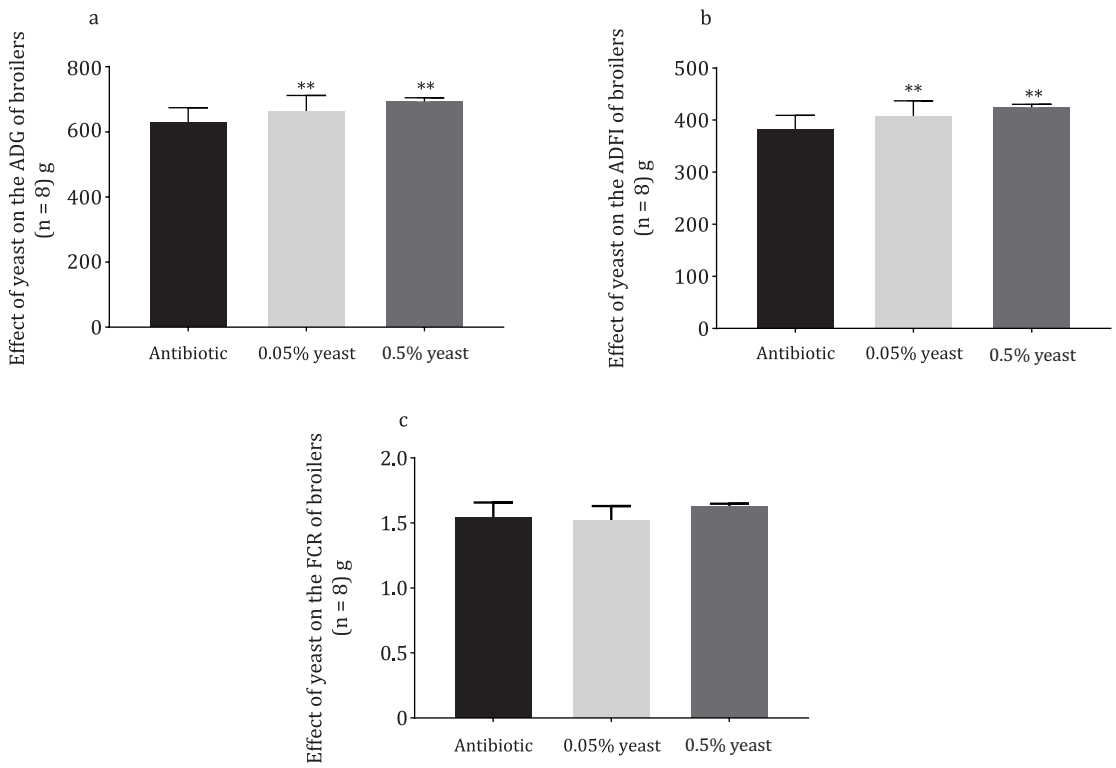
$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij}, \quad (1)$$

in which Y_{ij} = dependent variable, μ = variable mean, β_i = fixed effect of i–th broilers of the treatment, and ε_{ij} = experimental error associated with observation Y_{ij}. Significance was defined as $P<0.05$ and $0.05 < P<0.10$ as a trend. All data are expressed as mean and standard error of the mean (SEM).

3. Results

3.1. Growth performance

Compared with the antibiotic control group, the addition of yeast to the diet significantly increased ADG and ADFI ($P<0.01$), but no effect on FCR ($P>0.05$) during the early stage (1~21 d) (Figure 1).



ADG - average daily weight gain; ADFI - average daily feed intake; FCR - feed conversion ratio.
0.05% yeast and 0.5% yeast = control supplemented with 0.05 or 0.5% yeast per kg of diet.
** Within each figure, the asterisk means that the difference is significant (P<0.01).
No symbols in the figure indicate that the difference is not significant (P>0.05).

Figure 1 - Effect of yeast on the growth performance of broilers at 1 to 21 d of age.

Lipopolysaccharide stimulation decreased ADG and ADFI and increased FCR in broilers from 21 to 28 d of age (P<0.01). The interaction between LPS stimulation and diet had a tendency to improve FCR in broilers (P = 0.084) (Figure 2).

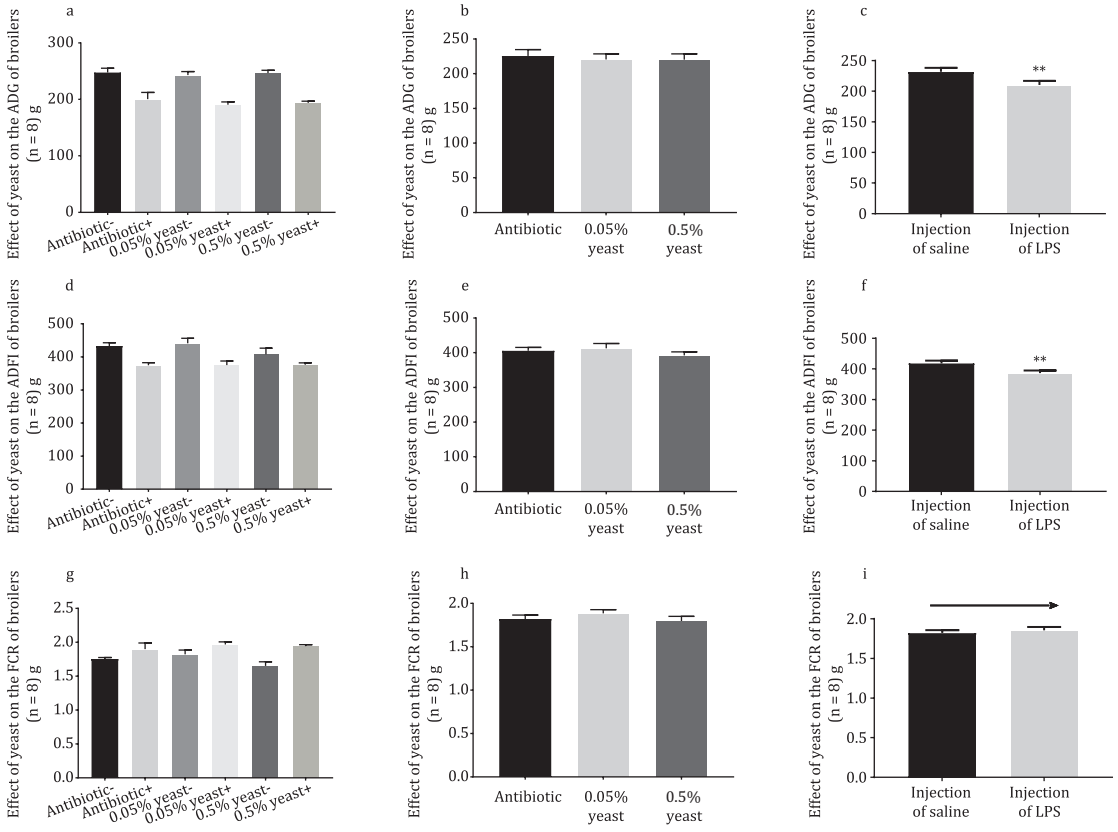
3.2. Relative weight of the spleen and liver

There was no interaction effect between LPS stimulation and diet on liver index and spleen index in broilers (Figures 3 and 4). Broiler spleen index decreased significantly (P<0.05) after LPS stimulation on day 27 at 3 and 12 h. Neither LPS nor diet had an effect on liver index.

3.3. Acute-phase proteins and immune globulin

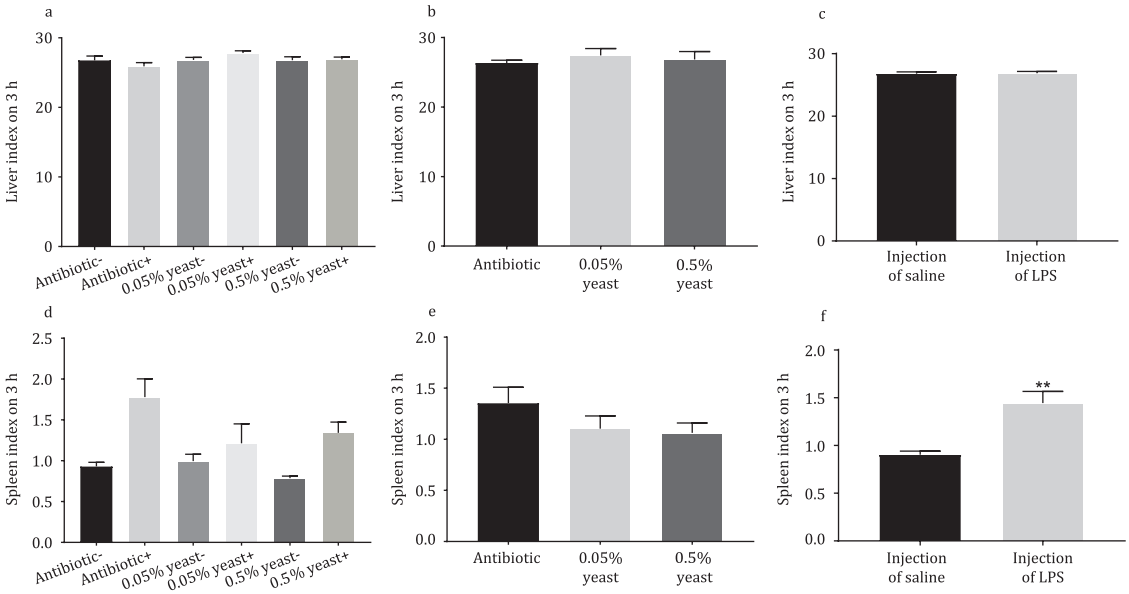
The levels of α-AGP at 3~12 h of the first time and 3 h of the final time, and the levels of HP at 3 h of the final time in serum were increased significantly (P<0.01) by LPS injection (Figures 5 and 6). All chickens fed diets supplemented with 0.5% yeast tended to have higher CRP levels at first LPS injection (P = 0.086, P = 0.091) compared with those fed diets with antibiotic. No significant changes in other serum acute-phase proteins were observed as a result of LPS stimulation. There was no interaction between LPS stimulation and diet on serum acute-phase protein levels (Figure 5).

There was no interaction effect between LPS stimulation and diets on serum immunoglobulin and lysozyme levels at 3 and 12 h on 21 and 27 d after LPS stimulation (P>0.05), but there was a tendency for IgM (P = 0.052) to decrease at 3 h on 27 d after injection and of lysozyme to increase (P = 0.092) at 12 h on 27 d after injection (Table 3). Serum IgG and IgM levels decreased significantly after LPS stimulation (P<0.01), and lysozyme levels increased significantly 3 h after the first injection (P<0.01), and IgG levels decreased significantly 12 h after the first injection (P<0.01). All chickens fed diets



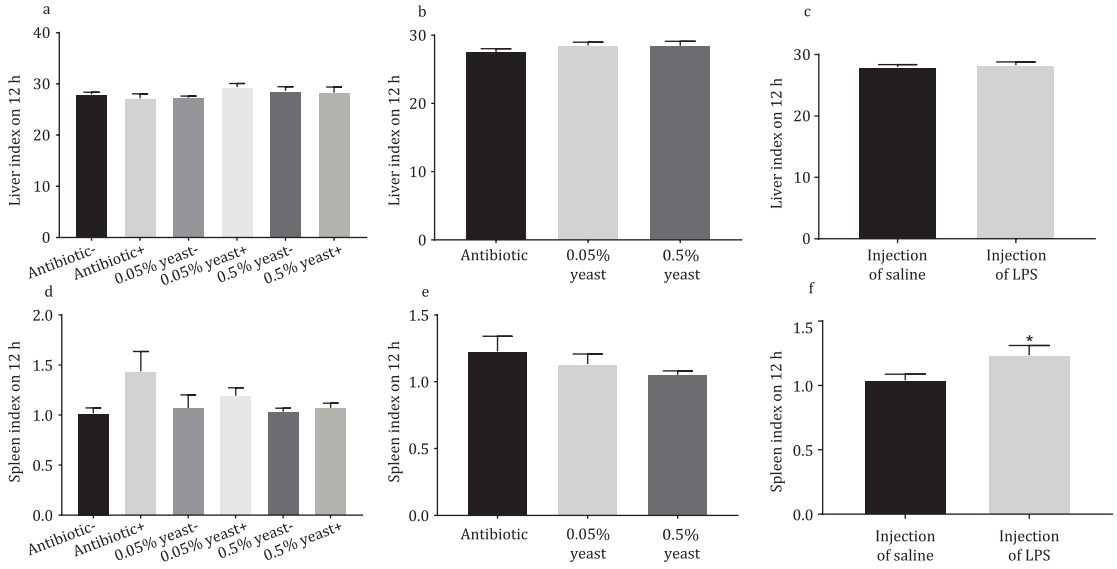
Lipopolysaccharide (LPS) was intra-abdominally injected at 1 mg/kg body weight or the same amount of saline at 21, 23, 25, and 27 d of age.
– mean injected with the saline; + mean injected with LPS.
0.05 and 0.5% yeast = control supplemented with 0.05 or 0.5% yeast per kg of diet.
** Within each figure, the asterisk means that the difference is significant (P<0.01).
"→" Within each figure, the arrow means that there is a trend (P = 0.084).
No symbols in the figure indicate that the difference is not significant (P>0.05).

Figure 2 - Effect of yeast on the growth performance of broilers from 21 to 28 d of age.



[1] Lipopolysaccharide (LPS) was intra-abdominally injected at 1 mg/kg body weight or the same amount of saline at 21 d of age.
– mean injected with the saline; + mean injected with LPS.
0.05 and 0.5% yeast = control supplemented with 0.05 or 0.5% yeast per kg of diet.
** Within each figure, the asterisk means that the difference is significant (P<0.01).
No symbols in the figure indicate that the difference is not significant (P>0.05).

Figure 3 - Effect of yeast on the organ index of broilers after lipopolysaccharide (LPS) injection[1] on d 21 (n = 8).



[1] Lipopolysaccharide (LPS) was intra-abdominally injected at 1 mg/kg body weight or the same amount of saline at 21, 23, 25, and 27 d of age.
– mean injected with the saline; + mean injected with LPS.
0.05 and 0.5% yeast = control supplemented with 0.05 or 0.5% yeast per kg of diet.
* Within each figure, the asterisk means that the difference is significant (P<0.05).
No symbols in the figure indicate that the difference is not significant (P>0.05).

Figure 4 - Effect of yeast on the organ index of broilers after lipopolysaccharide (LPS) injection[1] on d 27 (n = 8).

supplemented with 0.5% yeast had higher serum levels of IgA (P = 0.094) and lysozyme (P = 0.067) at 12 h after the first injection compared with chickens fed antibiotic diets (Table 3).

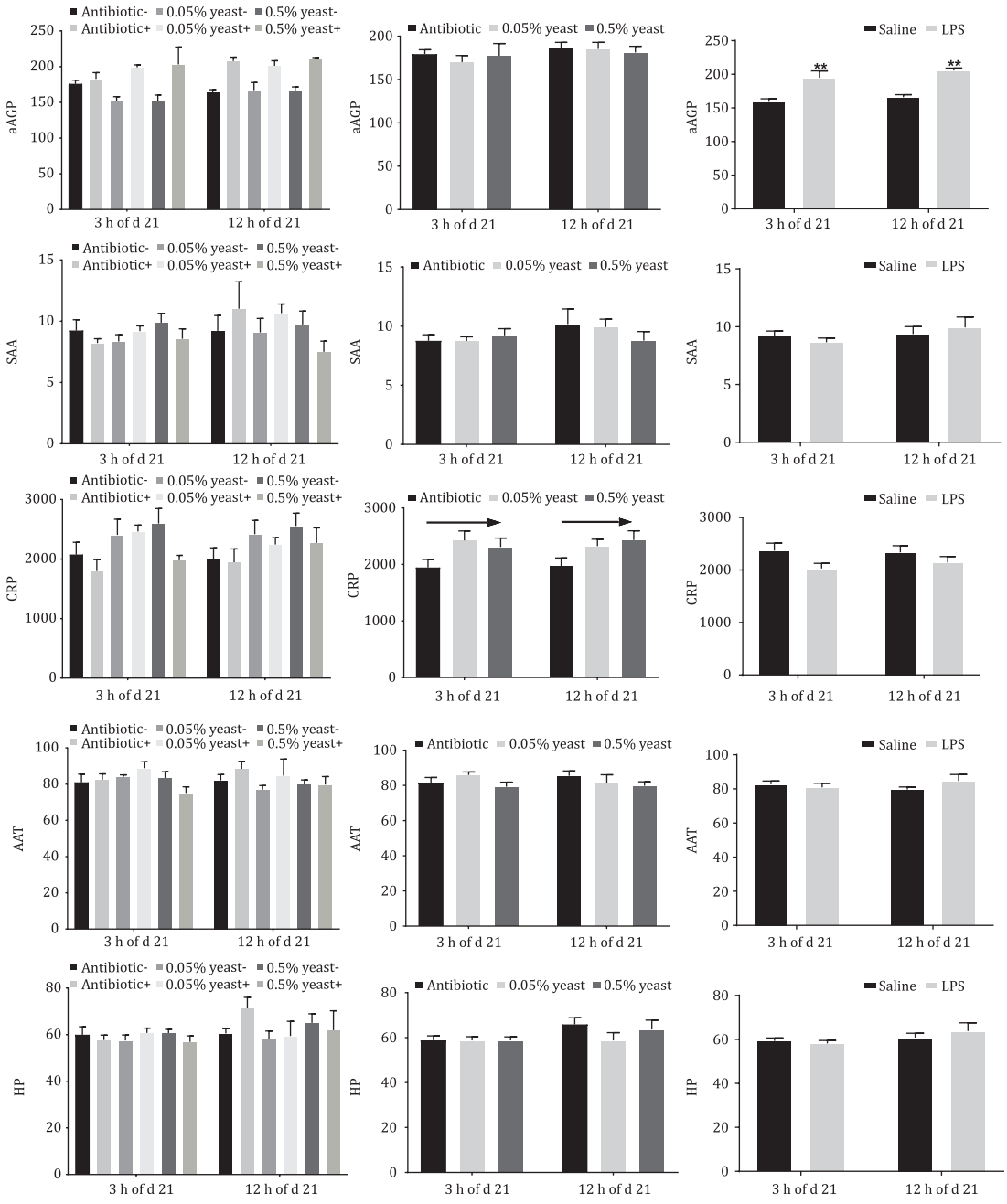
3.4. Serum cytokines and mRNA expression associated with inflammatory factors

For the expression analysis of all immune target genes, compared with the antibiotic control group, there was a trend toward lower IFN-γ (P = 0.092) 3 h after first injection and a trend toward higher IL-4 (P = 0.095), IL-10 (P = 0.084), IFN-γ (P = 0.097), and TGF-β (P = 0.086) 12 h after first injection. Lipopolysaccharide injection greatly increased serum cytokine levels. At the time of the first injection, all chickens fed diets supplemented with 0.5% yeast showed a significant decrease in TGF-β levels at 3 h (P<0.05), a trend toward higher levels of IL-1β at 3 h (P = 0.085), and a trend toward higher levels of TGF-β at 12 h (P = 0.065), as compared with chickens fed diets with antibiotic (Table 4).

There was an interaction between LPS stimulation and diets, which had a tendency to reduce IL-10 (P = 0.078), TGF-β (P = 0.075), and IFN-γ (P = 0.061) levels at 27 d. Multiple injections of LPS significantly increased serum levels of IL-1β, IL-4, and IL-6 (P<0.05) at 3 h compared with saline-injected chickens. Compared to the diet with antibiotic, diets supplemented with 0.05 or 0.5% yeast significantly increased TFN-α levels (P<0.05) at 12 h and tended to increase IFN-γ (P = 0.070) and decrease IL-10 (P = 0.053) at 3 h (Table 4).

3.5. mRNA expression related to inflammatory factors in liver and spleen

At 12 h after LPS stimulation, there was a significant interaction (P<0.05) between diet and immune challenge on IL-1β transcript levels in the liver (Table 5). In LPS-injected chickens, the diet supplemented with 0.5% yeast significantly decreased IL-1β transcript levels at 3 h (P<0.05) and had a tendency to increase TGF-β transcript levels (P = 0.077) and decrease IL-10 transcript levels at 3 h (P = 0.076), compared with the diet with antibiotic. Compared with saline injection, LPS stimulation significantly increased the transcript levels of IL-1β, IL-2, IL-4, TNF-α, and TGF-β at 3 h and 12 h, as well as IFN-γ at 3 h after repeated injections in chicken livers (P<0.05).



AGP - acid glycoprotein; SAA - serum amyloid A protein; CRP - C-reactive protein; AAT - α1-antitrypsin; HP - haptoglobin.
[1] Lipopolysaccharide (LPS) was intra-abdominally injected at 1 mg/kg body weight or the same amount of saline at 21 d of age.
– mean injected with the saline; + mean injected with LPS.
0.05 and 0.5% yeast = control supplemented with 0.05 or 0.5% yeast per kg of diet.
** Within each figure, the asterisk means that the difference is significant (P<0.01).
"→" Within each figure, the arrow means that there is a trend (P = 0.086, P = 0.091).
No symbols in the figure indicate that the difference is not significant (P>0.05).

Figure 5 - Effect of yeast on the levels of serum acute-phase protein of broilers after lipopolysaccharide (LPS) injection[1] at 3 and 12 h of d 21 (n = 8).

There was no interaction effect between diets and LPS stimulation on the expression of mRNA related to inflammatory factors in spleen (Table 5). Compared with saline injection, LPS stimulation significantly increased the levels of IL-1β, IL-2, IL-4, TNF-α, and TGF-β transcripts in the liver at 3 and 12 h, as well as the levels of IFN-γ transcripts at 3 h after repeated injections, and significantly increased the levels of IL-1β, TNF-α, and IFN-γ transcripts in the spleen at 3 and 12 h, as well as the levels of IL-6, IL-10, and TGF-β transcripts at 3 h after repeated injections. Lipopolysaccharide stimulation

Table 3 - Effect of yeast on the levels of serum immunoglobulin and lysozyme of broilers after lipopolysaccharide (LPS) injection[1]

Item			Antibiotic		0.05% yeast		0.5% yeast		SEM	P-value		
LPS/Saline			–	+	–	+	–	+		Diet	LPS	Interaction
First	3 h	IgG (mg/mL)	2.44	1.20	2.02	1.12	1.99	1.05	0.11	0.192	<0.001	0.569
		IgM (μg/mL)	351.03	249.62	330.32	275.33	326.23	244.27	8.53	0.404	<0.001	0.307
		IgA (mg/mL)	3.99	3.43	3.36	3.87	3.23	3.44	0.10	0.258	0.788	0.100
		Lysozyme (U/mL)	179.82	194.22	157.59	171.99	154.66	193.62	4.82	0.141	0.016	0.434
	12 h	IgG (mg/mL)	2.43	1.22	1.90	1.04	1.75	1.48	0.12	0.362	<0.001	0.185
		IgM (μg/mL)	355.35	398.86	354.82	376.04	326.10	348.87	8.77	0.173	0.097	0.837
		IgA (mg/mL)	3.99	3.14	2.56	2.98	3.31	3.36	0.15	0.094	0.669	0.212
		Lysozyme (U/mL)	189.82	206.05	207.59	216.59	200.66	200.23	2.69	0.067	0.114	0.416
Final	3 h	IgG (mg/mL)	2.52	1.98	1.64	2.25	1.41	1.95	0.16	0.367	0.52	0.280
		IgM (μg/mL)	363.83	328.09	315.68	342.06	339.31	324.11	5.37	0.354	0.429	0.052
		IgA (mg/mL)	2.04	1.74	1.82	2.14	2.17	2.24	0.07	0.148	0.836	0.159
		Lysozyme (U/mL)	155.29	166.27	153.36	154.6	145.6	160.70	3.68	0.713	0.196	0.849
	12 h	IgG (mg/mL)	1.74	2.36	1.68	1.72	1.68	2.15	0.19	0.614	0.203	0.705
		IgM (μg/mL)	362.58	323.54	341.93	364.21	339.31	381.78	8.17	0.683	0.602	0.118
		IgA (mg/mL)	2.16	1.93	1.8	2.06	2.24	1.97	0.06	0.47	0.483	0.122
		Lysozyme (U/mL)	182.69	192.17	185.12	172.5	167.02	192.05	6.87	0.545	0.298	0.092

IgG - immunoglobulin G; IgM - immunoglobulin M; IgA - immunoglobulin A; –: injected with the saline; +: injected with LPS.
[1] Lipopolysaccharide was intra-abdominally injected at 1 mg/kg BW or the same amount of saline at 21, 23, 25, and 27 d of age.

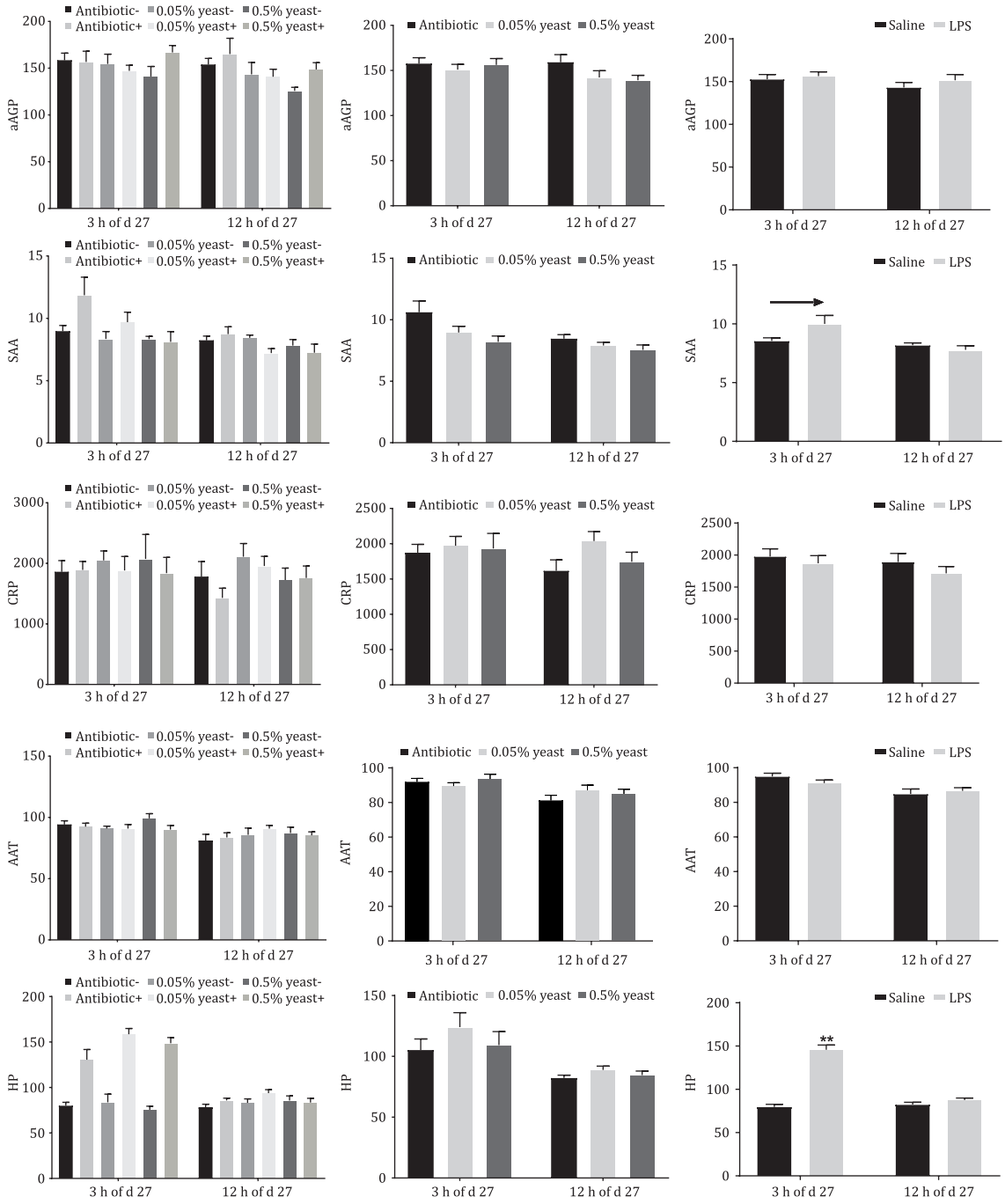
significantly increased the levels of IL-6, IL-10, and TGF-β transcript levels in the spleen at 3 h after repeated injections (P<0.05). There were no differences (P>0.05) in cytokine levels in the livers and spleens of chickens fed diets supplemented with 0.05 or 0.5% yeast compared with LPS-injected chickens fed antibiotic diets.

Lipopolysaccharide increased the expression of TLR-2, TLR-4, and β-defensin 1 in the liver at 3 h (P<0.05), and there was a trend to increase the level of TLR-4 transcripts at 12 h (P = 0.093). There was no interaction between LPS stimulation and diet on the expression of inflammatory factor-associated mRNAs in the liver (Table 6).

HSP70, TLR-2, TLR-4, and β-defensin 1 transcript levels were significantly increased at 3 h and TLR-4 and β-defensin 1 transcript levels were significantly increased at 12 h after LPS stimulation. Chickens fed 0.05 or 0.5% yeast tended to have higher transcript levels of TLR4 (P = 0.074) at 3 h and β-defensin 1 (P = 0.065) at 12 h in spleens compared with chickens fed the antibiotic diet. There was no interaction between LPS stimulation and diet on the expression of inflammatory factor-associated mRNAs in spleens (Table 6).

4. Discussion

The present study was designed to investigate whether the addition of 0.05 or 0.5% live yeast to the diet affects growth performance, organ weights, and inflammatory immune activity when the organisms are in a state of immune stress or in a normal physiological state. The results of this paper showed a significant increase in BWG and FI in chickens fed diets supplemented with 0.05 or 0.5% yeast compared with chickens fed antibiotics for the first three weeks, which is similar to previously described results (Kompiang, 2002; Bai et al., 2013). The improved performance of broiler chickens may be related to the fact that yeast enters the gut and remains active. On the one hand, yeast is rich in nutrients such as proteins, minerals, and vitamins, and the yeast cell wall can absorb mycotoxins and pathogens to protect intestinal health. On the other hand, protease and amylase are the products of yeast metabolism, which can effectively help the intestinal tract to degrade nutrients and promote



AGP - acid glycoprotein; SAA - serum amyloid A protein; CRP - C-reactive protein; AAT - α1-antitrypsin; HP - haptoglobin.
[1] Lipopolysaccharide (LPS) was intra-abdominally injected at 1 mg/kg body weight or the same amount of saline at 21, 23, 25, and 27 d of age. – mean injected with the saline; + mean injected with LPS.
0.05 and 0.5% yeast = control supplemented with 0.05 or 0.5% yeast per kg of diet.
** Within each figure, the asterisk means that the difference is significant (P<0.01).
"→" Within each figure, the arrow means that there is a trend (P = 0.077).
No symbols in the figure indicate that the difference is not significant (P>0.05).

Figure 6 - Effect of yeast on the levels of serum acute-phase protein of broilers after lipopolysaccharide (LPS) injection[1] at 3 and 12 h of d 27 (n = 8).

nutrient absorption. Meanwhile, yeast contains some unknown growth factors that can promote the growth of intestinal bacteria and improve the nutritional metabolism of animals. After injection of LPS, FI and body weight gain were reduced and FCR was increased in the fourth week compared with the control group, similar to the previous results (Virden et al., 2007; Yang et al., 2011; Feng et al., 2012).

Table 4 - Effect of yeast on the levels of serum cytokines of broilers after lipopolysaccharide (LPS) injection[1]

Item			Antibiotic		0.05% yeast		0.5% yeast		SEM	P-value		
LPS/Saline			–	+	–	+	–	+		Diet	LPS	Interaction
First	3 h	IL-1β	20.17	21.19	20.91	21.48	20.91	24.62	0.43	0.085	0.03	0.197
		IL-2	2.11	2.36	2.13	2.45	2.14	2.30	0.04	0.742	0.004	0.707
		IL-4	11.82	54.33	11.07	57.08	11.38	48.59	3.82	0.685	<0.001	0.671
		IL-6	20.12	64.49	19.31	68.95	19.51	60.24	3.77	0.498	<0.001	0.477
		IL-10	2.08	2.57	1.95	2.54	2.21	2.57	0.09	0.802	0.009	0.867
		TNF-α	60.07	56.65	59.27	72.15	38.71	69.88	4.02	0.482	0.092	0.188
		IFN-γ	59.65	41.72	43.98	50.27	61.38	43.83	2.57	0.651	0.052	0.092
		TGF-β	0.42	1.73	0.48	2.13	0.39	1.23	0.13	0.035	<0.001	0.105
	12 h	IL-1β	20.07	23.83	19.12	24.53	20.94	25.03	0.51	0.431	<0.001	0.672
		IL-2	1.89	2.06	1.65	1.90	1.56	2.26	0.09	0.651	0.044	0.445
		IL-4	12.50	33.09	11.31	43.04	11.38	36.25	2.22	0.204	<0.001	0.095
		IL-6	20.12	38.49	19.31	63.75	19.51	59.78	3.78	0.136	<0.001	0.106
		IL-10	2.38	2.45	2.55	2.66	2.43	2.47	0.08	0.659	0.673	0.982
		TNF-α	54.29	57.85	50.52	60.9	48.01	57.74	0.92	0.122	<0.001	0.084
		IFN-γ	42.46	51.61	45.67	48.93	45.32	54.41	0.85	0.125	<0.001	0.097
		TGF-β	0.43	0.99	0.44	1.72	0.49	2.95	0.21	0.065	<0.001	0.086
Final	3 h	IL-1β	19.37	26.35	19.95	24.31	20.42	25.7	0.52	0.433	<0.001	0.233
		IL-2	1.84	2.05	2.07	1.70	1.62	1.91	0.09	0.706	0.831	0.305
		IL-4	11.5	13.39	14.48	13.32	15.3	13.45	0.88	0.198	0.016	0.173
		IL-6	18.74	20.71	19.21	19.89	19.20	20.26	0.21	0.912	0.003	0.391
		IL-10	2.23	2.58	2.25	2.17	2.76	2.08	0.09	0.587	0.442	0.078
		TNF-α	52.96	55.78	52.11	55.49	50.03	52.63	0.77	0.250	0.061	0.977
		IFN-γ	35.97	38.16	45.95	65.18	52.83	52.5	3.47	0.070	0.302	0.434
		TGF-β	0.33	0.33	0.36	0.26	0.33	0.32	0.01	0.672	0.103	0.075
	12 h	IL-1β	19.09	20.29	20.48	19.96	21.26	20.45	0.25	0.163	0.932	0.210
		IL-2	2.22	1.7	2.28	2.04	1.75	2.19	0.10	0.622	0.568	0.114
		IL-4	11.00	12.71	12.73	11.73	13.31	12.9	0.45	0.436	0.903	0.368
		IL-6	15.79	16.79	15.03	15.05	15.19	16.47	0.47	0.571	0.442	0.857
		IL-10	3.79	7.88	2.98	3.40	2.83	3.89	0.29	0.053	0.061	0.260
		TNF-α	39.95	39.68	64.60	73.21	56.95	83.28	5.65	0.047	0.295	0.601
		IFN-γ	31.67	49.53	46.18	39.86	55.04	38.53	3.00	0.689	0.779	0.061
		TGF-β	0.37	0.41	0.39	0.41	0.38	0.37	0.01	0.651	0.438	0.617

IL - interleukin; TNF-α - tumor necrosis factor-alfa; IFN-γ - interferon-gamma; TGF-β - transforming growth factor beta; –: injected with the saline; +: injected with LPS.
[1] Lipopolysaccharide was intra-abdominally injected at 1 mg/kg BW or the same amount of saline at 21, 23, 25, and 27 d of age.

There were no significant differences in the effects of adding 0.05 or 0.5% yeast on FI, body weight gain, and FCR of yellow-feathered broilers compared with antibiotics. This indicates that yeast and antibiotics had similar effects on the performance of yellow-feathered broilers after LPS stimulation, but there was no interaction between diet type and immune stress. These results suggest that the addition of yeast can play an important role in the nutritional modulation of immune stress to alleviate it.

The liver has metabolic and immune functions, and the spleen is the largest peripheral immune organ in broilers, participating in systemic cellular and humoral immunity (Pozo et al., 2009). It is generally accepted that the relative weights of immune organs in domestic animals reflect the strength of immune function, but the weights and indices of immune organs in broiler chickens as a measure of immune function are not clearly defined (Cai et al., 2022). In the present study, there was no significant difference in liver indices by LPS stimulation, but spleen indices were significantly decreased, which is consistent

Table 5 - Effect of yeast on the expression of mRNA related to inflammatory factors in liver and spleen of broilers after repeated lipopolysaccharide (LPS) injection[1]

Item			Antibiotic		0.05% yeast		0.5% yeast		SEM	P-value		
LPS/Saline			–	+	–	+	–	+		Diet	LPS	Interaction
Liver	3 h	IL-1β	1.00	36.46	2.67	35.88	5.06	33.60	14.04	0.953	<0.001	0.289
		IL-2	1.00	15.28	2.98	25.01	1.41	20.75	3.23	0.085	<0.001	0.271
		IL-4	1.00	11.90	1.02	11.30	0.63	12.32	5.51	0.959	<0.001	0.834
		IL-6	1.00	7.19	2.68	3.10	1.27	2.72	2.89	0.550	0.106	0.298
		IL-10	1.00	3.97	2.40	1.48	0.92	2.55	0.49	0.819	0.239	0.302
		TNF-α	1.00	9.08	1.97	13.53	2.43	7.48	1.64	0.243	<0.001	0.229
		IFN-γ	1.00	2.06	0.78	1.69	0.87	2.38	0.59	0.440	0.001	0.606
		TGF-β	1.00	0.53	0.05	3.13	1.20	3.47	0.22	0.144	0.019	0.077
	12 h	IL-1β	1.00d	18.19a	2.67cd	15.27ab	4.93c	13.11b	5.79	0.851	<0.001	0.010
		IL-2	1.00	12.22	3.15	14.35	1.54	14.98	1.69	0.243	<0.001	0.603
		IL-4	1.00	10.21	1.64	8.99	1.17	7.59	4.08	0.587	<0.001	0.514
		IL-6	1.00	7.24	0.33	4.42	0.71	6.60	2.67	0.158	<0.001	0.437
		IL-10	1.00	3.71	2.18	0.43	0.95	1.39	0.39	0.371	0.525	0.076
		TNF-α	1.00	6.20	1.96	3.09	2.40	4.05	0.80	0.714	0.029	0.277
		IFN-γ	1.00	0.33	0.33	0.72	0.84	0.84	0.43	0.641	0.735	0.303
		TGF-β	1.00	2.96	0.04	2.80	1.51	2.22	0.14	0.509	<0.001	0.157
Spleen	3 h	IL-1β	1.00	6.15	1.11	2.86	1.57	4.42	0.89	0.142	<0.001	0.108
		IL-2	1.00	1.54	3.15	1.05	1.82	0.87	0.14	0.78	0.896	0.683
		IL-4	1.00	1.04	1.17	1.59	1.22	1.70	0.14	0.231	0.160	0.656
		IL-6	1.00	3.51	1.73	3.49	1.42	3.09	0.35	0.754	0.001	0.669
		IL-10	1.00	831	1.17	9.10	1.29	8.99	1.47	0.281	0.005	0.171
		TNF-α	1.00	25.79	2.96	20.46	3.16	23.38	1.83	0.95	0.001	0.826
		IFN-γ	1.00	13.27	0.32	9.22	0.42	7.80	11.89	0.572	0.002	0.703
		TGF-β	1.00	2.36	0.86	2.15	0.79	2.01	0.58	0.913	0.032	0.995
	12 h	IL-1β	1.00	2.60	1.51	3.89	2.11	3.06	0.38	0.295	0.005	0.503
		IL-2	1.00	4.58	2.43	2.45	2.73	2.72	0.28	0.946	0.215	0.220
		IL-4	1.00	1.85	0.98	6.25	4.17	3.13	0.45	0.579	0.400	0.426
		IL-6	1.00	1.27	1.13	1.32	0.98	1.37	0.43	0.981	0.467	0.976
		IL-10	1.00	1.64	1.56	1.42	1.58	1.52	0.15	0.806	0.619	0.499
		TNF-α	1.00	23.88	2.96	22.27	3.39	25.93	1.73	0.873	<0.001	0.917
		IFN-γ	1.00	15.58	3.53	10.42	2.30	13.58	2.23	0.864	<0.001	0.340
		TGF-β	1.00	1.52	0.88	0.91	1.29	1.60	0.15	0.155	0.216	0.654

IL - interleukin; TNF-α - tumor necrosis factor-alfa; IFN-γ - interferon-gamma; TGF-β - transforming growth factor beta; –: injected with the saline; +: injected with LPS.
[1] Lipopolysaccharide was intra-abdominally injected at 1 mg/kg BW or the same amount of saline at 21, 23, 25, and 27 d of age.

with the results in weaned piglets (Li et al., 2022) and quail (Yu et al., 2017). Matur et al. (2010) also reported that the addition of yeast extract had no effect on liver index in hen diet. It was also found that there was no significant difference in liver index and spleen index between LPS-stimulated and non-LPS-stimulated chickens with or without the addition of yeast, which may be related to the amount of yeast added and broiler diet, or the effect of yeast on liver and spleen has not yet been shown on the organ lever.

Acute-phase proteins such as CRP, HP, SAA, α-AGP, and AAT are transiently altered in animal serum when stimulated by trauma, inflammation, or infection, and their potential application in animal health status is of great importance (Cray et al., 2009). This study showed that LPS stress increased the level of serum α-AGP at 3 and 12 h after the initial injection, which was similar to that of many scholars on livestock and poultry (Han et al., 2014; Li et al., 2018). The α-AGP is synthesized in the

Table 6 - Effect of yeast on the expression of mRNA related to inflammatory factors in liver and spleen of broilers after repeated lipopolysaccharide (LPS) injection[1]

Item			Antibiotic		0.05% yeast		0.5% yeast		SEM	P-value		
LPS/Saline			–	+	–	+	–	+		Diet	LPS	Interaction
Liver	3 h	HSP70	1.00	8.24	2.53	2.61	1.05	2.27	1.47	0.412	0.142	0.246
		TLR-2	1.00	5.65	1.51	4.75	1.25	2.68	1.82	0.437	0.005	0.373
		TLR-4	1.00	2.30	1.07	2.99	1.03	4.20	1.03	0.474	0.005	0.485
		β-defensin 1	1.00	2.27	1.62	2.02	1.72	1.85	0.16	0.776	0.020	0.136
	12 h	HSP70	1.00	1.69	2.95	0.96	1.16	0.68	0.44	0.464	0.394	0.294
		TLR-2	1.00	2.54	4.08	0.35	1.38	0.19	1.46	0.505	0.277	0.136
		TLR-4	1.00	2.09	1.43	2.36	1.31	1.82	0.47	0.790	0.093	0.871
		β-defensin 1	1.00	3.62	0.82	2.27	0.62	1.18	0.56	0.515	0.137	0.689
Spleen	3 h	HSP70	1.00	3.30	0.10	1.85	0.12	3.09	0.4	0.304	0.002	0.704
		TLR-2	1.00	5.43	0.17	4.44	0.17	5.36	0.89	0.779	0.001	0.928
		TLR-4	1.00	3.79	1.74	9.06	5.73	7.03	0.69	0.074	0.014	0.198
		β-defensin 1	1.00	3.29	0.34	2.97	1.03	5.47	0.40	0.230	0.001	0.453
	12 h	HSP70	1.00	0.54	0.10	0.47	0.12	0.56	0.12	0.184	0.604	0.225
		TLR-2	1.00	1.04	0.17	0.80	0.17	1.32	0.25	0.582	0.167	0.560
		TLR-4	1.00	3.29	1.09	4.49	2.29	3.54	0.60	0.619	0.005	0.449
		β-defensin 1	1.00	1.68	0.44	1.79	1.19	2.86	0.18	0.065	0.001	0.394

HSP70 - heat-shock protein 70; TLR - toll-like receptor; −: injected with the saline; +: injected with LPS.
[1] Lipopolysaccharide was intra-abdominally injected at 1 mg/kg BW or the same amount of saline at 21, 23, 25, and 27 d of age.

liver, is mediated by IL-1β, IL-6, and TNF-α (Drazan et al., 1996), and is an anti-inflammatory factor that inhibits the activity of neutrophils and natural killer cells. In addition, α-AGP binds and transports substances such as histamine and steroids, enhancing LPS clearance or neutralizing its toxicity by binding directly to LPS (Waititu et al., 2016). In this study, there was no significant change in serum CRP detected after LPS injection, which may be related to sampling time or LPS dose. C-reactive protein is one of the earliest changes in acute-phase response protein. The peak of CRP in serum appeared at 1 and 24 h after stress, while the two sampling times of the present test were not included at this period, so it may be the reason why no significant changes of CRP was detected. Compared with chickens fed antibiotic diets, all chickens fed diets supplemented with 0.05 or 0.5% yeast had higher CRP levels at the time of the first LPS injection, and all chickens fed diets supplemented with 0.05% yeast had higher CRP levels at the time of the last 12 h of LPS injection. The effect of adding 0.5% yeast was consistent with that of antibiotics for many hours after LPS injection. The results showed that yeast had no significant effect on serum acute-phase protein levels in broilers in the absence of stress and in the presence of LPS stress, similar to the antibiotic group. Humoral immunity is an important aspect of animal specific immunity, which is mainly realized through immunoglobulin levels and specific antibody levels. In this study, we found that after the first injection of LPS, serum IgG and IgM levels of piglets decreased significantly at 3 h. Serum IgG level also decreased significantly, which was consistent with the results of the study on weaned piglets (Wang et al., 2019). However, serum IgA, IgG, and IgM levels were not affected by multiple stimulation with LPS. This may be due to the strong initial humoral immune response of the organism, the rapid action of serum IgG and IgM on the antigen, the increase in protein catabolism, and the inability of immunoglobulin synthesis to satisfy the needs of the stress state of the organism in a short period. Until 12 h, serum IgM returned to normal levels, but serum IgG levels remained deficient. In this study, all chickens fed diets supplemented with yeast had lower serum IgA levels and higher lysozyme levels at 12 h of the first injection compared with those fed diets with antibiotic. The effects of yeast and antibiotics were similar.

Initially, LPS stimulation induces the release of cytokines *in vivo*. In addition, LPS stimulation is involved in the synthesis of acute-phase proteins and the regulation of the immune system. Changes in cytokines affect not only signaling and the increase of specific cell populations, but also the metabolism

and redistribution of nutrients in the body (Klasing, 2007). Due to the high dose of LPS (1 mg/kg), serum cytokines were altered at 3 and 12 h after the initial immunization, and the results were similar to those of Williams et al. (2009). In this study, serum cytokine levels were dynamically monitored after two LPS injections. It was found that anti-inflammatory and pro-inflammatory factors showed corresponding changes, suggesting that the organism has a feedback regulatory role in maintaining the dynamic balance of inflammatory response. At 3 h after the first injection, 0.5% yeast reduced serum TGF-β levels, but had no effect on IL-4 and IL-10 levels, indicating that yeast can prevent excessive inflammatory response of the organism and avoid immune imbalance.

There was an interaction effect between LPS stimulation and diets on IL-1β transcript levels in the liver at 12 h after repeated LPS injections. Compared with saline-injected chickens, LPS stimulation significantly reduced the transcript levels of IL-1β, IL-2, IL-4, and TNF-α in the liver at 3 and 12 h as well as IFN-γ at 3 h after repeated injections, and significantly reduced the transcript levels of IL-1β, TNF-α, and IFN-γ in the spleen at 3 and 12 h as well as IL-6, and IL-10 at 3 h after repeated injections. At the same time, the results showed that the changes in the expression of inflammatory factors in the liver and spleen were consistent with the changes in the expression of inflammatory cytokines in the serum. In LPS-injected flocks, there were no differences in serum immunoglobulin and lysozyme levels when fed diets supplemented with 0.05 or 0.5% yeast compared with those fed antibiotic diets.

5. Conclusions

The injection of LPS significantly increases the inflammatory response in broiler chickens, while the addition of 0.05 or 0.5% yeast to broiler diets can effectively alleviate this inflammatory response, which has a good prospect of application as an alternative to antibiotics.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Zhang, L. and Fang, Y. **Data curation:** Sun, H.; Tao, H. and Xue, S. **Formal analysis:** Fang, Y. **Methodology:** Ding, Y. **Writing – original draft:** Zhang, L. **Writing – review & editing:** Fang, Y.

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