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Effects of Artemisia argyi Aqueous Extract on Serum Cytokine Levels and Small Intestinal Inducible Nitric Oxide Synthase mRNA Expression in Broiler Chickens (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different levels of Artemisia argyi aqueous extract on serum cytokine contents and small intestinal inducible nitric oxide synthase (iNOS) mRNA expression levels in broiler chickens. A total of 192 healthy 1-day-old Arbor Acres (AA) broiler chickens were randomly allocated into 4 groups with 6 replicates per group and 8 chickens per replicate. The control group was fed a basal diet, while the treatment groups were fed experimental diets supplemented with 500, 1,000, and 2,000 mg/kg Artemisia argyi aqueous extract, respectively. The experimental period lasted 42 days. The results showed: 1) Compared with the control group, at 21 days of age, serum interleukin-1β (IL-1β), interleukin-2 (IL-2), and interleukin-4 (IL-4) contents in the 2,000 mg/kg group were significantly increased ($P<0.05$), serum IL-4 content in the 500 mg/kg group and serum IL-2 content in the 1,000 mg/kg group were extremely significantly increased ($P<0.01$); at 42 days of age, serum IL-2 content in the 500 mg/kg group was significantly increased ($P<0.05$). 2) Compared with the control group, at 21 days of age, there were no significant differences ($P>0.05$) in serum nitric oxide (NO) content and iNOS activity and iNOS mRNA expression levels in the duodenum, jejunum, and ileum in the 500 mg/kg group, serum NO content and ileal iNOS mRNA expression level in the 1,000 mg/kg group were significantly increased ($P<0.05$), and ileal iNOS mRNA expression level in the 2,000 mg/kg group was extremely significantly increased ($P<0.01$); at 42 days of age, serum NO content and iNOS activity and jejunal iNOS mRNA expression level in the 500, 1,000, and 2,000 mg/kg groups were significantly or extremely significantly increased ($P<0.05$ or $P<0.01$). In conclusion, dietary supplementation with Artemisia argyi aqueous extract can promote the secretion of immune cytokines

and production of NO in broiler chickens, which is associated with enhanced iNOS mRNA expression.

Full Text

Abstract

This experiment was conducted to study the effects of dietary supplementation with Artemisia argyi aqueous extract (AAE) on serum cytokine content and small intestine inducible nitric oxide synthase (iNOS) mRNA expression level in broilers. A total of 192 one-day-old Arbor Acre (AA) broilers were randomly divided into four groups with six replicates per group and eight chickens per replicate. Broilers in the control group were fed a basal diet, while the other groups were fed the basal diet supplemented with 500, 1,000, and 2,000 mg/kg AAE, respectively. The experiment lasted for 42 days. The results showed as follows: 1) Compared with the control group, at 21 days of age, the contents of interleukin-1β (IL-1β), interleukin-2 (IL-2), and interleukin-4 (IL-4) in serum of the 2,000 mg/kg supplementation group were significantly improved ($P<0.05$), and the serum IL-4 content of the 500 mg/kg supplementation group and the serum IL-2 content of the 1,000 mg/kg supplementation group were significantly improved ($P<0.01$); at 42 days of age, the serum IL-2 content of the 500 mg/kg supplementation group was significantly increased ($P<0.05$). 2) Compared with the control group, at 21 days of age, the nitric oxide (NO) content and iNOS activity in serum and iNOS mRNA expression level in duodenum, jejunum, and ileum of the 500 mg/kg supplementation group showed no significant difference ($P>0.05$); the serum NO content and ileum iNOS mRNA expression level of the 1,000 mg/kg supplementation group were significantly increased ($P<0.05$); the ileum iNOS mRNA expression level of the 2,000 mg/kg supplementation group was significantly increased ($P<0.01$); at 42 days of age, the NO content and iNOS activity in serum and jejunum iNOS mRNA expression level of the 500, 1,000, and 2,000 mg/kg supplementation groups were significantly improved ($P<0.05$ or $P<0.01$). The results suggested that dietary supplementation of AAE can improve the secretion of cytokines and the production of NO, and it is related to the expression of iNOS mRNA.

Keywords: Artemisia argyi aqueous extract; broilers; cytokines; inducible nitric oxide synthase; mRNA

1. Materials and Methods

1.3 Animal Management

The broilers were housed in a temperature-controlled facility with adjustable ventilation. The housing environment was maintained according to standard broiler management practices. The temperature and humidity were controlled appropriately throughout the experimental period. Animal welfare protocols were followed, and all procedures were approved by the institutional animal

care committee.

1.4 Sample Collection

At 21 and 42 days of age, two chickens were randomly selected from each replicate (totaling 12 chickens per group) for blood collection. Blood samples were drawn from the wing vein and allowed to clot at room temperature for 1 hour before centrifugation at 3,000 r/min for 10 minutes to separate serum. The serum was stored at -20°C for subsequent analysis. Following blood collection, the chickens were euthanized and tissue samples from the duodenum, jejunum, and ileum were immediately collected. Approximately 1.5 cm segments from each intestinal region were excised, flushed with physiological saline, and preserved in 1.5 mL EP tubes. These samples were snap-frozen in liquid nitrogen and stored at -80°C until RNA extraction.

1.5 Detection Methods

1.5.1 Serum Cytokine Content Detection Serum levels of IL-1β, IL-2, IL-4, IL-6, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer’ s instructions. All assays were performed in duplicate.

1.5.2 Serum NO Content and iNOS Activity Detection Serum NO content and iNOS activity were measured using commercial detection kits according to the manufacturer’ s protocols. The assays were conducted with strict adherence to standard operating procedures for biochemical analysis.

1.5.3 iNOS mRNA Expression Level Detection 1.5.3.1 RNA Extraction and Reverse Transcription

Total RNA was extracted from intestinal tissue samples using Trizol reagent (Takara, Japan) following the manufacturer’ s protocol. RNA quality and quantity were assessed using a spectrophotometer, with A260/A280 ratios between 1.8 and 2.1 considered acceptable. RNA integrity was verified by electrophoresis on 2% agarose gels. Reverse transcription was performed using PrimeScript RT Master Mix to synthesize cDNA, which was stored at -80°C until further analysis.

1.5.3.2 Real-time Quantitative PCR

Primers for iNOS and β-actin (internal control) were designed based on GenBank sequences (Table 2). Real-time quantitative PCR was performed using SYBR Premix Ex TaqTM II in a total reaction volume of 20 µL, containing 10 µL SYBR Premix, 0.4 µL each of forward and reverse primers, 2 µL cDNA template, and 7.2 µL RNase-free water. The thermal cycling conditions were: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s, with a final

extension at 72°C for 20 s. A melting curve analysis was performed from 70°C to 95°C to verify product specificity. Relative gene expression was calculated using the 2-ΔΔCt method.

1.6 Statistical Analysis

All data were analyzed using SAS 2000 software. One-way analysis of variance (ANOVA) was performed to determine treatment effects, and Duncan's multiple range test was used for post-hoc comparisons. Significance was declared at $P<0.05$, and highly significant differences were indicated at $P<0.01$. Results are presented as means with standard errors.

2. Results

2.1 Effects of AAE on Serum Cytokine Content

As shown in Table 3, at 21 days of age, dietary supplementation with 500 mg/kg AAE significantly increased serum IL-4 content ($P<0.01$), while 1,000 mg/kg AAE significantly increased serum IL-2 content ($P<0.01$). The 2,000 mg/kg AAE group showed significant increases in serum IL-1β, IL-2, and IL-4 contents ($P<0.05$). At 42 days of age, the 500 mg/kg AAE group exhibited significantly elevated serum IL-2 content ($P<0.05$). No significant differences were observed in IL-6, IFN-γ, or TNF-α levels among treatments at either time point (data not shown).

2.2 Effects of AAE on Serum NO Content and iNOS Activity

The effects of AAE on serum NO content and iNOS activity are presented in Table 4. At 21 days of age, the 1,000 mg/kg AAE group showed significantly increased serum NO content ($P<0.05$), while the 500 and 2,000 mg/kg groups showed no significant differences compared with the control ($P>0.05$). Serum iNOS activity was not significantly affected by AAE supplementation at this time point. At 42 days of age, all AAE-supplemented groups (500, 1,000, and 2,000 mg/kg) exhibited significantly increased serum NO content ($P<0.05$ or $P<0.01$) and iNOS activity ($P<0.05$ or $P<0.01$) compared with the control group.

2.3 Effects of AAE on iNOS mRNA Expression in Small Intestine

As illustrated in Table 5, at 21 days of age, iNOS mRNA expression in the duodenum and jejunum was not significantly affected by AAE supplementation ($P>0.05$). However, the 1,000 mg/kg group showed significantly increased iNOS mRNA expression in the ileum ($P<0.05$), and the 2,000 mg/kg group exhibited a highly significant increase in ileal iNOS mRNA expression ($P<0.01$). At 42 days of age, all supplementation levels significantly increased iNOS mRNA expression in the jejunum ($P<0.05$), while no significant effects were observed in the duodenum or ileum.

3. Discussion

3.1 Effects of AAE on Serum Cytokines

Cytokines are crucial mediators of immune responses, including inflammatory reactions, cellular immunity, humoral immunity, and NK cell activity. IL-1, IL-2, IL-4, IL-6, IFN-γ, and TNF-α play pivotal roles in regulating immune function. IL-1 promotes T lymphocyte proliferation and IL-2 production, thereby enhancing cellular immunity. IL-2 stimulates T cell proliferation and differentiation, promoting LAK cell activity. IL-4, produced by Th2 cells, regulates humoral immunity and IgE-mediated immune responses, while IFN-γ, secreted by Th1 cells, antagonizes IL-4 activity. IL-6 supports B cell differentiation and antibody production. TNF-α, produced by activated macrophages, participates in inflammatory responses and immune regulation.

The present study demonstrated that AAE supplementation increased serum IL-1β, IL-2, and IL-4 levels in broilers, particularly at 21 days of age. These findings align with previous research showing that AAE can modulate immune function by enhancing cytokine secretion. The immunomodulatory effects of AAE may be attributed to its bioactive compounds, which can stimulate T/B lymphocyte proliferation, macrophage activity, and NK cell function. The differential effects observed at 21 versus 42 days suggest that younger broilers may be more responsive to AAE supplementation, possibly due to the developmental stage of their immune system.

3.2 Effects of AAE on NO Production, iNOS Activity, and iNOS mRNA Expression

NO is a key signaling molecule produced by activated macrophages that plays diverse roles in immune responses. iNOS is the enzyme responsible for NO synthesis during immune activation, and its expression is regulated at the transcriptional level by various stimuli including cytokines and lipopolysaccharide. The upregulation of iNOS mRNA leads to increased iNOS protein synthesis and subsequent NO production, which contributes to antimicrobial defense and immune regulation.

Our results showed that AAE supplementation enhanced serum NO content and iNOS activity, particularly at 42 days of age. The increased iNOS mRNA expression in the small intestine, especially in the jejunum, suggests that AAE may modulate local mucosal immunity. The finding that iNOS expression was more pronounced in the ileum at 21 days but shifted to the jejunum at 42 days indicates a dynamic, age-dependent effect of AAE on intestinal immune function. These results are consistent with studies demonstrating that plant extracts can activate the NF- B signaling pathway, leading to iNOS transcription and NO production.

The dose-dependent effects observed in this study suggest that AAE supplementation at 1,000–2,000 mg/kg may be optimal for enhancing immune function in

broilers. The ability of AAE to simultaneously improve cytokine secretion and NO production while upregulating iNOS mRNA expression indicates its potential as a natural immunomodulator in poultry production.

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