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Effects of *Echinacea* extract on the performance, antibody titres, and intestinal histology of layer chicks

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Abstract 1. This research was conducted to determine the effect of diet supplementation with *Echinacea* extract (cichoric acid) on the growth performance, antibody titres and intestinal tissue histology of layer chicks.
2. White, 1-d-old, Hy-Line hybrid chicks ($n = 540$) were divided into three treatments, each consisting of 6 groups of 30 chicks ($n = 180$): (1) control; (2) 2·5 mg/kg cichoric-acid-fed; and (3) 5 mg/kg cichoric-acid-fed. The trial lasted 60 d.
3. While the growth performance of the chicks was depressed between d 1 and 45, it was found to improve between d 45 and 60.
4. Feed consumption was lower in both of the cichoric-acid-fed groups than in the control group between d 1–15 and 15–30, but was higher between d 30 and 45. Overall, mean feed consumption did not differ between the control and cichoric-acid-fed groups during the 60 d study period.
5. During the 60 d evaluation period, live weight gain, feed utilisation rate and final live weight were higher in the control group than in both of the cichoric-acid-fed groups.
6. Antibody titres against infectious bronchitis and infectious bursal disease did not differ between the three groups, but those for Newcastle disease were higher in the 2·5 mg/kg cichoric-acid-fed group than in the control group after 45 d.
7. Height and width of the jejunal villus and the thickness of the muscle layer were lower in the 5 mg/kg cichoric-acid-fed group than in both the control and the 2·5 mg/kg cichoric-acid-fed groups. The height of the ileal villus was also lower in the 5 mg/kg cichoric-acid-fed group than in the other two groups.
8. *Echinacea* extract supplementation for layer chicks appears not to benefit growth performance and intestinal histology during the growing period.

INTRODUCTION

The growth performance of animals is influenced mainly by their genetic characteristics, health, immune status and nutrition. Daily weight gains are lower in animals under stress and those with immune suppression (Iben, 2000; Roth-Maier *et al.,* 2005). Therefore, the application of immune-stimulating substances to increase the immune status can improve performance (Roth-Maier *et al.,* 2005). Paramunity inducers or immune modulators, such as inactivated viruses or plant extracts, activate the innate immune system. One herbal stimulant already being used in human medicine for the immune system is the plant *Echinacea purpurea* (L.) MOENCH. *Echinacea* preparations are known to stimulate various non-specific variables such as phagocytosis or the activity of lymphocytes (Wagner *et al.,* 1986). These effects are partly

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attributable to the content of cichoric acid and alkamids (Bauer and Wagner, 1991). *Echinacea* is most commonly used in human medicine as a pressed juice or ethanol plant extract.

However, there is little understanding of the effects of *Echinacea* on productive livestock, do the objective of this study was to determine the effect of *Echinacea* as a feed additive on the growth performance, antibody titres, and intestinal histology of layer chicks.

MATERIALS AND METHODS

Experimental design and animals

One-d-old layer chicks (Hy-Line W-36; $n = 540$) were randomly allocated to three experimental treatments with 6 replications (30 birds per replicate, giving 180 chicks per experimental group). The chicks were housed in metal cages (108·5 × 65 × 38 cm) on an experimental farm in a controlled environment, with 30 birds in each cage. Feed and water were provided *ad libitum*. The experimental period lasted 6 d. Chicks and feed were weighed on d 1, 15, 30, 45, and 60 to determine daily weight gain, feed intake and feed efficiency.

Diets and feeding

The chicks were fed a complete diet formulated to meet their requirements, which comprised 12·55 MJ ME/k and 200 g/kg crude protein, according to the Hy-Line W-36 Commercial Management Guide (Table 1); the diet was unchanged throughout the experimental period. The diets in the two experimental groups were supplemented with 0·17% (2·5 mg/kg cichoric acid) *Echinacea* extract or 0·34% (5·0 mg/kg cichoric acid) *Echinacea* extract. The dosage of *Echinacea* supplementation was determined according to the content of cichoric acid.

Vaccination

Chicks were vaccinated against Newcastle disease on d 1, 7, and 21, against infectious bronchitis on d 1, and against infectious bursal disease on d 18, 21, 25, and 32.

Feed analyses

Feeds were analysed for dry matter, ash, crude protein, ether extract, crude fibre, calcium, and total phosphorus (AOAC, 1984).

Production of *Echinacea* extract

The above-ground parts of the *E. purpurea* plant were cultivated in Konya, Turkey, and harvested during the full-blooming period. Approximately 10% pure juice was obtained from each fresh plant by cold pressing the fresh-cut plant. The squeezed plant juice was kept below −20°C until cichoric acid analysis, conducted using high-performance liquid chromatography (European Pharmacopoeia, 2008). The cichoric acid content of the juice was determined to be 148 mg/100 ml.

Table 1. *Components and chemical composition of the diet*[1]

Variables	Diet
Components (g/kg mixture)	
Maize	604·9
Soybean meal	190·0
Full-fat soybean	80·0
Fish meal	40·0
Sunflower soapstock	25·0
Sunflower meal	24·0
Dicalcium phosphate	14·0
Limestone	12·0
Salt	3·0
Vitamin and mineral premix[2]	2·5
Methionine	1·2
Soda	1·0
Toxin binder	1·0
Phytase enzyme	0·9
Anticoccidials (lasalocid sodium)	0·5
Calculated metabolisable energy (MJ/kg)[3]	12·55
Estimated on the basis of chemical analysis of components and mixtures	
Dry matter (g/kg)	875·0
Ash (g/kg)	60·9
Crude protein (g/kg)	200·0
Crude fat (g/kg)	68·7
Crude fibre (g/kg)	33·9
Calcium (g/kg)	10·0
Total phosphorus (g/kg)	7·0

[1]Cichoric acid was added at 0, 2·5, and 5·0 mg/kg as 0, 0·17, and 0·34 mL/100 kg *Echinacea* extract, respectively, which replaced sunflower meal in the diet.
[2]Added per kg of diet: retinylpalmitate, 6·0 mg; cholecalciferol, 0·05 mg; DL-α-tocopherylacetate, 25 mg; menadione, 3 mg; thiamin, 2·5 mg; riboflavin, 4·5 mg; pyridoxine, 4 mg; cyanocobalamin, 0·015 mg; nicotinic acid, 25 mg; Ca-pantothenate, 8 mg; folic acid, 1·2 mg; choline chloride, 450 mg; Mn, 74 mg as MnO; Fe, 30 mg as $Fe_2SO_4 \cdot H_2O$; Zn, 45 mg as ZnO; Cu, 4 mg as CuO; Co, 0·4 mg as $CoSO_4$; iodine, 0·3 mg as KI.
[3]Calculated according to European Table of Energy Values for Poultry Feedstuffs (Janssen, 1989).

Immunological analyses

At 1, 15, 30, and 45 d of age, three birds were randomly selected from each replication (18 chicks per experimental group) and blood samples were obtained by cardiac puncture for determining antibody titres for Newcastle disease, infectious bronchitis and infectious bursal disease. Newcastle disease titres were measured by conventional haemagglutination-inhibition test (Thayer and Beard, 1998). A commercial enzyme-linked immunosorbent assay kit (Idexx Laboratories, Roswell, GA, USA) was used to

Table 2. *Performance of layer chicks with (2·5 or 5·0 mg/kg cichoric acid) and without (control)* Echinacea *extract supplementation*[1]

Variable (d)	Control	Cichoric acid		SEM	*P* value
		2·5 mg/kg	5·0 mg/kg		
Daily weight gain, mean g/d					
1–15	4·23[a]	3·27[b]	3·17[b]	0·128	0·000
15–30	7·29[a]	5·13[b]	4·89[b]	0·268	0·000
30–45	10·64[a]	7·39[b]	7·05[b]	0·411	0·000
45–60	11·22[b]	13·14[a]	12·83[ab]	0·362	0·050
1–60	8·34[a]	7·23[b]	6·98[b]	0·160	0·000
Feed intake, mean g/d					
1–15	11·34[a]	10·55[b]	10·53[b]	0·133	0·009
15–30	22·98[a]	20·63[b]	21·20[ab]	0·464	0·050
30–45	34·92[b]	42·09[a]	44·51[a]	1·194	0·000
45–60	62·93	52·82	52·92	2·405	0·143
1–60	33·04	31·52	32·29	0·104	0·628
Feed efficiency, mean g feed/g					
1–15	2·70[b]	3·23[a]	3·34[a]	0·079	0·000
15–30	3·15[c]	4·03[b]	4·34[a]	0·140	0·000
30–45	3·29[c]	5·69[b]	6·35[a]	0·330	0·000
45–60	5·64[a]	4·02[b]	4·16[b]	0·258	0·008
1–60	3·69[b]	4·24[a]	4·55[a]	0·103	0·000
Mortality, mean %					
1–15	3·89	3·89	3·89	0·819	1·000
15–30	0·62	0·0	0·0	0·205	0·391
30–45	0·0	0·76	1·43	0·396	0·360
45–60	0·0	0·0	0·0	0·000	1·000
1–60	1·13	1·16	1·33	0·279	0·956
Initial weight, mean g/chick	35·87	36·19	35·69	0·141	0·361
Final weight, mean g/chick	503·26[a]	441·10[b]	417·87[c]	9·32	0·000

[1]Data are mean values for 180 chicks for each treatment.
[a–c]Means within the same row bearing different superscripts differ significantly ($P < 0·05$).

analyse the infectious bronchitis and infectious bursal disease titres in serum samples.

Intestinal histomorphometry

At the end of the trial (60 d), two birds per replicate (12 chicks per experimental group) were randomly selected and killed by cervical dislocation to collect tissue samples such as duodenum, jejunum, ileum, and caecum. The samples were kept in 10% neutral buffered formalin and processed using routine histological methods and mounted in paraffin blocks. Six-micrometer-thick sections were cut and stained with Masson's trichrome (Culling *et al.,* 1985). All specimens were examined under a light microscope [Nikon Eclipse E-400 equipped with a digital camera head (DS-5M) and camera control unit (DS-L1), Nikon, Japan]. Villus height and width, crypt depth, and muscle-layer thickness were measured using an image-analysis system (BS200 PRO, 2005, BAB Ltd Şti, Turkey).

Statistical analyses

Data were subjected to one-way analysis of variance using SPSS (2006) software. Significant effects of dietary treatments on experimental groups were evaluated by *t* test. Statements of statistical significance are based on a probability of $P < 0·05$. Experimental procedures were approved by the Selcuk University, Veterinary Faculty Ethics Commission.

RESULTS

Growth performance

Daily weight gain was higher in the control group than in both of the cichoric-acid-fed groups throughout the experimental period, except between d 45 and 60. Data for the 60 d evaluation period are presented in Table 2. The overall daily weight gain of the chicks was higher ($P < 0·05$) in the control group than in the cichoric-acid-fed groups. The two cichoric-acid-fed groups had similar daily weight gains after the 60 d trial. Final body weight was higher ($P < 0·05$) in the control group (503·3 g) than in both the 2·5 mg/kg (441·1 g) and 5 mg/kg (417·9 g) cichoric-acid-fed groups.

Feed intake

During d 1–15 and 15–30, feed intake was higher in the control than in both of the cichoric-acid-fed

Table 3. *Antibody titres of layer chicks with (2·5 or 5·0 mg/kg cichoric acid) and without (control)* Echinacea *extract supplementation*[1]

Condition (d)	Control	Cichoric acid		SEM	*P* value
		2·5 mg/kg	5·0 mg/kg		
Infectious bursal disease, geometric mean titre					
0	7103·21	7141·34	6724·33	363·58	0·663
15	2237·06	1950·89	1808·78	197·37	0·415
30	5240·18	4113·93	5815·20	433·71	0·136
45	9935·28	8975·78	9393·00	376·57	0·337
Infectious bronchitis, geometric mean titre					
0	5889·42	7766·92	6379·50	687·09	0·306
15	4836·67	4120·56	4531·89	238·23	0·256
30	5571·72	4415·67	5049·67	210·97	0·079
45	5224·94	6599·56	4059·06	468·62	0·084
Newcastle disease, $\log_2$ HI[2]					
0	7·50	8·25	8·00	0·16	0·073
15	8·78	9·28	9·28	0·13	0·152
30	10·00	10·28	9·89	0·13	0·260
45	9·83[b]	10·72[a]	9·83[b]	0·16	0·025

[1]Data are mean values for 18 chicks for each treatment.
[2]HI = hemagglutination-inhibition.
[a–b]Means within the same row bearing different superscripts differ significantly ($P < 0·05$).

groups. However, during d 30–45 feed intake was lower ($P < 0·05$) in the control than in the cichoric-acid-fed groups. There were no differences in feed intake during d 45–60 and 1–60 (the entire study period) (Table 2).

Feed conversion efficiency

Feed efficiency was higher ($P < 0·05$) in the control group than in the two cichoric-acid-fed groups throughout the experimental period except during d 45–60, during which it was higher ($P < 0·05$) in both cichoric-acid-fed groups than in the control (Table 2).

Mortality rate

The mortality rate throughout the trial did not differ ($P > 0·05$) among treatments (Table 2).

Antibody titres

Table 3 shows the effect of feeding different inclusion rates of cichoric acid on antibody titres. The infectious bursal disease and infectious bronchitis titers did not differ ($P > 0·05$) among the three treatments during the experimental period. In addition, there were no differences ($P > 0·05$) in Newcastle disease titres among the three groups on d 0, 15, and 30; however, Newcastle disease titres were higher ($P < 0·05$) in the 2·5 mg/kg cichoric-acid-fed group than in the control and the 5 mg/kg cichoric-acid-fed group.

Intestinal histomorphometry

Table 4 shows the effects on intestinal histomorphometry. The height and width of the jejunal villi and the thickness of the muscle layer were lower ($P < 0·05$) in the 5 mg/kg cichoric-acid-fed group than in the control and 2·5 mg/kg cichoric-acid-fed groups. The height of the ileal villi was lower ($P < 0·05$) in the 5 mg/kg cichoric-acid-fed group than in the other two groups.

DISCUSSION

The addition of *Echinacea* extract containing cichoric acid at 2·5 and 5 mg/kg depressed the growth performance of chicks during the first 45 d of the 60 d trial period. However, between d 45 and 60, the *Echinacea* extract appeared to improve growth. A study involving broiler and layer chicks (Roth-Maier *et al.,* 2005) found that the growth performance was lower in all *Echinacea*-fed groups than in control animals. None of the other variables in the present study can be compared with the findings of previous studies because there are currently no other published data regarding the usage of *Echinacea* extract in layer chicks. A study involving pigs (Maass *et al.,* 2005) found that adding *Echinacea* extract affected the growth performance equally in all groups. However, a study involving rats (Skaudickas *et al.,* 2004) found that diet supplementation with *Echinacea* extract decreased the live weight relative to controls.

Table 4. *Intestinal histomorphometry of layer chicks with (2·5 or 5·0 mg/kg cichoric acid) and without (control)* Echinacea *extract supplementation*[1]

Parameters	Control	Cichoric acid		SEM	*P* value
		2·5 mg/kg	5·0 mg/kg		
Duodenum					
Villus width (μm)	175·64	163·57	163·69	6·22	0·648
Villus height (μm)	1490·60	1387·60	1536·25	33·91	0·287
Muscle-layer thickness (μm)	127·17	118·77	126·33	5·02	0·796
Crypt depth (μm)	189·04	183·75	208·96	8·02	0·507
Villus height/crypt depth	8·11	7·68	7·95	0·39	0·911
Jejunum					
Villus width (μm)	145·14[a]	154·73[a]	133·17[b]	3·65	0·043
Villus height (μm)	844·51[a]	929·85[a]	645·64[b]	31·30	0·001
Muscle-layer thickness (μm)	140·53[a]	140·54[a]	110·69[b]	5·19	0·037
Crypt depth (μm)	118·86[b]	157·54[a]	152·12[ab]	7·07	0·030
Villus height/crypt depth	6·96[a]	6·09[ab]	4·49[b]	0·450	0·050
Ileum					
Villus width (μm)	132·28	122·56	137·68	3·24	0·252
Villus height (μm)	478·57[a]	392·61[ab]	300·65[b]	23·13	0·002
Muscle-layer thickness (μm)	277·44	267·78	263·83	10·22	0·854
Crypt depth (μm)	137·43	146·22	152·50	6·13	0·604
Villus height/crypt depth	3·85[a]	2·81[ab]	2·08[b]	0·290	0·033
Caecum					
Muscle-layer thickness (μm)	304·33	323·66	295·52	12·33	0·724
Crypt depth (μm)	161·85	171·60	184·95	11·14	0·715

[1]Data are mean values for 12 chicks for each treatment.
[a,b]Means within the same row bearing different superscripts differ significantly ($P < 0·05$).

The addition of *Echinacea* extract to layer-chick rations had no positive effect on growth performance, feed intake or feed efficiency. However, when the data for the 60 d period were examined in total, live weight gain, feed efficiency, and live weight were higher in the control group than in both of the *Echinacea*-extract-fed groups.

While feed consumption was lower in groups fed *Echinacea* extract between d 1 and 15, it is not clear why consumption decreased between d 30 and 45 in the controls. However, when d 1–60 were evaluated in total, no differences were found between the control and the *Echinacea*-extract-fed groups. Roth-Maier *et al*. (2005) reported that feeding *Echinacea* extract at different concentrations to broilers did not affect feed consumption. However, the same study found that with layer chicks, while feed consumption was lower in the *Echinacea*-extract-fed group between weeks 1 and 4, there was an increase in feed consumption after week 5, which is similar to the findings of the present study.

Stabuc-Starevic and Kumer (2003) found that *Echinacea* extract decreased the death rates of piglets in the short term, but no difference was found in terms of death rates between the groups during the entire experimental period.

Some studies have shown that the titre of specific antibodies increases with the dosage of *Echinacea* extract (Chaves *et al.,* 2007; Ma *et al.,* 2009). In the present study, Newcastle disease titres at 45 d were higher in the 2·5 mg/kg cichoric-acid-fed group than in both the control and the 5 mg/kg cichoric-acid-fed group.

Histological investigation of the chicks' intestinal mucosa can reveal useful information on intestinal function. An increase in villus height suggests an increased surface area that would be capable of greater absorption of the available nutrients (Iji *et al.,* 2001). Because early functioning of the gastrointestinal tract is vital for the growth performance of chickens, it is desirable to optimise the development and functional capacity of the intestine (Uni and Ferket, 2004). Crypt development is a step in intestinal maturation (Uni *et al.,* 2000), and the villus height/crypt depth ratio is a useful criterion for estimating the digestive capacity of the small intestine (Montagne *et al.,* 2003).

In the present study, villus width and height, and muscle-layer thickness in the jejunum, as well as villus height in the ileum were all lower in the 5 mg/kg cichoric-acid-fed group than in both the 2·5 mg/kg cichoric-acid-fed and control groups, which would have affected intestinal absorption. In line with this is the finding of a reduction in body weight in the 5 mg/kg cichoric-acid-fed group compared to the 2·5 mg/kg cichoric-acid-fed and control groups at the end of the 60 d period.

The effect of *Echinacea* extract on intestinal mucosal histology is probably attributable to certain active ingredients in *Echinacea* extract, such as phenolic compounds (chichoric acid and caffeic acid), polysaccharides, and glycoproteins. These active ingredients may act directly on the gut tissues (Bone, 1997). In the present study, intestinal mucosal histology was affected by the higher dose of *Echinacea* extract, which might have reduced intestinal absorption and growth.

Because no previous study has investigated the effects of *Echinacea* extract on chicks, the dosages in the present study were calibrated on the basis of human studies (Blumenthal, 2000). The *Echinacea* extract given to the chicks caused negative effects, particularly during the early period and on some performance data in the group fed *Echinacea* 5 mg/kg cichoric acid extract. As the chicks grew older, these negative effects declined. These results suggest that while the dosage may have initially depressed performance, *Echinacea* extract may be tolerated better as chicks grow.

Future studies should investigate the effects of *Echinacea* extract and its active ingredients on growth, digestive physiology and metabolism.

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