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Full Length Research Paper

Effects of using different forms and levels of *Echinacea purpurea L. on* immune responses of broiler chicks

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This experiment was conducted to examine the effects of using different forms and levels of *Echinacea purpurea* L. (EP) in comparison with an antibiotic growth promoter (flavophospholipol) on performance, carcass characteristics and immune responses of broiler chicks. In this trial, 336 one-day-old broiler chicks (Ross 308) were weighted and randomly assigned to the 7 treatment groups, each with 4 replicates and with 12 broilers in each replicate. The 7 treatments were as follows: Basal diet (control), Basal diet + antibiotic (4.5 mg flavophospholipol/kg diet), Basal diet + dried aerial part powder of EP continuously (5 g/kg diet), Basal diet + the dried aerial part powder of EP continuously (10 g/kg diet), Basal diet + the ethanolic extract continuously (0.25 gr/kg diet), Basal diet + the dried aerial part powder of EP with 3-days application followed by 11 application free days intermittently (5 g/kg diet), and Basal diet + the dried aerial part powder of EP with 3-days application followed by 11 application free days intermittently (10 g/kg diet). The contents of flavonol-o-glycosides as quescetin in the *Echinacea* dried aerial part and the ethanolic extract were determined, and the amount of flavonol-o- glycosides as quescetin in the 5 g/kg diet EP continuously to the ethanolic extract was equal. At the 28th and 31st day, blood samples were taken and analyzed for immune responses. The results showed that the use of the 5 g/kg diet EP continuously led to the highest DFI, DWG and antibody titres against sheep red blood cell (SRBC) and Newcastle virus when compared to other groups. The intermittent application of 10 g/kg diet EP had significant effect in the grower period on DWG, FCR and immune responses against SRBC ($p<0.05$). DFI and DWG were increased by 5 g/kg diet EP continuously than the ethanolic extract significantly ($p<0.05$). The percentage weight of carcass traits were not affected by dietary treatments except for the percentage of the small intestine that decreased in the intermittent groups ($p<0.05$). In conclusion, performance and immune responses of broiler chicks were improved by continuous application of 5 g/kg diet EP.

Key words: Broilers, *Echinacea purpurea* L., performance, immune responses, carcass characteristics.

INTRODUCTION

For the past several decades, different strategies have been applied to improve poultry productivity and profitability. The most important of them were always directed towards maintaining health, reducing disease outbreak and improving general immunity. Antibiotics growth promoter feed additives have been successfully used at subtherapeutic doses in poultry production to promote growth, protect health and maximize the genetic potential of poultry (Eyssen and Desomer, 1963; Miles et al., 1984; Harms et al., 1986; Rosen, 1996; Engberg et al., 2000). Antibiotic growth promoters (AGP) were supposed to increase growth rate as a result of improved gut health, resulting in better nutrients utilization and

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improved feed conversion (Visek, 1978). Today, the non-prescription use of antibiotics in poultry feeds has been eliminated or severely limited in many countries because of the potential risks associated with their use and development of resistant strains of bacteria, mainly in humans. A complete ban on antibiotics in poultry feeds was brought into force on January 1st by European Union (EU); thus, all of the antibiotics used at sub-therapeutic doses for growth promotion were withdrawn (Nollet, 2005; Cervantes, 2006; Michard, 2008; Toghyani et al., 2010b). Now, as a result of the ban on use of antibiotics in EU, increasing demand of antibiotic free products in other parts of the world and demand for organic animal products, alternative substances are required to create a safety margin in animal production against unexpected hazards and stressful conditions (Nasir and Grashorn, 2006).

Phytogenic feed additives which is also called phytobiotics are plant derived products, used in animal feeding to improve performance through amelioration of feed properties, promotion of production performance, and improving the quality of animal origin food (Windisch, 2008; Toghyani et al., 2011). The medicinal herb *Echinacea purpurea L.* (E.P) is commonly known as an immune stimulating substance which is most widely used as herbal medicines throughout Europe and North America for the prevention or treatment of infectious diseases (Akhtar et al., 2003; Nasir and Grashorn, 2006). *Echinacea* and its different derivatives contain a variety of active substances like alkamides, glycoproteins, phenolic compounds, cinnamic acid, essential oil and flavonoids (Barrett, 2003). These substances are effective in treatment of various ailments and proved to be beneficial in improving immunity (Bauer, 1999). However, there have been few reports on the effect of the herb in chickens.

Schranner et al. (1989) studied the effects on the humoral immune response of a complex drug containing *Echinacea angustifolia* homoeopatic mother tincture and *E. angustifolia* mother tincture alone on normal and immunodeficient chickens. The administration of the complex drug enhanced humoral immune parameters in both types of chickens, but *E. angustifolia* mother tincture alone had no significant effects. Continuous in-feed application of EP aerial parts to healthy broiler and layer chickens, have not shown beneficial effect on feed intake and growth performance of the poultry (Roth-Maier et al., 2005). On the other side, Allen (2003) showed that feed supplementation with powdered EP root during the starter period of breeding, for the processed broiler chickens that were given a live oocyst vaccine of multiple coccidia species, enhanced growth before coccidian challenged chickens up to a certain extent against weight gain suppression and development of intestinal lesion. Jurcic et al. (1989) reported that continuous application of *Echinacea* derivatives may lead to immune suppression due to over stimulation and they recommended that *Echinacea* derivatives should be applied intermittently, and the interval between applications should be two to three times the duration of application.

In past literature, there was no datum available to compare testing of effects of continuous in-feed application and intermittent in-feed application of EP and its derivatives on broiler health, performance and carcass characteristics. Therefore, the aim of this study was to investigate the effects of supplementation of EP ethanolic extract, continuous application of different levels of dried aerial parts powder of EP, intermittent application of different levels of dried aerial parts powder of EP and comparative effects of continuous in-feed application with intermittent in-feed application of EP in comparison with antibiotic growth promoter on performance, carcass characteristics and some of the immune responses in broiler chickens.

MATERIALS AND METHODS

Plant material and extraction

Echinacea was cultivated in Esfahan city (Iran) and the aerial parts of EP were used for this study. These parts were collected during the flowering season and dried under standard conditions (45°C). The dried aerial parts of EP plants were pressed to cobs to provide optimal storage conditions and were added to experimental diets of broilers after carefully grinding them. The ethanolic extract was extracted using a percolation method at room temperature with 70% ethanol. The extract was concentrated under reduced pressure (bath temp. 50°C) and dried in a vacuum desiccator. The residue was dissolved in distilled water and filtered, after which the filtrate was evaporated to dryness (Chattopadhyay, 1999).

Quantification of flavonoids

The content of the flavonol-o- glycosides as quescetin in the aerial parts and the ethanolic extract were established by using the spectrophotometric method at 430 nm through creating a complex with $AlCl_3$ according to the European Pharmacopoeia (2005). The flavonol-o-glycosides content of the dried aerial part and the ethanolic extract of EP were 20 ± 2 mg/100 g and 394 ± 5 mg/100 g dry weight (DW), respectively. The cobs were added to the experimental diets of broilers after carefully grinding them.

Bird, diets and management

A total of 336 one-day-old broiler chicks of mix sexes (Ross-308) were weighted and randomly assigned to the 7-treatment group, each with 4 replicates with 12 broiler chickens in each replicate. A commercial antibiotic growth promoter and feed additives were supplemented to the no additive added basal diet. The 7 treatments are as follows:

i. Basal diet (control).
ii. Basal diet + antibiotic (4.5 mg flavophospholipol/kg diet).
iii. Basal diet + dried aerial part powder of EP continuously (5 g/kg diet).
iv. Basal diet + dried aerial part powder of EP continuously (10 g/kg diet).
v. Basal diet + ethanolic extract continuously (0.25 g/kg diet).
vi. Basal diet + dried aerial part powder of EP with 3-days application followed by 11 application free days intermittently (5 g/kg diet).

Table 1. The ingredient and chemical composition of basal starter, grower and finisher diets.

Ingredients (g/kg)	Starter	Grower	Finisher
Corn	537.3	533	561.5
Soybean meal	400	396	370
Soybean oil	20	35	35
DCP	19.3	17.1	15.6
$Caco_3$	10.5	8.7	8.5
NaCl	3.5	3	3
Mineral-Premix[1]	2.5	2.5	2.5
Vitamin-Premix[2]	2.5	2.5	2.5
DL-Methionine	3.1	2	1.4
L-Lysine	1.3	-	-
Calculated composition			
M. energy (kcal/kg)	2870	2980	3000
Crude protein (g/kg)	221	220	210
Calcium (g/kg)	8.6	7.5	7
Av. phosphorus (g/kg)	4.9	4.4	4.1
Meth.+cysteine (g/kg)	10.1	8.9	8
Lysine (g/kg)	13.3	11.9	11.3
Sodium (g/kg)	1.5	1.3	1.3
Threonine (g/kg)	8.3	8.3	6.3
Tryptophan (g/kg)	3.2	3.2	3

1-To provide the following per kg of diet: Vit A 10,000 IU, vitamin D3 2000 IU, vitamin E 5 IU, vitamin K 2 mg, riboflavin 4. 20 mg; vitamin B12 0.01 mg; pantothenic acid 5 mg; nicotinic acid 20 mg; folic acid, 0.5 mg. 2- To provide the following per kg of diet: choline 3 mg; Mg 56 mg; Fe 20 mg; Cu, 10 mg; Zn 50 mg; Co 125 mg; Iodine 0.8 mg.

vii. Basal diet + dried aerial part powder of EP with 3-days application followed by 11 application free days intermittently (10 g/kg diet).

So, the amounts of flavonoids in the dried aerial part (5 g/kg EP continuously) of the ethanolic extract were equal. The trial comprised three 2-weeks periods. In the intermittent treatments, each period was divided into 3 days of application of EP followed by 11 days without application.The birds were fed a starter diet from day 1 to 14, a grower diet from day 14 to 28 and a finisher diet from day 28 to 42 (Table 1). The diets were formulated to meet the nutrients requirements of broilers as recommended by Ross (2007). All of the dietary feed additives were added at the expense of saw dust. Birds were allowed free access to feed and water during the 42 days of growth period.The lighting cycle was 23 h/day and was maintained at all breeding times.The ambient temperature in experimental house was maintained at 32°C during the first week and after then it was gradually decreased by 3°C in the second and third week, and finally fixed at 22°C thereafter.Chicks were vaccinated against Infectious Bursal Disease at days 14 and 21, New Castle Disease (B_1) and Avian Influenza (oil emulsion H9N2 vaccine) at day 9 and New Castle Disease (Lasota) at day 21.

Analytical procedures

Growth performance of broilers was evaluated by recording the daily body weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) during the 42 days of experimental period. Individual body weights (BW) of the broiler chicks were recorded at the beginning and on days 14, 28 and 42 of the trial period. Feed intakes of birds were recorded per pen on days 14, 28 and 42 of the experiment.

FCR was calculated as the amount of feed consumed per unit of body weight gain on days 14, 28 and 42. Also, FCR was calculated as DFI per unit of DWG and was adjusted for weight of chicks at the first day of the experiment. Mortality was recorded as it occurred and was used to adjust the total number of birds to determine the total feed intake per bird and FCR. At 28 days of age, two male broilers from each replicate of treatments were randomly selected, and blood samples were taken by puncture of the brachial vein for analysis of antibody titres against Newcastle Disease Virus (NDV) and Avian Influenza (AI). Serum antibody titers against NDV and AI were measured by the hemagglutination inhibition test (HI). HI antibodies were then converted into $\log_2$ (Cunningham, 1971). At 25 days of the experiment, 2 birds per each group were injected in the right wing vein with 1 mL and 1% sheep red blood cell (SRBC). One week after injection, blood samples were taken and the plasma was collected. The antibody titer against SRBC was done by HA method (Amponsem et al., 2000).

At the end of the study, 2 female birds from each pen were selected, based on the average weight of the group, and were then sacrificed. Carcass yield was calculated by dividing the eviscerated weight by the live weight. Empty proventriculus, empty gizzard, liver, empty small intestine, abdominal fat pad and heart were also removed and weighted, after which the percentage of live weight was calculated. However, the small intestine length and ceca length was measured.

Statistical analysis

All the data were subjected to ANOVA using the General Linear Models procedure of SAS software (SAS Institute, 1991). The mean differences among different treatments were separated by Duncan's multiple range tests. Consequently, a level of ($P < 0.05$)

Table 2. The effect of dietary inclusion of feed additives on Serum antibody titers (IgG) against NDV, AI and SRBC in broilers supplemented.

Treatments	Antibody titer		
	NDV[1] (log_2)	AI[1] (log_2)	SRBC[2] (log_2)
Control	3.00[b]	2.75	6.57[b]
Flavophospholipol	2.85[b]	2.57	7.62[ab]
Ethanolic extract	3.37[ab]	2.71	8.57[a]
5 g/kg EP continuously	4.57[a]	2.25	9.12[a]
10 g/kg EP continuously	2.71[b]	2.75	3.42[c]
5 g/kg EP intermittently	4.00[ab]	2.71	7.57[ab]
10 g/kg EP intermittently	3.14[b]	2.57	8.71[a]
SEM	0.41	0.17	0.57

[a-c]Mean values followed by the same letters in the column do not differ according to Duncan test. [1]Antibody titers by the hemagglutination inhibition test (log_2). [2]Antibody titers by the hemagglutination test (log_2).

was used as the criterion for statistical significance.

RESULTS AND DISCUSSION

The results for serum antibody titers against NDV, AI and SRBC in broilers are presented in (Table 2). The antibody titer against SRBC significantly decreased in groups treated with 10 g/kg doses of EP diet continuously than other treatments ($p<0.05$). The highest SRBC antibody titers were seen in the groups that received 5 g/kg diet EP continuously. The SRBC antibody titers, obtained in birds fed diet containing 5 g/kg diet EP continuously, were higher followed by birds fed control diet and 10 g/kg diet EP continuously ($p<0.05$). The antibody titer against SRBC decreased in groups treated with 10 g/kg diet doses of EP continuously followed by birds fed control diet and 10 g/kg EP intermittently ($p<0.05$). The SRBC antibody titers obtained in birds fed diet containing ethanolic extract of EP was higher than groups fed control diet and 10 g/kg diet EP continuously ($p<0.05$).

There was no significant response to the AI vaccination, although NDV vaccination increased antibodies in groups treated with 5 g/kg diet EP continuously than other treatments. This was higher than birds fed control diet, flavophospholipol, 10 g/kg diet EP continuously and 10 g/kg diet EP intermittently ($p<0.05$). In addition, the use of 5 g/kg diet EP continuously in broiler diets promotes better immune responses than other treatments. According to previous studies, the effect of stimulating the immune system of EP has been proven. While the immune system improvement was known by adaptation, the vaccine stimulated by increased proliferation of polyclonal antibody producing cells and hence increased resistance to disease, will be considered (Barrett, 2003).

Allen (2003) showed that EP may potentiate the immune response to live vaccination and may provide protective immunostimulation in the presence of natural coccidia population in the litter. In other studies conducted in Swiss mice, Frieier et al. (2003) showed that EP may increase the humoral immune response. These results showed that continuous application of EP obviously has the potential to increase the humoral immune response. The result of this study showed that the use of 10 g/kg EP intermittently had a significant effect on immune responses against the sheep red blood cell of broiler chickens. Moreover, the use of 5 g/kg diet EP intermittently had no significant effect on the antibody titer against SRBC.

From the use of EP in humans, it was well documented that intermittent application has a significant effect on immunity because immunity over stimulation was eliminated. Therefore, in humans it was recommended that the interval between applications should be two to three times the duration of application (Jurcic et al., 1989). Based on this general knowledge, in the present study, the dried aerial parts of EP were supplemented intermittently for a limited time period (3 days), followed by three times (11 days) EP free application. The application of the dried aerial parts of EP was repeated three times (on 1 to 3, 14 to 16 and 28 to 30 days) during 42 days of the rearing period. These results show that intermittent application of EP obviously has the potential to increase the humoral immune response, as antibody titers are being used as an indicator of the immune response (Abdel-Fattah et al., 2008). The present findings are in accordance with previous observations that *Echinacea* application improved serum immunoglobulin concentration, especially IgG concentration (Schranner et al., 1989). In a study conducted by Bohmer et al. (2008), positive effects were observed on the health and immunity in layers by 2 days of application of EP juice followed by 12 days without application. Positive effects of the intermittent EP application on immunoglobulins were also observed in sows and piglets (Kuhn et al., 2005). These findings suggest that in poultry, immunostimulatory and immunopotentiating

Table 3. The effect of dietary inclusion of feed additives on DWG, DFI, BW and FCR of broilers supplemented.

	Control	Flavophospholipol	Ethanolic extract	5 g/kg EP continuously	10 g/kg EP continuously	5 g/kg EP intermittently	10 g/kg EP intermittently	SEM
				DWG [1]				
0-14 d	17.7[ab]	18.3[ab]	18.2[ab]	19.5[a]	17.9[ab]	17.5[b]	18.1[ab]	0.65
14-28 days	48.4[bc]	52.3[a]	51.1[ab]	52.7[a]	45.3[c]	48.0[bc]	52.1[a]	1.07
28-42 d	68.9[ab]	64.4[ab]	62.6[ab]	68.9[a]	60.6[b]	64.3[ab]	65.4[ab]	2.23
0-42 d	44.3[b]	44.9[ab]	44.0[b]	46.9[a]	41.3[c]	43.2[bc]	45.2[ab]	0.83
				DFI[2]				
0-14 d	28.5[ab]	28.9[ab]	29.6[ab]	30.5[a]	26.6[ab]	28.0[ab]	26.6[b]	0.87
14-28 d	93.7[bc]	94.5[abc]	96.7[ab]	99.5[a]	93.5[bc]	89.3[c]	92.9[bc]	1.82
28-42 d	151.4[b]	153.0[b]	153.7[b]	164.7[a]	154.5[b]	154.0[b]	159.0[ab]	3.34
0-42 d	91.2[b]	92.2[b]	93.3[b]	97.7[a]	92.0[b]	90.4[b]	92.5[b]	1.83
				FCR[3]				
0-14 d	1.53	1.57	1.62	1.56	1.60	1.59	1.53	0.04
14-28 d	1.90[ab]	1.80[bc]	1.88[bc]	1.88[bc]	2.02[a]	1.85[bc]	1.78[c]	0.04
28-42 d	2.27[b]	2.38[ab]	2.45[ab]	2.38[ab]	2.53[a]	2.39[ab]	2.43[ab]	0.08
0-42 d	2.07[b]	2.04[b]	2.11ab	2.07[b]	2.21[a]	2.08[b]	2.04[b]	0.04
				BW[4](g)				
14 da	303.5	300.5	299.5	315.5	295.0	290.5	298.7	8.94
28 day	982.3	1033.8	1015.3	1053.3	929.0	962.8	1029.0	16.67
42 day	1907.0[ab]	1936.5[ab]	1892.2[b]	2019.0[a]	1777.7[c]	1863.0[bc]	1945.0[ab]	35.54

[a-c]Mean values followed by the same letters in a row do not differ according to Duncan test. 1. Daily Weight Gain (g per bird per day). 2. Daily Feed Intake (g per bird per day). 3. Feed Conversation Ratio (g/g). 4. Body Weight.

properties of *Echinacea* can be obtained by intermittent application.

The results of growth performance in broilers are presented in (Table 3). In the starter, grower and finisher periods and in the total part of the trial, the highest amount of DFI was seen in the groups that received 5 g/kg diet EP continuously ($p<0.05$). The worst FCR in the starter period was obtained in broilers fed diet containing 10 g/kg diet EP continuously, but not significantly, while the worst FCR in grower period was seen in broilers fed diet containing 10 g/kg diet EP continuously followed by broilers fed diet containing flavophospholipol, ethanolic extract of EP, 5 g/kg diet EP continuously, 5 g/kg diet EP intermittently and 10 g/kg diet EP intermittently ($p<0.05$). In the finisher period, the use of 10 g/kg diet EP led to worst FCR than broilers fed with the control diet significantly ($p<0.05$). During the total trial, in terms of FCR index, the 10 g/kg diet EP continuous group had the worst treatment than all other treatments. The BW obtained in broilers fed diet containing 5 g/kg diet EP continuously was highest than all treatments in the 14 and 28 days, but the height was not significant. However, the highest BW in the 42 days experiment was obtained in broilers fed diet containing 5 g/kg diet EP continuously, followed by birds fed 5 g/kg diet EP intermittently, 10 g/kg diet EP continuously and ethanolic extract of EP ($p<0.05$). In the starter

Table 4. The effect of dietary inclusion of feed additives on carcass characteristics of broiler chickens.

Characteristics	Control	Flavophospholipol	Ethanolic extract	5 g/kg EP continuously	10 g/kg EP continuously	5 g/kg EP intermittently	10 g/kg EP intermittently	SEM
Carcass yield (%)[1]	76.2	75.7	73.8	75.1	73.8	74.2	75.5	0.764
Abdominal fat pad (%)	0.90	0.85	0.92	1.120	0.88	0.95	1.10	0.113
Liver (%)	1.92	1.93	1.83	2.00	1.98	2.03	1.92	0.106
Gizzard (%)	2.53	2.52	2.65	2.28	2.28	2.76	2.51	0.165
Heart (%)	0.57	0.55	0.51	0.54	0.54	0.51	0.56	0.025
Proventriculus (%)	0.40	0.46	0.45	0.38	0.42	0.39	0.41	0.027
Small intestine (%)	4.87[a]	4.28[ab]	4.95[a]	4.02[ab]	4.13[ab]	3.92[b]	3.67[b]	0.302
Ceca length (cm)	43.37	40.25	44.25	42.50	40.62	41.12	42.75	1.296
Small Intestine length (cm)	199.8	185.0	194.8	185.0	194.1	191.3	197.0	4.800

[a-b]Mean values followed by the same letters in a row do not differ according to Duncan test. 1. Percentage of live weight.

period, the DWG obtained in broilers fed diet containing 5 g/kg diet EP continuously was higher, followed by birds fed 5 g/kg diet EP intermittently and significantly ($p<0.05$). In the grower period, the DWG decreased in groups treated with 10 g/kg diet doses of EP continuously than all treatments significantly ($p<0.05$). However, the highest DWG in this period was seen in the groups that received 5 g/kg diet EP continuously. In the finisher period, the use of 5 g/kg diet EP continuously led to a higher DWG when compared to the 10 g/kg diet EP ($P<0.05$). Overall, a part of the trial statistical analysis of data on DWG revealed significant difference among the treatment groups due to dietary inclusion of 5 g/kg diet EP continuously (Table 3).

The herb EP is commonly known as an immune stimulating substance. Whilst the performance of animals is influenced mainly by the health and immune status, a stressed or weak immune system with a load of infectious diseases causes low DWG. On the other hand, an enhanced immune system allows maximum performance. Therefore, the application of immune stimulating substances to increase the immune status can result in increased performance (Iren, 2000). The significant increase in DWG in the group fed 5 g/kg diet EP continuously may be due to the optimum immune status of *E. purpurea L.* that led to better DWG. The other trial with immune stimulating substances application of inactivated poxvirus to calves resulted in better performance of the treated animals (Hanschke, 1997), and this is agreement with the study's findings. In contrast to the study's observations, Roth-Maier et al. (2005) observed no significant effect on Application of 5 g/kg diet EP continuously seems to have beneficial effect when compared to the 10 g/kg diet EP continuously because of the weak immune system, which can be better achieved by 5 g/kg diet EP continuously. Also, Toghyani et al. (2010a) reported that high dosage of medicinal plants in the diet may have an adverse effect on some beneficial microbial populations, such as lactobacillus, preventing the herb from exhibiting its positive influence on performance. It can be concluded that the supplementation of broiler diets with aerial performance parameters by continuous application of EP cobs through feed to broilers. parts powder of the EP had beneficial effect on growth performance and is useful as a replacement of feed antibiotics. There are appetizing effects which have been demonstrated with the dried aerial part of the EP. The hypothesis that supplementation with EP could result in better performance, as it was shown with fattening pigs (Maass et al., 2005), can be confirmed with the present experiment.

The result of this study showed that the use of 10 g/kg diet dried aerial part powder of EP intermittently had significant effect on DWG, FCR in grower period and immune responses against SRBC of broiler chickens ($p<0.05$). Intermittent application of *Echinacea* preparations has also been reported to be beneficial in improving health by modulation of the immune system (Jurcic et al., 1989). Bohmer et al. (2008) reported some positive effects of intermittent application (2-days application and 12-application free days) on the health and immunity of the EP layer, while Kuhn et al. (2005) observed immune stimulating effects in piglets by a repeated application of EP juices for 5 days. During the present experiment, 3-days application of EP followed by 11 application free days showed positive effects in terms of broiler performance and antibody titer against SRBC.

Table 4 shows the relative weight means (as a percentage of live weight at slaughter) of digestive and non-digestive organs, and the absolute small intestine length as a function of treatments. There is a significant difference in the intestine relative weight between the control and the intermittent groups (P<0.05), but there is no significant difference in the intestine relative weight between the control and continuous groups. Birds fed the control diet presented significantly heavier intestine as compared to the intermittent groups (P<0.05), which was the lowest among all treatments. All other parameters did not present any significant difference among treatments. These results are consistent with those observed by Hernández et al. (2004) who did not find any difference among the control treatments and those containing antibiotic or mixtures of plant extracts for organ weight of the 42-day-old broilers. In conclusion, continuous application of 5 g/kg diet EP seems to be more beneficial as compared to the intermittent application, as *Echinacea* has been reported to trigger the weak immune system, which can be better achieved by continuous application. The present study shows that some positive effects can be achieved by application of EP for 3 days, followed by 11 treatment free days. However, there might be some other treatment regimen that can show more positive effects, even though there is need for some further researches.

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