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RESEARCH ARTICLE

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Effects of *Echinacea purpurea* extract on the immunological response to infectious bursal disease vaccine in broilers

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Abstract *Echinacea purpurea* is among the most widely used herbal medicines throughout Europe and North America for the prevention or treatment of infectious diseases. However, there have been few reports on the effect of the herb in chickens. In order to investigate the immunomodulatory effect of *Echinacea purpurea* in fowls, 150 seven-day-old broilers were randomly divided into five groups (30 in each group). Birds in Groups A, B, and C were orally given *Echinacea purpurea* extract once a day at low (0.1 g, Group A), medium (0.5 g, Group B), and high (1 g, Group C) doses for seven days, while Group D and Group E, assigned as control Group I and control Group II, respectively, were given distilled water in the same amount as Groups A, B, and C. Broilers in Groups A, B, C, and D were normally immunized with infectious bursal disease (IBD) vaccine at 14-day-old, whereas Group E was neither treated with *Echinacea purpurea* extract nor vaccinated. Results indicated that antibody titers were higher ($P < 0.01$) in the three *Echinacea purpurea* extract treated groups (Groups A, B, and C) compared with Group D on days 21, 28, 35, and 42 after treatment. The antibody titer raised more strikingly in groups treated with higher doses of *Echinacea purpurea* extract (0.5 g and 1 g) than lower dose (0.1 g). The IL-2 level in peripheral blood was significantly higher in Groups B and C compared with Group D ($P < 0.01$ on day 21 and $P < 0.05$ on days 28 and 35). No significant difference was observed between Group A and Group D. The TNF-α content in Group B was significantly higher than that of Group D ($P < 0.01$ on day 21 and day 28, $P < 0.05$ on day 35). Birds in Group C also showed a higher TNF-α content than Group D ($P < 0.05$ at the three measuring dates). These results indicated that *Echinacea purpurea* extract significantly enhanced IL-2 and TNF-α production and antibody titers to the IBD vaccine. The *Echinacea purpurea* extract was also found to increase the feed conversion ratio.

Keywords *Echinacea purpurea* extract, antibody, IL-2, TNF-α, infectious bursal disease, broiler

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1 Introduction

With the development of the chicken farming industry in China, infectious diseases of fowls occur more frequently than ever before and often cause severe damage to the industry. Vaccination is the major consideration in controlling infectious conditions up to date. However, for some infectious diseases such as IBD, the birds often do not respond to the vaccine well enough as expected, i.e., there are still some infected cases with IBD. The reasons for this phenomenon may be that there are more and more altered virus strains or some immunosuppressive factors (Jackwood and Sommer, 1987).

Extracts from *Echinacea purpurea* are among the most widely used herbal medicines throughout Europe and North America for the prevention or treatment of common cold, coughs, bronchitis, and other upper respiratory infections (Sharma et al., 2006a). There are several plant species used therapeutically including *Echinacea angustifolia*, *Echinacea pallida* and *Echinacea purpura*. These species differ somewhat in their chemical makeup, containing multiple substances, of which polysaccharides, caffeic acid derivatives (cichoric acid), alkamides, and glycoproteins are the most important in activity. Cichoric acid is an appropriate marker of the quality of *E. purpurea* containing product, because it has immune stimulatory effects and it is susceptible to degradation (Mancek and Kreft, 2005; Liu et al., 2006).

Echinacea purpurea was successfully introduced into China a few years ago, and its products have been applied increasingly in clinical veterinary. In medical practice, a herbal preparation containing *E. purpurea* was effective in treating human acute respiratory infection (Narimanian

et al., 2005) or canine upper respiratory tract infections (Reichling et al., 2003). However, there have been few reports on what effect the herb exerts in chickens. We hypothesize that the herb has an immunoenhancing effect on chickens because studies indicate the herb stimulates the immunoglobulin and antibody synthesis in chickens immunized with human serum albumin (Schranner et al., 1989). The experiment was designed to elucidate the immunomodulatory effect of *Echinacea purpurea* extract on immunity response of broilers to the challenge with the infectious bursal disease vaccine.

2 Materials and methods

2.1 Reagents

Echinacea purpurea extract was obtained from Huisheng Medicinal Company Ltd. in Shanxi Province, Cat. No. LYZZJ-060136. The extract was prepared from the whole grass of *Echinacea purpurea*, extracted in chloroform. The extraction rate was 0.33%, i.e., 3.3 g of extract obtained from 1 kg of whole plant. According to the manufacturer's instructions, high performance liquid chromatography (HPLC) results revealed that 1 g *E. purpurea* extract contained 20 mg cichoric acid, 2 mg caffeic acid, 23 mg echinacoside, and 3 mg chlorogenic acid. The extract was diluted in a solution at $1\ g \cdot mL^{-1}$ extract with distilled water. The ELISA Kits for measuring the infectious bursal disease antibody was from Biochek Corporation Ltd, UK. The IL-2 ELISA Kit was from BIOWEN CO. LTD., CANADA (Cat. No. JND-12308 ELISA). The ELISA Kits of TNF-α was from BIOWEN CO., LTD. CANADA (Cat. No. JND-35471 ELISA). The infectious bursal disease vaccine was from Ruipu Biological Company, Tianjin, China (Cat. No. 070606003).

2.2 Animals and treatments

One-day-old Ross 308 broilers were acclimatized for 7 days, then 150 birds were randomly divided into Groups A, B, C, D, and E with 30 birds in each group. Each bird in Group A was treated with 0.1 g of *Echinacea purpurea* extract. Birds in Group B were given 0.5 g of the extract per bird, and birds in Group C were treated with 1 g of the extract. The extracts were given to the birds orally in Groups A, B, and C daily from the age of day 8 to day 14 consecutively for 7 days. Broilers in Groups D and E were given the same volume of distilled water. Birds in Groups A, B, C, and D were immunized with infectious bursal disease vaccine by nasal or eye drops at double dosages as recommended at day 14. Group E was double blind control, receiving neither vaccination nor *E. purpurea* extract.

Animal care and handling procedure are performed in compliance with Chinese national guidelines.

2.3 Immunity status and body weight gain analysis

Blood samples were collected aseptically at days 14, 21, 28, 35, and 42, respectively. Blood plasma was obtained by centrifugation at $3000\ r \cdot min^{-1}$ for 15 min and stored at −80°C for the detection of antibody titers to IBD vaccine. The cytokine levels of IL-2 and TNF-α in peripheral blood were analyzed at days 21, 28, and 35, respectively. Feed consumption and body weight gain were recorded every two weeks to calculate the feed conversion ratio according to the following formula:

Feed conversion ratio = quantity of feeding(g)/body weight(g).

2.4 Statistical analysis

All data were expressed as mean ± SE. The differences between groups were analyzed by one-way analysis of variance (ANOVA). All statistical analysis was carried out using SPSS 11.0 for Windows.

3 Results

3.1 Changes of IBD vaccine antibody titers

All the broilers in Groups A, B, C, and D were vaccinated at day 14. No significant difference in antibody titers was observed between the four groups. However, higher antibody titers were calculated ($P < 0.01$) in the three *Echinacea purpurea* extract treated groups compared with the distilled water treated group (Group D) on days 21, 28, 35, and 42, respectively. Significant differences were also observed in Groups B and C in comparison with Group A ($P < 0.01$) measured at days 21, 28, and 42, respectively. At day 35, the antibody titer in Group C was higher ($P < 0.05$) than that in Group A, while Group B had much higher ($P < 0.01$) antibody titer than Group A (Table 1).

Group E as the control Group II was treated with neither *E. purpurea* extract nor vaccination. The antibodies remained at the same low level during the course of the experiment. Each bird in Groups A–C was treated with 0.1 g, 0.5 g, and 1 g *E. purpurea* extract, respectively, with Group D treated with vaccination only.

3.2 Changes of IL-2

The IL-2 level in peripheral blood was significantly higher in Groups B and C compared with Group D (the control Group I) ($P < 0.01$ on day 21, $P < 0.05$ on days 28 and 35). No significant difference was observed between Group B and Group A or between Group C and Group A. When compared with the control Group I (Group D), the IL-2 level was not significantly higher in Group A. In the control Group II (Group E), IL-2 content was not detectable (Table 2).

Table 1 Changes of IBD antibody (log10) titers in each group ($n = 20$)

group	day 14	day 21	day 28	day 35	day 42
A	2.392 ± 0.030^{A}	3.287 ± 0.015^{B}	3.280 ± 0.026^{B}	3.267 ± 0.015^{Bb}	3.200 ± 0.010^{B}
B	2.391 ± 0.015^{A}	3.463 ± 0.080^{A}	3.530 ± 0.106^{A}	3.533 ± 0.114^{A}	3.510 ± 0.125^{A}
C	2.385 ± 0.027^{A}	3.477 ± 0.080^{A}	3.477 ± 0.100^{A}	3.413 ± 0.057^{ABa}	3.453 ± 0.055^{A}
D	2.403 ± 0.012^{A}	3.057 ± 0.057^{C}	3.100 ± 0.025^{C}	3.027 ± 0.112^{C}	2.427 ± 0.025^{C}
E	2.391 ± 0.012^{A}	2.731 ± 0.116^{D}	2.143 ± 0.049^{D}	1.820 ± 0.026^{D}	1.384 ± 0.006^{D}

Note: Values followed by different lowercases or capitals within the same column are significantly different at 0.05 and 0.01 probability level, respectively.

Table 2 Changes of IL-2 ($pg\cdot mL^{-1}$) level in each group ($n = 10$)

group	day 21	day 28	day 35
A	62.35±14.10	55.75±12.82	59.65±26.55
B	75.95±20.07**	64.50±10.60*	69.25±21.27*
C	78.40±14.71**	63.85±19.28*	62.75±28.49*
D	55.45±29.01	52.60±12.40	50.32±16.63
E	—	—	—

Note: Compared with Group D (the control Group I), * means significantly different at $P < 0.05$, ** means significantly different at $P < 0.01$.

3.3 Changes of TNF-α

The TNF-α content in Group B was significantly higher than that in Group D ($P < 0.01$ on day 21 and day 28, $P < 0.05$ on day 35). Birds in Group C also showed a higher TNF-α content than Group D ($P < 0.05$) at three measuring dates. There was no significance in the TNF-α contents between Groups A and D. The TNF-α content in control Group II (Group E) was too low to be detected (Table 3).

Table 3 Changes of TNF-α ($pg\cdot mL^{-1}$) level in each group ($n = 10$)

group	day 21	day 28	day 35
A	26.50±11.51	37.13±18.31	34.07±16.67
B	57.83±25.56**	47.02±19.78**	43.30±16.72*
C	37.52±21.97*	40.15±13.00*	42.67±2.80*
D	27.35±15.68	30.74±4.57	25.02±16.73
E	—	—	—

Note: Compared with Group D (the control Group I), * means significantly different at $P < 0.05$, ** means significantly different at $P < 0.01$.

3.4 Changes of feed conversion ratio

All the birds were weighed on day 1, and there was no significant difference between each group. Gavage with *Echinacea purpurea* extract in Groups B and C significantly reduced the feed conversion ratio on days 14, 28, and 42 compared with Group D (control Group I) (Table 4).

Table 4 Feed conversion ratio of each group ($n = 20$)

group	day 1	day 14	day 28	day 42
A	1.130±0.231	1.155±0.121**	1.622±0.103	1.896±0.325*
B	1.135±0.452	1.131±0.113**	1.462±0.467**	1.821±0.462**
C	1.132±0.536	1.153±0.312**	1.593±0.362*	1.911±0.251*
D	1.136±0.352	1.205±0.423	1.679±0.156	2.040±0.674
E	1.134±0.436	1.245±0.325	1.803±0.025	2.286±0.856

Note: Compared with Group D (the control Group I), * means significantly different at $P < 0.05$, ** means significantly different at $P < 0.01$.

4 Discussion

With the increasing worry over food contamination by drug residues and the abuse of antibiotics, more and more health practitioners are focusing on fortifying the immune system to fight off potential infections rather than just treating the infection after it has developed. Therefore, a natural antibiotic with virtually no side-effects is one of the best choices. *Echinacea* is one of several herbs that possess antibacterial, antiviral, and antifungal properties. Our present study is designed to investigate whether *Echinacea purpurea* extract exerts the immunoenhancing properties in broilers. The results reveal significantly higher antibody titers in broilers treated with the herb extract (Table 1). Using female Swiss mice, Freier et al. (2003) observed an enhancement of the antibody forming cell response by oral gavage of the glycerine extract of *E. purpurea* following immunization with sheep red blood cells (SRBC) over naive controls, suggesting the potential for enhancement of humoral immune responses by *E. purpurea* extract. An aqueous-ethanolic extract of the mixed herbal drugs *Echinaceae purpureae radix* and *Echinaceae pallidae radix* and two other herbs caused a significant enhancement of the antibody response against sheep red blood cells, inducing an increase in the number of splenic plaque-forming cells and the titers of specific antibodies in the sera of the treated animals. The extract also brought the antibody response of the immunosuppressed mice to normal (Bodinet et al., 2002). Zhang (2005) reported that *E. purpurea* extract ($1\ g\cdot L^{-1}$ drinking water) used for five days significantly augmented the newcastle disease

antibody and IBD antibody production in chickens. The swine erysipelas antibodies showed a marked significance ($P < 0.05$) with regard of the level in both *Echinacea* supplemented groups (Maass et al., 2005). Our results were in agreement with the abovementioned reports.

Humoral immunity plays a vital role in immunosuppressive disease such as the infectious bursal disease (IBD). *Echinacea purpurea* extract significantly enhanced the IBD antibody levels in the blood in our experiment, and three different dosages of the extract had the similar effects, which indicated that *Echinacea purpurea* extract could exert the humoral immune boosting effect in broilers.

Though there are few reports on application of *Echinaceae purpureae* in chickens, there are some studies in mice. The aqueous-ethanolic extract of *E. purpureae* and other herbs induced spleen cells in mice to produce higher amount of IL-2, IFN-γ, and GM-CSF ex vivo. The application of the herbal extract also triggered the production of IL-1 and TNF-α by peritoneal macrophages ex vivo (Bodinet et al., 2002). Sharma et al. (2006b) reported that rhinovirus infection of epithelial cells resulted in a dramatic increase in more than 30 transcription factors (TF), including the 12 proinflammatory factors examined, such as NF-κB, AP-1, AP-2, and STATs 1–6. Treatment with *Echinacea* extracts reduced TFs to low levels. Goel et al. (2002) reported that *Echinacea* stimulated macrophage function in the lung and spleen of normal rats. Phagocytic activity of alveolar macrophage was increased with increasing concentrations of the *Echinacea* components. An enhanced release of cytokines (such as TNF-α and IFN-γ) in response to *Echinacea* components, was also apparent in the rat's spleen macrophage. These results suggested that the *Echinacea* preparations containing optimal concentration of cichoric acid, polysaccharides, and alkylamides are potentially effective in stimulating an *in vivo* nonspecific immune response in normal rats. Purified polysaccharide of *Echinacea purpurea* can stimulate macrophages to produce TNF-α, IL-1, and IFN-β (Stimpel et al., 1984; Luettig et al., 1989). Mishima et al. (2004) reported that *Echinacea purpurea* could activate macrophages to stimulate IFN-γ production in association with the secondary activation of T lymphocytes. In addition, it is reported that the activated macrophages in association with the secondary T lymphocyte activation can increase IFN-γ production and stimulate the proliferation of cytotoxic T cells and suppressor T cells. An *in vitro* study revealed normal human peripheral blood macrophages cultured with *Echinacea* juice produced significantly higher levels of IL-1, TNF-α, IL-6, and IL-10 ($P < 0.05$) than unstimulated cells (Burger et al., 1997).

Currier and Miller (2000) recently showed that daily dietary administration of *Echinacea purpurea* root extract to normal mice for merely one week resulted in a significant increase in the number of natural-killer (NK) cells. Such boosting of this fundamental immune cell population suggested a prophylactic role for this herb in normal animals. Another study indicated that *Echinacea-purpurea*-treated leukemic mice had a 2.5-fold increase in the absolute number of NK cells in their spleens (Currier and Miller, 2001).

The above mentioned studies indicated that *Echinacea purpurea* may exert an immunostimulating effect in many aspects, enhancing cytokine production, stimulating macrophages and T lymphocytes, and elevating NK cells. These findings can explain why the oral administration of *Echinacea purpurea* significantly enhanced IL-2 and TNF-α contents in broilers (Tables 2 and 3) in our present study. Flow cytometry analysis reveals a significant augment in CD4+ and CD8+ lymphocytes in peripheral blood in *Echinacea purpurea* treated broilers (results to be reported later).

It has been reported that feed conversion of *Echinacea* supplemented group was significantly ($P < 0.05$) better than that of the control group (2.44 vs. 2.51), suggesting that *Echinacea purpurea* might be used as a feed additive to increase feed-to-gain-conversion (Maass et al., 2005). In this experiment, *E. purpurea* extract significantly lowered the feed conversion efficiency in broilers (Table 4). There still exist some controversies on this issue. Some reports claim that *Echinacea purpurea* as a feed additive for broilers and layers is not beneficial for growth or layer performance (Roth-Maier et al., 2005). How the herb facilitates feed conversion still needs larger studies.

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