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Full Length Research Paper

Enhancement of broiler performance and immune response by *Echinacea purpurea* supplemented in diet

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The objective of the present study was to compare short and long term application of *Echinacea purpurea* root powder on growth performance and immunity response of broiler chicks. Three replicate trials involving a total of 600 day-old Ross chicks were used in this study. In each trial, a total of 200 chicks were randomly allocated into 5 groups. Each group consisted of 4 pens with 10 chicks in each pen. The birds in group A received control mash diet during the experiment, but those in groups B and C were given control diet supplemented with 0.1% (w/w) and 0.5% (w/w) *E. purpurea* root powder, respectively. The chicks in groups D and E received control diet supplemented with 0.1% (w/w) and 0.5% (w/w) *E. purpurea* root powder, respectively, just for one week and fed control diet afterwards. The results showed that *E. purpurea* consumption for six weeks changed the total counts of white blood cells (WBCs), number of lymphocytes and heterophils, feed conversation ratio, and antibody titers against newcastle and avian influenza diseases ($p < 0.05$). In conclusion, this result suggests that feeding *E. purpurea*, particularly for long time, may improve feed conversion, change blood cells number and enhance immunity response in broilers.

Key words: *Echinacea purpurea*, broilers, feed conversion, immunity response.

INTRODUCTION

It has been suggested that herbal medicines can be good alternative for antibiotics as therapeutic and growth promoting agent. Therefore, herbal medicines have widespread use all over the world (Alexander and Chettle, 1977). One of the most important and popular medical herb is *Echinacea purpurea* (Barrett, 2003). This herbal medicine has been used from long time ago for a variety of purposes including treatment, growth enhance-ment and immunostimulation (Percival, 2000; Barrett, 2003).

It has been reported that *E. purpurea* has an interferon (IFN) like effect, activating macrophages and inducing the production of interleukin (IL)- 1 and IFN (Rininger et al., 2000). *E. purpurea* has been shown to have non-specific immuno stimulatory properties *in vitro* (Bauer and Wagner, 1991), including increased phagocytosis (Stotzem et al., 1992), increased cytokine production (Burger et al., 1997), and natural killer cell activity (See et al., 1997). However, due to: 1) the use of different plant parts (herb, roots, or both), 2) different methods of extraction, 3) the soil type in which the plant is grown and 4) the phase of plant development at harvest; many products commonly summarized under the name *E. purpurea* can be chemically completely different preparations. Depending on these factors, Echinacea products can contain highly variable amounts of a variety of bioactive ingredients including caffeic acids, alkyl-amides, polysaccharides and glycoproteins (Bauer and Wagner, 1991). However, results on the *in vivo* efficacy of Echinacea products have been controversial. While, Rehman et al. (1999) showed an increase in primary and secondary immunoglobin G response in rats treated with *E. purpurea*, animal and human studies have shown that *E. purpurea* had generally little or no effect on existing serum immunoglobin levels or on specific antibody production (Melchart et al., 1998; Grimm and Müller, 1999; Turner et al., 2000).

There are also some controversies on the consumption period of *E. purpurea*. Skaudickas et al. (2003) observed

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significant elevation in the number of lymphocytes in rat receiving *E. purpurea* extract for at least 8 weeks. Improvement in feed conversion was reported by Maass et al. (2005) in pig receiving *E. purpurea* cobs supplementation for at least two weeks. Meanwhile, Currier and Miller (2000) showed that daily dietary administration of *E. purpurea* root extract to normal mice for one week resulted in a significant increase in the number of natural-killer (NK) cells. Ma et al. (2009) in addition showed that administration of *E. purpurea* extract for one week significantly enhanced the infectious bursal disease antibody levels in the broiler's blood.

Therefore, our objectives were: a) using *E. purpurea* root powder instead of *E. purpurea* extract. b) determining the effects of dietary *E. purpurea* root powder on growth performance and immunity responses of broiler chicks, and c) comparing the effects of two different consumption periods of *E. purpurea* root powder.

MATERIALS AND METHODS

Two years old plants of *E. purpurea* were harvested in September 2010 and the roots were cleaned, washed and sun-dried. The roots were ground and sifted, then mixed with basal diets. Determination of phenolic contents such as caftaric acid, chlorogenic acid, cichoric acid, and echinacoside in the dried, powdered *E. purpurea* root was performed by high-pressure liquid chromatography. The *E. purpurea* used in this study contained 1.54% cichoric acid, 0.4% caftaric acid, around 0.01% chlorogenic acid and about 0.01% echinacoside.

Three replicate trials involving a total of 600 day-old Ross chicks were used in this study. In each trial, a total of 200 chicks were randomly allocated into 5 groups. Each group consisted of 4 pens with 10 chicks in each pen. These chicks were floor reared. The birds in group A received control mash diet during the experiment, but those in groups B and C were given control diet supplemented with 0.1% (w/w) and 0.5% (w/w) *E. purpurea* root powder, respectively. The chicks in groups D and E received control diet supplemented with 0.1% (w/w) and 0.5% (w/w) *E. purpurea* root powder, respectively, just for one week and fed control diet afterwards.

The gross energy of *Echinacea* was estimated to be 3,240 kcal/kg. Using the gross energy value of *Echinacea* and values from the NRC (1994), the diet was adjusted (NRC, 1994). So, soy oil was added with the *Echinacea*, and equal amounts of corn were removed. All chicks were fed for 6 weeks on broiler diet formulated (Table 1) to meet NRC requirements of broiler chicks (NRC, 1994). This diet along with water was available *ad libitum* throughout the experiment. According to laboratory recommendations, at 13 days of age, all birds were vaccinated with a bivalent oil emulsion of an inactivated vaccine containing both ND (Lenthogen) and AI (H_9N_2) viruses, subcutaneously.

Ten chicks from each group were randomly weighted and bled via wing vein on days 21, 28, 35 and 42 of experiment. After weighing and collecting blood (aseptically) from chicks, they were marked with leg bands, to avoid reusing for blood collection. Blood samples (approximately 5 ml/sample) were collected in tubes either containing EDTA for hematological investigations or no anticoagulant agents for serological studies. White blood cell (WBC) counts and differentiation were assayed by Medonic -precision instrument for hematology research (CA620). Blood samples containing no anticoagulant agent were allowed to clot. They were then centrifuged at 4000 *g* for 10 min. Sera were then separated and stored at -20°C until the end of the experiment. The serum samples were tested for antibodies against newcastle disease virus (NDV) and avian influenza virus (AIV) . The AIV and NDV specific antibodies levels were measured by Hemagglutination inhibition test according to Alexander and Chettle (1977) and Allan and Gough (1974), respectively.

Feed intake and body weight were determined and their feed conversion ratio (FCR) was calculated. At the end of the experiment, all birds were slaughtered to determine the weight of carcass, bursa of fabricius, thymus and spleen. All samples were analysed and the results were assessed statistically using one way analysis of variances (ANOVA). Data were presented as mean ± SE and values differing at $p < 0.05$ were considered statistically significant. All calculations were made using SPSS 11.0 software.

RESULTS

As shown in Table 2, chicks in experimental groups showed better production performance than chicks in control group. There were no significant differences on feed intake and weight gain between control group (group A) and the experimental groups ($p > 0.05$). Birds receiving *E. purpurea* for 6 weeks had a greater weight gain and lower feed consumption in comparison to chicks receiving *E. purpurea* for one week. However, these differences were not significant ($p > 0.05$). *E. purpurea* consumption reduced feed conversion, but this reduction was only significant in groups B and C compared to group A ($p < 0.05$). Differences in the feed conversion between experimental groups were not significant ($p > 0.05$).

As shown in Table 3, the total count of WBCs and number of lymphocytes in chicks receiving *E. purpurea* for 6 weeks was significantly higher than in the control group at days 21, 28, 35 and 42 ($p < 0.05$). Moreover, at age of 21 days, there was a significant increase in the number of lymphocytes in group D in comparison with group A ($p < 0.05$). As presented in Table 3, at ages of 21, 28 and 42 days, there were significant increase in number of heterophils in group C (receiving 0.5% *E. purpurea* for 6 weeks), compared to control group ($p < 0.05$). Furthermore, at the age of 21 and 42 days, there was a significant increase in number of these cells in group B (receiving 0.1% *E. purpurea* for 6 weeks), when compared to control group ($p < 0.05$). There were no significant differences in the hematological parameters between B, C, D and E groups ($p > 0.05$).

As shown in Table 4, antibody titers against NDV were increased in all supplementation groups in comparison to the control group. However, this elevation was only significant for experimental groups B and C ($p < 0.05$). No significant difference in antibody titers was detected among the different modes of treatments ($p > 0.05$). More also, according to Table 5, antibody titers against AIV were significantly affected by supplementation of *E. purpurea* for 6 weeks compared with group A ($p < 0.05$). There was no significant difference in the antibody titer among the experimental groups receiving *E. purpurea* ($p > 0.05$). Although percentage of bursa of fabricius,

Table 1. Composition and calculated analysis of the experimental diets.

Ingredient	Starter (%) (1 to 21 days)	Finisher (%) (22 to 42 days)
Corn	46.71	56.11
Soybean meal (44%)	37.21	31.24
Sunflower oil	7.7	6.21
Fish meal (64%)	4.95	3
Oyster	1.22	1.19
Dicalcium phosphate	1.04	1.06
Vitamin premix	0.3	0.3
Mineral premix	0.3	0.3
Salt (NaCl)	0.22	0.26
Methionine	0.15	0.03
Vitamin E	0.1	0.1
Vitamin D_3	0.1	0.1
Vitamin K	-	0.1
Total	100	100
Calculated analysis		
ME (kcal/kg)	3200	3200
Crude protein	23	20
C/P ratio	139	160
Calcium	1	0.9
Available phosphorus	0.45	0.4
Sodium	0.15	0.15
Methionine + cysteine	0.93	0.72
Methionine	0.57	0.4
Lysine	1.44	1.19
Arginine	1.63	1.41

thymus and spleen to live body weight were greater in experimental groups compared with control group (Table 6), these elevations were not significant ($p > 0.05$).

DISCUSSION

FCR and body weight gain are sensitive indicators of non-specific body response against any substances used in live animals. Based on the results presented in Table 2, broilers in the experimental groups B and C receiving *E. purpurea* for 6 weeks showed an improvement in mean feed conversion ratio compared to the control group ($p < 0.05$), although there was no significant change in condition factor between these experimental groups and control group. The mode of action of the herb mixtures on feed conversion is through the enhancement of the digestive functions (Przybilla and Weiss, 1998). The improvement of feed conversion ratio with feeding *E. purpurea* is in agreement with the findings of Maass et al. (2005) who also reported that *E. purpurea* botanicals(herbs and/or spices), supplementation as feed additive improved feed conversion. Meanwhile, there are some controversies on the effect of *E. purpurea* extract on feed conversion. While, Ma et al. (2009) reported that *E. purpurea* extract significantly lowered the feed conversion efficiency in broilers, Roth-Maier et al. (2005) claimed that *E. purpurea* extract as a feed additive for broilers and layers is not beneficial for growth or layer performance. It has also been shown that *E. purpurea* increases the non-specific activity of the immune system. This includes increased phagocytosis (Stotzem et al., 1992), increased cytokine production (Burger et al., 1997), and natural killer cell activity (See et al., 1997). In this study, the significantly increased total count of WBCs was associated with the increase in lymphocytes and heterophils (Table 3). This may explain the efficacy of *E. purpurea* in terms of the health status and non-specific immune response. The significant increase in lymphocytes might also indicate the specific and non-specific immune stimulant role of *E. purpurea*. Bauer (1996) found *in vitro* and *in vivo* pharmacological effects

Table 2. Effect of *E. purpurea* on production performance of broiler chicks (M ± SE).

Parameter	Group A (control)	Group B (6 weeks 0.1%)	Group C (6 weeks 0.5%)	Group D (1 week 0.1%)	Group E (1 week 0.5%)
Initial body wt. (g)	47.04 ± 1.1	46.52 ± 2.5	47.87 ± 2.6	45.63 ± 3.3	46.58 ± 2.3
Final body wt. (g)	1579.6 ± 70	1618.8 ± 72	1622.7 ± 47	1597.9 ± 99	1605.3 ± 51
Total gain (g)	1532.56 ± 64	1572.28 ± 39	1574.83 ± 55	1552.27 ± 76	1558.72 ± 45
Feed consumption (g)	3484.47	3441.91	3416.89	3476.32	3470.76
Feed Conversion (g/g)	2.20	2.12*	2.10*	2.17	2.16

* = $p < 0.05$ vs. Group A.

Table 3. Effect of *E. purpurea* on some hematological parameters of chicks (M ± SE).

Day	Parameter (ml)	Group A (control)	Group B (6 weeks 0.1%)	Group C (6 weeks 0.5%)	Group D (1 week 0.1%)	Group E (1 week 0.5%)
	WBC ($\times 10^3$)	24.16 ± 1.3	30.65 ± 1.3*	30.86 ± 1.1*	27.34 ± 1.6	28.26 ± 1.7
	Heterophil	809.36 ± 12	1034.43 ± 19*	1043.68 ± 11*	918.62 ± 16	955.18 ± 15
21	Lymphocyte	1401.28± 23	1800.68 ± 24*	1817.65 ± 25*	1604.85 ± 32	1656.03 ± 26*
	Monocyte	193.28± 8	216.08 ± 2	211.69 ± 3	198.21 ± 5	203.47± 7
	Eosinophil	12.08 ± 0.6	13.79 ± 0.9	12.96 ± 0.7	12.3 ± 0.2	11.30 ± 0.3
	WBC ($\times 10^3$)	24.74 ± 1.8	30.18 ± 1.6*	31.11 ± 1.3*	27.74 ± 1.6	27.77 ± 1.5
	Heterophil	816.42 ± 12	1001.97 ± 29	1039.96 ± 34*	920.96 ± 19	920.57 ± 13
28	Lymphocyte	1459.66± 56	1793.69 ± 16*	1854.15 ± 86*	1647.75 ± 80	1646.76 ± 69
	Monocyte	183.07± 8	208.24 ± 6	203.31 ± 4	191.40 ± 2	195.77± 9
	Eosinophil	14.84 ± 0.4	15.09 ± 0.8	13.99 ± 0.4	13.87 ± 0.15	13.88 ± 0.9
	WBC ($\times 10^3$)	25.39 ± 1.2	30.81 ± 1.8*	31.18 ± 1.9*	28.56 ± 1.2	29.15 ± 1.3
	Heterophil	842.94 ± 24	1033.67 ± 20	1048.27 ± 12	953.90 ± 74	976.52 ± 36
35	Lymphocyte	1485.31± 78	1823.95 ± 34*	1855.20 ± 85*	1685.04 ± 60	1719.85 ± 15
	Monocyte	198.04± 3	209.50 ± 7	200.48 ± 4	203.63 ± 3	205.50± 4
	Eosinophil	12.69 ± 0.5	13.86 ± 0.3	14.03 ± 0.6	13.42 ± 0.7	13.11 ± 0.7
	WBC ($\times 10^3$)	25.23 ± 1.5	30.51 ± 1.1*	30.72 ± 1.5*	27.26 ± 1.1	28.12 ± 1.3
	Heterophil	837.63 ± 21	1023.91 ± 10*	1032.19 ± 28*	911.84 ± 37	942.02 ± 16
42	Lymphocyte	1470.90± 53	1793.98 ± 76*	1812.48 ± 49*	1597.43 ± 79	1647.83± 85
	Monocyte	201.84± 7	218.45 ± 6	213.19 ± 9	203.08 ± 3	208.08± 5
	Eosinophil	12.61 ± 0.5	14.64 ± 0.6	14.13 ± 0.6	13.63 ± 0.9	14.06 ± 0.3

* = $p < 0.05$ vs. Group A.

Table 4. Effect of *E. purpurea* on antibody titer (log_2 HI titer) against Newcastle virus at different ages (M ± SE).

Day	Group A (control)	Group B (6 weeks 0.1%)	Group C (6 weeks 0.5%)	Group D (1 week 0.1%)	Group E (1 week 0.5%)
21	2.08 ± 0.48	2.17 ± 1.26*	2.15 ± 0.67*	2.11 ± 0.48	2. 15 ± 0.53
28	1.96 ± 0.23	2.21 ± 0.41 *	2.37 ± 0.43*	2.11 ± 0.64	2.08 ± 0.25
35	2.16 ± 0.46	2.30 ± 0.34*	2.55 ± 0.37*	2.28 ± 0.80	2.08 ± 0.99
42	2.11 ± 0.55	2.19 ± 0.81*	2.48 ± 0.58*	2.14 ± 0.43	2.13 ± 0.54

* = $p < 0.05$ vs. Group A.

associated with extracts from the aerial parts of *E. purpurea* and the alcoholic extracts of the roots of *E. purpurea, E. angustifolia* and *E. pallida*. The effects were mainly linked to a modulation of the non-specific cellular immune system by polysaccharides, glycoproteins, caffeic acid derivatives and alkylamides. Moreover, the various immune cells (macrophages, monocytes and natural killer cells) were stimulated *in vitro* by *Echinacea* extract (Bauer, 1998, 1999; Sun et al., 1999; Rininger et al., 2000).

The results of this study generally indicate that *E. purpurea* increased total counts of WBCs and the number of heterophils and lymphocytes. This is in agreement with Cundell et al. (2003) who found a significant increase of lymphocytes in rats fed with dried *Echinacea* preparations. It has been reported that ethanolic juice of *Echinacea* increased the number of lymphocytes and total leucocytes significantly ($p < 0.05$) in hens and pigs (Bohmer et al., 2009). Jurkstine et al. (2004) reported that *E. purpurea* extract from root were more effective phytoimmunostimulators than those from above-ground parts. *E. purpurea* extract from root significantly increased *in vivo* the number of leucocytes and lymphocytes. It is reported that *Echinacea* activates rat immune system. It could increase the number of lympho-cytes too. However, elevation in the number of lymphocytes can be statistically reliable only if rats are fed *Echinacea* for at least eight weeks (Skaudickas et al., 2003). ND and AI HI-antibody titers were routinely examined to evaluate the effect of *E. purpurea* on humoral immune response of the chickens. Some statis-tical differences between the experimental and control groups were observed at different days of age; however, mean HI- antibody titers in all groups were above the protective level and the titers were considered uniform, which means that the chickens in all groups were properly immunized by vaccine (Tables 4 and 5). The results of the present study revealed that the *E. purpurea* consumption neither for 1 nor 6 weeks had effect on lymphoid organs weight (Table 6), but increased antibody titers against NDV and AIV (Tables 4 and 5). Scientific studies indicate that *Echinacea* derived polysaccharides; alkylamides and cichoric acid each possess health- promoting properties. Since the *Echinacea* used in this study is a complex mixture, any or several of the components could be responsible for the effects seen. Although the exact mode of action of *Echinacea* is still not clearly understood, it is possible that its stimulatory activities allow it to exhibit properties comparable to those of an immunological adjuvant. In accordance to present study, Rehman et al., (1999) reported that *Echinacea* administration for six weeks increased IgG pro-duction in the early to middle term in rats. A rapid and strong elevation in the NDV antibody titer in the layers treated with *Echinacea* was reported by Bohmer et al. (2009). Elevated antibody titer against NDV was reported in broiler chicks with supplement of (1 $g \cdot L^{-1}$ drinking water) *E. purpurea* as well (Zhang, 2005) . This investi-gator reported also that *E. purpurea* extract (1 $g \cdot L^{-1}$ drinking water) used for five days, significantly augmented the infectious bursal disease antibody production in chickens (Zhang, 2005). Ma et al. (2009) reported that antibody titer against infectious bursal disease was improved in broiler chicks fed 0.1 to 1 g *E. purpurea*.

The results of the present study demonstrate that feed supplementation with *E. purpurea* results in non-significantly lower feed consumption and higher weight gain, which indicate the beneficial effects of this herb on feed intake and weight gain. Based on the results of the current study, feeding *E. purpurea* for 6 weeks, particularly at concen-tration of 0.5%, had the most positive effects on performance parameters. Whereas, increasing the *E. purpurea* supplementation length had significant effects on total count of WBC and number of lymphocytes and heterophils. The study revealed that *E. purpurea* supplementation particularly for 6 weeks also enhanced the specific

Table 5. Effect of *E. purpurea* on antibody titer (log_2 HI titer) against Avian Influenza virus at different ages (M ± SE).

Day	Group A (control)	Group B (6 weeks 0.1%)	Group C (6 weeks 0.5%)	Group D (1 week 0.1%)	Group E (1 week 0.5%)
21	2.75 ± 0.81	3.37 ± 0.32*	3.73 ± 0.50*	3.25 ± 0.49	3.33 ± 0.88
28	2.83 ± 0.52	3.5 ± 6.82*	3.91 ± 0.42*	3.14 ± 0.76	3.06 ± 0.73
35	3.00 ± 0.73	3.41 ± 0.37*	3.55 ± 0.74*	3.23 ± 0.98	3.03 ± 0.9
42	3.00 ± 1.08	3.33 ± 1.01*	3.62 ± 0.71*	3.18 ± 0.92	3.12 ± 1.5

*= $p < 0.05$ vs. Group A.

Table 6. Effect of *E. purpurea* on lymphoid organs (expressed by percentage of live weight) of broiler chicks at 42 days of age (M ± SE).

Organ	Group A (control)	Group B (6 weeks 0.1%)	Group C (6 weeks 0.5%)	Group D (1 week 0.1%)	Group E (1 week 0.5%)
Bursa of Fabricius	0.085 ± 0.016	0.094 ± 0.018	0.098 ± 0.011	0.090 ± 0.014	0.092 ± 0.012
Thymus	0.370 ± 0.063	0.401 ± 0.055	0.471 ± 0.032	0.371 ± 0.037	0.397 ± 0.032
Spleen	0.105 ± 0.01	0.126 ± 0.009	0.123 ± 0.013	0.108 ± 0.01	0.114 ± 0.008

humoral immune response of broiler chicks.

Conclusion

E. purpurea root powder supplementation could therefore be used in broilers diet to improve performance and to potentially enhance the protective immune response.

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