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Dietary *Artemisia ordosica* extract alleviating immune stress in broilers exposed to lipopolysaccharide

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ABSTRACT

This study was conducted to investigate the effect of *Artemisia ordosica* extract (AOE) on broilers challenged with lipopolysaccharide (LPS). 96 one-day-old Arbour Acres broilers were assigned in 2×2 factorial design, including two dietary treatments (0 or 1000 mg/kg AOE) and two immunological challenge (saline or LPS). on d 14, 16, 18 and 20, broilers were injected intra-abdominally with LPS solution (the LPS was dissolved in sterile saline at a concentration of 100 μg/mL) at 500 μg/kg of base weight, or an equivalent amount of sterile saline. Blood samples were collected on d 21 and 28. During LPS-challenged periods (days 15–21), AOE alleviated the compromised average daily gain and average daily feed intake ($p < .05$) in broilers challenged with LPS. On day 21, the LPS challenge increased ($p < .05$) serum adrenocorticotropic hormone, corticosterone, interleukin-1, interleukin-2, interleukin-6, immunoglobulin G and immunoglobulin A, decreased ($p < .05$) the content of serum growth hormone and insulin-like growth factor-1. However, diet supplemented with AOE reduced the elevation of serum corticosterone ($p = .054$), interleukin-2 ($p < .05$), immunoglobulin A ($p < .05$) and immunoglobulin G ($p = .079$) caused by LPS on day 21. After a week's recovery, on d 28, AOE reduced the serum interleukin-6 content ($p < .05$). It may be that AOE exert its beneficial effect on broilers challenged with LPS by lessening the inflammatory cytokines and stress hormone, weakening the over activated immune system and finally, improving the growth performance.

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Artemisia ordosica extract; immune stress; growth performance; blood parameter; broiler

Introduction

Activated immune system can resist the damage to the animal body from exogenous microbes, but excessive activation of immune system will produce immunological stress. There are many kinds of stress factors (environment, diseases, psychological factors and feeding management) in broiler breeding, which can directly or indirectly change the immune system, and induce the immune stress. (Mashaly et al. 2004; Shini & Kaiser 2009; Wilkinson et al. 2011) Previous studies showed that a number of different stressors result in the activation of the Hypothalamic–Pituitary–Adrenal axis (HPA axis) and the release of adrenocorticotropic hormone (ACTH), corticosterone (CORT) and the stressors can also act on immune system to influence organ growth, profiles of inflammatory cytokines and the immune response of antigen to antibody (Marketon & Glaser 2008; Shini & Kaiser 2009; Shini et al. 2010). When under immune stress, animal body protein and fat anabolism will be weakened, while catabolism will be enhanced in order to satisfy the requirement of nutrients to synthesise immune effector molecules, and then animal will consume large amount nutrients, resulting in lower feed utilisation and decreased growth performance (Johnson 1997; Jacobi et al. 2006). Immune stress always brings great economic losses to animal husbandry. Therefore, it is important to avoid immune stress though improving environmental conditions and balancing nutrition (Roura et al. 1992; Takahashi et al. 2002).

Artemisia plants contain rich nutrients and bioactive components, most of which can be used as medicine and their leaf powder or extract also can be used as a natural Chinese herbal medicine feed additive (Bhakuni et al. 2001; Brisibe et al. 2008; Gouveia & Castilho 2013; Zhang et al. 2013). Studies have shown that Artemisia plants can promote animal growth performance, improve the quality of animal products, enhance the immune function and antioxidant activity

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(Kim et al. 2002, 2012; Gholamrezaie Sani et al. 2013). *Artemisia ordosica* is a semi-shrubby plant of the genus Artemisia, mainly distributed in north and northwest sandy grassland of China, which not only contains rich nutrients – its flowering crude protein content reached 17.94%, crude fat content is 7.74%, but is also rich in polysaccharides, flavonoids, organic acids and other bio-active compositions, and the whole plant can be used as medicine. In recent years, studies about the effect of Artemisia plants on animals are more concentrated in wormwood and *Artemisia annua* L. (Li et al. 2015; Wan et al. 2016), but the impact of *Artemisia ordosica* is rarely reported.

Our research group has completed the initial research regarding the effect of *Artemisia ordosica* extract (AOE) in broilers, and the result showed that AOE could promote the growth performance and improve the immune function of broilers (Yue et al. 2015a, 2015b). Based on previous studies, the present experiment was conducted to investigate the effect of AOE on broilers under immune stress induced by injecting LPS, the major composition of the cell wall of Gram-negative bacteria, which can stimulate the body cell to synthesise and release many cytokines, such as tumour necrosis factor-α (TNF-α), interleukin (IL) and nitric oxide (NO). These bio-active molecules can induce animals' immune stress responses through the neuron-endocrine-immune network (Mangoni et al. 2008; Gomes et al. 2010; Murray & Smale 2012).

Materials and methods

Materials

Escherichia coli lipopolysaccharide (LPS, O55:B5), purchased from Sigma-Aldrich (L2880), was dissolved in sterile saline at a concentration of 100 μg/mL. *Artemisia ordosica* was collected from Erdos (Inner Mongolia, China) in July. The whole plant was washed with distilled water and placed in the shade to dry at room temperature. The dried plant was extracted in distilled water at 100 °C, for 3 times with 30 min per time, then the extract was concentrated and lyophilised to prepare the powder, and stored at −20 °C.

Experimental design

The animal protocol for the present study was approved and conducted under the guidelines of Animal Care and Use Committee of Inner Mongolia Agricultural University. A total of 96 broilers were raised for 28 days in a 2 × 2 factorial design, comparing 2 challenges [sham (−) or infected (+)] and two different dietary treatments. The basal diet supplemented with (1) no additive (CTR), or (2) *Artemisia ordosica* extract (AOE). All birds were randomly assigned to four treatment groups with six replicates, four birds in each replicate. The broilers in Treatment 1 and 2 were fed with the basal diet, and those in Treatment 3 and 4 were fed with the experimental diets (add 1000 mg/kg AOE). On d 14, 16, 18 and 20, broilers in both Treatment 1 and 3 were injected intra-abdominally with LPS solution at 500 μg LPS/kg of base weight (the LPS was dissolved in sterile saline at a concentration of 100 μg/mL), and those in Treatment 2 and 4 were injected intra-abdominally with equal amount of 0.9% sterile saline. Maize-soybean-based basal diet (Table 1) was formulated referring to the NRC (1994) nutrient requirement of broilers. During the entire experimental period, all birds were housed in a temperature-controlled cage maintained about 33 °C from d 1 to 3 which was then gradually reduced to 21 °C at the rate of 3 °C per week and then kept constant thereafter. The lighting programme was 23 h light/1 h dark for the first three days, followed by 18 h light/6 h dark for the rest of the trial period, and the relative humidity was maintained at 50 to 70%. Feed and water were provided *ad libitum* throughout the experiment.

Table 1. Composition and nutrient levels of basal diet (dry matter basis, %).

Ingredients	d 1–14	d 15–28
Maize	51.68	58.49
Soybean meal	41.00	34.30
Soybean oil	3.00	3.00
Dicalcium phosphate	1.90	1.80
Limestone	1.10	1.20
Salt	0.37	0.37
Lysine (98%)	0.05	0.03
Methionine	0.19	0.10
Premix[a]	0.71	0.71
Total	100	100
Nutrients levels[b]		
ME, MJ/kg	12.62	12.87
Crude Protein	21.84	19.95
Calcium	1.00	1.00
Available phosphorus	0.48	0.46
Lysine	1.40	1.20
Methionine	0.56	0.44
L-Cystine	0.40	0.37

[a]Premix provided the following per kilogram of diet: vitamin A 6141.5 IU; vitamin D_3 1789.2 IU; vitamin E 7.99 mg; vitamin K 1.82 mg; vitamin B_1 0.65 mg; vitamin B_2 3.93 mg; vitamin B_6 2.08 mg; vitamin B_{12} 0.01 mg; niacin 18.06 mg; calcium pantothenate 6.65 mg; folic acid 0.59 mg; biotin 0.07 mg; choline chloride 332.28 mg; Fe 60.91 mg; Cu 6.01 mg; Zn 65.75 mg; Mn 62.3 mg; I 0.9 mg; Se 0.21 mg.

[b]Crude Protein was measured value, while others were all calculated values.

Sampling and measurements

Growth performance parameters

Broilers were weighed at day 1, 14, 21, 28, and feed intake on each replicate basis was recorded at 14, 21 and 28d of age to calculate average daily weight gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR).

Serum parameters

On days 21 and 28, six broilers per treatment (one bird/replicate) were chosen, about 5 mL of blood sample were obtained from the wing vein, and collected in vacuum blood vessel. The serum samples were separated by centrifuged at 1790 × *g* for 10 min at room temperature, and then removed into microtubes and stored at −20 °C until analysis for immune and endocrine parameters. The concentration of drenocorticotropic hormone (ACTH), corticosterone (CORT), growth hormone (GH) and insulin-like growth factor-1 (IGF-1) interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM) were measured with commercial ELISA kit employs the quantitative sandwich enzyme immunoassay technique (R & D Systems China, Shanghai, China) according to the mamusfacturer's instructions.

Statistical analysis

All data were analysed using the GLM procedure of SAS 9.0 software (SAS Institute Inc. 2003) as 2 × 2 factorial arrangement with dietary treatments of AOE and LPS challenge as main effects. If a significant treatment effect was observed, the significance between the treatment differences was identified by Duncan's multiple comparisons test, and $p < .05$ was considered to be statistically significant.

Result

Growth performance

As shown in Table 2, dietary AOE had no significant effects on ADG, ADFI and FCR in broilers before LPS challenge (d 1–14). During the LPS-challenged days (d 15–21), LPS challenge reduced the ADG ($p < .05$) and ADFI ($p < .05$) of broilers, and tended to increase the FCR of broilers ($p = .061$). In contrast, as a main effect, the ADG ($p < .05$) and ADFI ($p < .05$) of broilers fed with AOE were higher than the broilers fed with basal diet, but feeding AOE had no impact on FCR. The interactions between LPS and AOE were observed in ADG ($p < .05$) and ADFI ($p = .063$). During the recovery period (d 22–28), there was no significant effect on ADG, ADFI and FCR among broilers fed with AOE or LPS-challenged.

Endocrine parameters

As shown in Table 3, on d 21, as a main factor, LPS challenge increased the serum ACTH ($p < .05$) and CORT content ($p < .05$), but feeding AOE tended to decreased the serum CORT content ($p = .054$), moreover, there were AOE × LPS interactions in ACTH ($p = .091$) and CORT ($p = .093$). When fed with basal diet, broilers challenged with LPS has higher serum ACTH ($p < .05$) and CORT ($p < .05$) content compared with those challenged with saline, but when fed with AOE diet, there was no significant difference in serum ACTH and CORT contents between the LPS group and saline group. On d 28, after a week of recovery, there was no significant difference in serum ACTH and CORT

Table 2. Effects of AOE on growth performance of broilers challenged with LPS.

	Control diet		AOE diet		SEM	*p*-Value		
	Saline	LPS	Saline	LPS		AOE	LPS	Interaction
ADG, g								
1–14 d	24.59	24.31	24.18	23.29	0.583	.449	–	–
15–21 d	43.10[a]	36.94[b]	42.76[a]	42.27[a]	1.398	.006	.042	.024
22–28 d	55.67	56.03	54.82	59.90	7.446	.327	.411	.742
ADFI, g								
1–14 d	40.51	37.51	38.60	38.92	1.152	.340	–	–
15–21 d	69.43[a]	59.32[b]	70.53[a]	68.90[a]	2.429	.012	.039	.063
22–28 d	120.57	119.38	118.88	119.13	5.467	.865	.935	.903
FCR								
1–14 d	1.65	1.55	1.60	1.67	0.049	.288	–	–
15–21 d	1.57	1.63	1.50	1.60	0.041	.321	.061	.339
22–28 d	1.86	1.84	1.78	1.76	0.046	.169	.566	.982

ADG: average daily gain; ADFI: average daily feed intake; FCR: feed/gain; SEM: standard error of the mean. In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > .05$), while with different letter superscripts mean significant difference ($p < .05$).

Table 3. Effects of AOE on stress and growth-related hormones of broilers challenged with LPS.

	Control diet		AOE diet			*p*-Value		
Item	Saline	LPS	Saline	LPS	SEM	AOE	LPS	Interaction
21d								
ACTH, μg/L	90.00^{b}	189.17^{a}	125.00^{ab}	147.50^{ab}	23.954	.878	.011	.091
CORT, μg/L	39.58^{b}	73.75^{a}	37.08^{a}	41.88^{a}	8.061	.054	.032	.093
GH, μg/L	12.73	8.07	13.79	8.17	2.498	.825	.049	.849
IGF-1, μg/L	17.54	13.86	16.33	13.54	1.399	.605	.037	.769
28d								
ACTH, μg/L	88.75	141.35	103.75	93.00	16.618	.349	.351	.118
CORT, μg/L	38.75	54.79	42.00	41.50	5.732	.373	.172	.178
GH, μg/L	12.23	11.60	21.89	14.52	3.864	.147	.342	.441
IGF-1, μg/L	16.95	19.11	23.16	17.49	2.056	.274	.401	.070

ACTH: adrenocorticotropic hormone; CORT: corticosterone; GH: growth hormone; IGF-1: insulin-like growth factor-1; SEM: standard error of the mean. In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > .05$), while with different letter superscripts mean significant difference ($p < .05$).

Table 4. Effects of AOE on serum cytokines of broilers challenged with LPS.

	Control diet		AOE diet			*p*-Value		
Item	Saline	LPS	Saline	LPS	SEM	AOE	LPS	Interaction
21d								
IL-1, ng/L	31.48^{b}	50.34^{a}	40.55^{ab}	40.36^{ab}	4.030	.909	.031	.028
IL-2, ng/L	45.56^{b}	56.67^{a}	43.06^{b}	45.83^{b}	2.675	.022	.036	.131
IL-6, ng/L	7.60^{b}	17.00^{a}	10.72^{ab}	12.81^{ab}	2.160	.816	.025	.133
28d								
IL-1, ng/L	35.08	38.06	34.38	34.65	1.334	.119	.216	.312
IL-2, ng/L	49.33	60.50	55.00	59.33	4.245	.629	.109	.465
IL-6, ng/L	14.22	14.70	10.55	10.62	1.644	.019	.860	.895

IL-1: interleukin-1; IL-2: interleukin-2; IL-6: interleukin-6; SEM: standard error of the mean. In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > .05$), while with different letter superscripts mean significant difference ($p < .05$).

contents among broilers fed with AOE or LPS-challenged.

On d 21, LPS stimulation reduced the contents of serum GH ($p < .05$) and IGF-1 ($p < .05$) in broilers, but, as a main factor, feeding AOE had no effect on GH and IGF-1 in broilers, and the interaction between LPS and AOE was not significant. On d 28, after a week of recovery, the tendency of interaction between LPS and AOE was observed in IGF-1 ($p = .070$).

Immune parameters

As shown in Table 4, On d 21, the contents of serum IL-1, IL-2 and IL-6 in broilers injected with LPS was significantly higher than the unchallenged broilers, while AOE, as a main factor, decreased the content of serum IL-2 ($p < .05$) in broilers, but had no impact on IL-1 and IL-6. The interaction between LPS stimulation and AOE treatment was statistically significant in serum IL-1, When fed with basal diet, broilers challenged with LPS showed significantly higher serum IL-1, IL-2 and IL-6 content compared with those injected with saline, but when fed with AOE diet, there was no significant difference in serum content of IL-1, IL-2 and IL-6 between the LPS groups and saline groups. On d 28, after a week of recovery, AOE significantly reduced the content of serum IL-6.

As shown in Table 5, on d 21, LPS challenge increased the serum IgA ($p < .05$), IgG ($p < .05$) contents compared with the saline treatment groups. However, diet supplemented with AOE reduced the content of serum IgG ($p < .05$) in broilers, and there was a significant interaction between LPS challenge and AOE treatment. From the statistical results of four treatment groups, in LPS-unchallenged group, there was no significant difference between AOE diet and basal diet. But in LPS-challenged group, the serum content of IgA and IgG in broilers fed with AOE was significantly lower than those fed with basal diet. On d 28, after a week of recovery, there was no significant difference in serum IgA, IgG and IgM contents among broilers fed with AOE or LPS-challenged.

Discussion

Growth performance

LPS model is a classical model of immune stress in broiler chickens. Undoubtedly, decreased feed intake, lower body weight gain and higher feed conversion efficiency are the common results of immune stress in

Table 5. Effects of AOE on serum immunoglobulin in broilers challenged with LPS.

	Control diet		AOE diet			*p*-Value		
Item	Saline	LPS	Saline	LPS	SEM	AOE	LPS	Interaction
21d								
IgA, ng/mL	57.75[b]	109.00[a]	42.33[b]	67.00[b]	14.379	.046	.016	.333
IgG, ng/mL	664.00[b]	930.00[a]	696.88[b]	701.67[b]	66.391	.079	.048	.044
IgM, μg/mL	4.45	3.83	4.74	3.63	0.587	.927	.187	.711
28d								
IgA, ng/mL	74.75	80.67	65.67	61.25	17.442	.571	.991	.828
IgG, ng/mL	323.50	314.50	377.00	397.00	60.313	.343	.935	.835
IgM, μg/mL	4.24	5.79	3.60	5.79	0.758	.142	.151	.542

IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; SEM: standard error of the mean. In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > .05$), while with different letter superscripts mean significant difference ($p < .05$).

different animals (Wright et al. 2000; Takahashi et al. 2003; Yang et al. 2011). Our experimental results were consisted with the previous studies. The present study showed that diet supplemented with AOE had no effect on growth performance of broilers unchallenged with LPS, but significantly increased the decline of ADG and ADFI caused by LPS. The reason to why AOE could relieve the growth inhibition of broilers may be that the bio-active compositions of AOE such as polysaccharides, flavonoids, could alleviate the inflammatory reaction and improve the growth performance (Liu et al. 2015).

Generally, the inflammatory response is a hallmark of immune stress. Previous studies have reported that the proinflammatory cytokines (IL-1, IL-6, TNF-α) can reduce the utilisation rate of nutrients (Feingold et al. 2008; Waggoner et al. 2009), and inhibit the growth performance (Sijben et al. 2001). In this study, LPS injection significantly increased the contents of serum IL-1, IL-2 and IL-6, meanwhile, diet supplemented with AOE significantly reduced the serum content of IL-2 (on d 21) and IL-6 (on d 28); there was an interaction on IL-1 in broilers between LPS and AOE. This observation suggested that AOE have beneficial effect on inflammatory reaction. It can be speculated that AOE could relieve the growth inhibition of broilers caused by LPS through decreasing the levels of serum inflammatory cytokines.

Inflammatory cytokines such as IL-1, IL-6 could activate the B cell and trigger its proliferation and humoral immune response. Studies have shown that increased humoral response is associated with inflammatory response (Yang et al. 2008, 2011). The present experiment showed that LPS stimulation significantly increased the content of serum IgA and IgG, which consist with the previous studies. The result illustrated that the immune system of broilers was over activated, which could result in the redistribution of nutrients away from growth and towards the immune reaction, and eventually led to the decline of the growth performance. Diet supplemented with AOE significantly inhibited the increased content of serum IgA and IgG, which indicated that AOE could weaken the excessive activation of immune system, and alleviate the immune stress, Currently, the effect of AOE on immune function in broilers rarely was reported, but many studies reported that the Artemisia plants could alleviate the inflammatory response induced by LPS, and the present study was corroborated by these research results (Jang et al. 2005; Yoon et al. 2010; Jang et al. 2015).

When under immune stress, the excessive inflammatory cytokines may lead to the activation of HPA axis, causing the secretion of ACTH and CORT, the levels of ACTH and CORT illustrate the degree of immune stress damaging animal body (Dorshkind & Horseman 2001; Dunn et al. 2004). In this study, LPS stimulation significantly increased the contents of serum ACTH and CORT; but AOE alleviated the increased serum CORT content, and the tended interaction between LPS and AOE was observed in ACTH and CORT (on d 21), associated with this result, AOE also slowed down the increase of inflammatory cytokines (IL-1 and IL-6) caused by LPS stimulation. Therefore, the effect of AOE on serum ACTH and CORT content may be related to its impact on the release of inflammatory cytokines.

GH and IGF-1 are the component parts of growth axis, and their main function is to regulate animal growth. Studies have reported that inflammatory cytokines can directly act on central nervous system, reducing the secretion of the growth promoting hormone such as GH and IGF-1 (Hasselgren 1993; Soto et al. 1998), and eventually affect the growth performance of animals. In this study, LPS stimulation significantly reduced the serum GH and IGF-1 content, which in line with the previous studies. However, the tended interaction between LPS and AOE was

observed in IGF-1 (on d 28), this may be due to AOE decreased the elevation of IL-6 induced by LPS (on d 28).

Conclusions

In conclusion, this may be because AOE exert its beneficial effect on broilers challenged with LPS by lessening the inflammatory cytokines, stress hormone and promoting the growth hormone and finally, improving the growth performance.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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