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Effects of intermittent application of different *Echinacea purpurea* juices on broiler performance and some blood parameters

Einfluss des intermittierenden Einsatzes von *Echinacea purpurea* Presssäften auf die Leistung und auf einige Blutparameter von Broilern

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Introduction

Use of phytogenic substances in human and animal nutrition is increasing globally in general, and especially in Europe. Since the ban on use of in-feed antibiotic in animal feeds in Europe in 2006, phytogenic substances are considered as one viable alternative due to their activity in different physiological systems. Phytogenic substances and extracts have wide range of activities in animals, as e.g. on digestive, immune and endocrine systems. Furthermore, they show physio-pathological (anti-inflammatory, anti-oxidative) and anti-microbial activities (static and cidal).

Echinacea purpurea (EP), commonly known as purple coneflower is extensively investigated in human and laboratory animals due to its immunostimulating effects. It belongs to Asteraceae family and contains a variety of active substances like alkamides, glycoproteins, polysaccharides, phenolic compounds, cinnamic acids, essential oils and flavonoids (Barrett, 2003; Sasagawa et al., 2006; Zhai et al., 2007; Liu et al., 2007; Nasir and Grashorn, 2009). These substances are effective in treatment of various ailments and proved to be beneficial in improving immunity (Woelkart and Bauer, 2007; Bauer, 1999). Solutions made from herb and root EP powders produced defined reproducible macrophage activity and proved anti-inflammatory and antioxidant properties (Rininger et al., 2000; Zhai et al., 2007). There is not enough data available on application of EP in livestock species, especially in poultry. According to BGA Commission E (1989) Echinacea should be used prophylactically for several weeks daily. Therefore, continuous in-feed application of EP in poultry diets have not shown beneficial effects on performance and meat quality either in cobs form or in dried herbal preparations (Roth-Maier et al., 2005; Gardzielewska et al., 2003; Koreleski and Swiatkiewicz, 2007). On the other side, dietary supplements of EP during starter phase seem to be useful adjuvants for live anticoccidial vaccines and provided weight gain advantage compared to live vaccination alone (Allen, 2003). The oral administration of a complex drug (Influex) and *Echinacea angustifolia* ethanolic extract to normal Leghorn chicken induced a rise in serum immunoglobulin concentration, as well as an increase in level and production of antibodies (Schranner et al., 1989). Jurcic et al. (1989) reported that continuous application of Echinacea may lead to overstimulation and they recommended that Echinacea preparations should be applied intermittently. Some positive effects of intermittent in-feed EP application on immunity were observed in sows (Kuhn et al., 2005; Böhmer et al., 2008) and in layers (Böhmer et al., 2008). But, Echinacea preparations may have more diverse effects on metabolism due to presence of a variety of active substances which may be visible in the activity of different heart and liver related enzymes and in blood biochemistry. Changes in activities of serum creatine kinase (CK) and lactate dehydrogenase (LDH) are indications of damage to cardiac muscles (Zhang et al., 2008). Changes in activities of alanine amino transferase (ALT), gamma-glutamyl transferase (γ-GT), alkaline phosphatase (ALP) and serum total proteins reflect liver functions, and levels of glucose and cholesterol may indicate stress.

Intermittent application of EP preparations through feed is laborious and there are also chances of binding of active sites of pharmacologically active substances by various feed ingredients, leading to reduced effectiveness. Oral administration of EP juices for a limited period through drinking water may be more suitable as compared to continuous feed supplementation. Therefore, the present experiments were conducted in view of a more practical application of EP in the field. It was assumed that a successful intermittent oral administration of EP juices would not only be beneficial for animals, but would also be cost and labour efficient in application. Therefore, the objectives of the present study were set to investigate the effects of intermittent application of EP fermented (EP-F) juice and EP juice on alcohol basis (EP-A) through drinking water on broiler performance and metabolism (serum protein, blood picture and some liver and heart associated enzymes in blood).

Material and methods

Animals, housing and diets

For all experiments, one-day old Ross 308 broilers were used. After arrival at the experimental station of Hohenheim University, broilers were feather-sexed and distributed to experimental boxes having equal sex ratio and 12 broilers were kept in one experimental box with about 1 m^2 of area. Birds were individually weighed and wing tagged. Experimental boxes were littered with wood shavings and were provided with nipple drinkers and trough feeders. Temperature and relative humidity of the room was according to the recommendations of broiler breeder

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company (AVIAGEN, 2002). A continuous 24-h lighting program was applied. Birds were vaccinated routinely against Newcastle Disease (ND) and Infectious Bronchitis (IB), but no type of medication was administered during the entire experimental period and no prophylaxis against coccidiosis was done.

Antibiotic free feed, in mash form, was offered *ad libitum* in two phases; starter (0–14 days) and grower diets (15–35 days) (Table 1). Feed was offered daily and residual feed was measured at weekly intervals. Daily fresh tap water at room temperature was offered *ad libitum* for drinking. The water of treatment groups was supplemented with EP juices according to treatment schedule.

Experimental treatments

Drinking water of treatment groups was supplemented with two different preparations of EP juices: EP-F and EP-A. These juices were obtained from Berghof-Kräuter GmbH (Berghof, Heilsbronn, Germany). These juices were extracted from above ground parts of EP plant and preserved either through fermentation or by addition of ethanol. Dry matter contents of EP-A and EP-F were 6.5% and 9.6%, respectively. Fermented juice was native pressed juice after fermentation for preservation (Chichoric acid 2.77 mg/ml; total alkamides 3.95 µg/ml), while ethanolic juice consisted of 80% (w/w) native juice and 20% (w/w) ethanol as preservative (Chicoric acid 2.06 mg/ml; total alkamides 27.7 µg/ml)[1]. There is no data available on dose level of EP preparation for poultry and other livestock species. Therefore, basic dosage of EP juices for broilers was adjusted on the basis of human medical recommendations at the rate of 0.25 ml/kg $BW^{0.75}$. EP juices were supplemented intermittently for a limited time period (3 days), followed by three times (9 days) EP free application. The application of EP juice was repeated three times (on 1–3, 13–15 and 25–27 days) during 35 days of rearing period.

[1] Results of analysis provided by Dr. Barbara Böhmer, TU München, Germany

Experimental design

Three experiments were performed using different treatment combinations and dose levels of EP juices. The dose levels and treatment schedule are listed in Table 2.

Experiment 1. This experiment was performed to compare the effects of two different preparations of EP juices (EP-F and EP-A) against a negative control (without any supplementation). In total, 108 one-day old broilers (Ross 308) were randomly divided into 9 experimental boxes (12 chicks/box; equal sex ratio). Each of two preparations, EP-F and EP-A, were applied (at the rate of 0.25 ml/kg $BW^{0.75}$) to 3 boxes each, randomly, while 3 boxes served as negative control.

Experiment 2. This experiment was performed to compare two different dose levels (0.25 ml/kg $BW^{0.75}$ and 0.50 ml/kg $BW^{0.75}$) of EP-F against a negative control. For this experiment, 72 one-day old broilers (Ross 308) were randomly divided into 6 boxes (12 chicks/box; equal sex ratio). Two experimental boxes were supplemented with EP-F juice at the rate of 0.25 ml/kg $BW^{0.75}$ (EP-F25), two experimental boxes with EP-F juice at the rate of 0.50 ml/kg $BW^{0.75}$ (EP-F50), while two remaining boxes served as negative control (without supplementation).

Experiment 3. The third experiment was performed to compare the effects of EP-F25 juice (0.25 ml/kg $BW^{0.75}$) against a negative control (without any supplementation). For this experiment 48 one-day old broiler chicks (Ross 308) were randomly divided into four groups. Two groups (having 12 birds each; equal sex ratio) received drinking water supplemented with EP-F juice at the rate of 0.25 ml/kg $BW^{0.75}$ (EP-F25), while other two groups received drinking water without supplementation and served as negative control.

Data collection and analyses

Birds were weighed individually at the start of experiment and at weekly intervals. During the experiments daily feed intake and weekly weight gains were recorded. Feed consumption was recorded on sub-group basis during a 35-day

Table 1a. Composition of the experimental diets
Zusammensetzung der Versuchsrationen

Ingredient (%)	Starter diet	Grower diet
Wheat	56.5	60.0
Soybean meal	33.5	29.3
Soybean oil	4.94	3.46
HPL 106 PALM	–	2.64
Limestone	1.12	1.05
Mono Ca Phosphate	2.12	1.45
SpurEleVor SG1	0.08	0.08
Cholincholride	0.20	0.20
Methionine	0.20	0.17
L-Lysin-HCl	0.35	0.30
$NaHCO_3$	0.28	0.28
NaCl	0.10	0.35
Luprosil	0.40	0.53
Loxidan TD 100	0.015	0.015
Vitamin and mineral premix[1]	0.20	0.20

Table 1b. Analyzed contents of nutrients (%)
Analysierte Inhaltsstoffe der Versuchsrationen (%)

Nutrient (%)	Starter diet	Grower diet
Dry matter	89.6	89.3
Crude protein	23.3	21.6
Crude fat	7.04	8.32
Crude ash	6.45	5.92
Crude fibre	2.65	3.05
Ca	0.97	0.91
P	0.87	0.66
Metabolizable energy (MJ/kg DM)[2]	12.4	12.7

[1] Supplements per kg of feed: Vit A 12600 I.U., Vit D_3 3150 I.U., Vit E 41 mg, Vit B_1 3 mg, Vit B_2 6 mg, Vit B_{12} 32 µg, Niacin 53 mg, Pantothenic acid 13 mg, folic acid 1050 µg, Biotin 105 µg, Fe 81 mg, Mn 108 mg, Zn 72 mg, Cu 14 mg, Iodine 1.44 mg, Selenium 0.45 mg.
[2] Calculation based on WPSA (1984)

Table 2. Treatment application plan for experiments 1, 2 and 3
Behandlungen in den Versuchen 1, 2 und 3

Experiment	Treatments			
	C	EP-F 25	EP-F 50	EP-A 25
	Control	EP fermented juice @ 0.25 ml/kg $BW^{0.75}$	EP fermented juice @ 0.50 ml/kg $BW^{0.75}$	EP alcoholic juice @ 0.25 ml/kg $BW^{0.75}$
1	X	X	–	X
2	X	X	X	–
3	X	X	–	–

experimental period and feed conversion ratio (FCR) was calculated (kg feed consumed/kg of live weight gain). Mortalities were recorded when occurred. At the end of the experimental period all birds were slaughtered to determine the carcass weight.

Blood samples of six birds per replicate (selected randomly) were collected during slaughtering. Two blood samples per bird were collected from jugular veins into two different tubes. Blood in EDTA tubes (having ethylene diamine tetra acetic acid (EDTA) as anti-coagulant) was collected for determination of blood picture and contents of cholesterol and glucose, while whole blood samples for determination of serum proteins and liver enzymes were collected without anticoagulant. Serum was separated after centrifugation of clotted whole blood at 3,500 rpm for 20 minutes. Serum and EDTA blood were kept at 4°C till further analysis. Blood samples were analysed by Vet Med Labor GmbH (Ludwigsburg 71611, Germany) to determine the contents of total protein, albumin, globulin, glucose, cholesterol, haemoglobin and hematocrit, levels of ALT, γ-GT, ALP, LDH, CK and numbers of leucocytes and erythrocytes. The blood was analysed photometrically for contents of proteins/enzymes by using Moduler (Roche). Quantitative determination of blood cells from un-coagulated blood (in EDTA tubes) was carried out by using the Coulter counter. Haemoglobin (Hb) was determined by HemoCue Hb 201+ and hematocrit by Microhematocrit centrifuge.

Statistical analysis

Data were subjected to one-way analysis of variance using JMP® 5.0.1 program (Sall et al., 2005). All data were tested for normal distribution before analysis. An one-way ANOVA model was used for all experiments. As treatment EP-F25 and control were included in all experiments a combined evaluation of data was done to test, whether or not EP-F25 treated groups show any beneficial effect on performance, blood parameters and health of birds. For the combined analysis experiments were considered as a covariate in the ANOVA model.

Significance of differences between group means was tested by Student's t-test. In the tables data are given as mean ± SEM (standard error of the mean). Values with different superscripts differ significantly ($P < 0.05$) between treatments.

Results

Experiment 1

Effects of EP-F25 and EP-A25 on broiler performance and blood parameters were studied during this experiment (Table 3). The results show that there was no significant difference between treatments on performance parameters. However, treatment groups consumed more feed and gained more weight as compared to control birds. FCR and dressing percentage of treatment groups was also numerically better than for control, with EP-F25 showing best results. During whole rearing period no bird died from EP-A25 groups, while 4 birds from control and one from EP-F25 died. Contents of ALP were significantly ($P < 0.05$) lower and serum globulin contents were near significantly ($P = 0.053$) higher in EP treated birds as compared to control. All other blood parameters showed non significant treatment effect.

Experiment 2

Effects of EP-F25 and EP-F50 on broiler performance and blood parameters were studied during this experiment (Table 4). The results show that there was no significant difference ($P > 0.05$) between treatments on FCR, WG, but average daily weight gain (ADWG) of EP-F treated groups was significantly ($P < 0.001$) higher as compared to control. Dressing percentage and FCR were also numerically better for EP-F treated groups as compared to control; with EP-F25 group showing best results. Three birds from control and one from EP-F50 group died during experiment, one bird from control group and one from EP-F25 group were culled on 33rd day due to leg abnormalities. Serum globulin contents of EP-F treated groups were significantly ($P < 0.05$) higher as compared to control. Levels of CK and ALP were numerically less in EP-F treated groups as compared to control.

Experiment 3

Results of experiment 3 are presented in Table 5. The table shows that no significant treatment effect was observed on performance parameters. However, numerically better (non significant) FCR was observed for EP-F25 treated groups (1.74) as compared to control (1.81). Results of blood analysis show that contents of total proteins and albumin in blood of EP-F25 treated birds were significantly ($P < 0.05$) lower as compared to control. Contents of serum globulin were numerically ($P > 0.05$) higher, while CK contents were significantly ($P = 0.039$) lower in EP-F25 treated birds as compared to control. Numbers of leucocytes were significantly ($P < 0.05$) lower in EP-F25 treated birds as compared to control.

Comparison of EP-F25 with control

Results of comparison of EP-F25 with control are presented in Table 6. The table shows that final weight, total weight gain, ADWG, FCR and dressing percentage were numerically better for EP-F25 treated birds as compared to control. Serum globulin contents were significantly ($P = 0.006$) higher for EP-F25 treated group as compared to control. The contents of CK were significantly ($P = 0.037$) lower for EP-F25 treated groups than for control. Near significant differences ($P = 0.053$) were observed in leucocytes numbers. The levels of all other parameters were not significantly ($P > 0.05$) different between treatments.

Discussion

Phytogenic substances are supposed to improve performance of the birds by stimulating secretion of digestive enzymes leading to improved nutrient digestion and ab-

Table 3. Effects of EP-F25 and EP-A25 supplementation on performance and blood parameters (experiment 1)
Einfluss der Zulage von EP-F25 und EP-A25 zum Trinkwasser auf die Leistung und auf einige Blutparameter (Versuch 1)

Parameter	Treatments			P value
	Control mean ± SEM	EP-F25 mean ± SEM	EP-A25 mean ± SEM	
Feed consumption (g)	2669 ± 58	2773 ± 45	2841 ± 46	0.701
Final weight (g)	1806 ± 62	1818 ± 39	1836 ± 41	0.904
FCR (kg feed/kg gain)	1.70 ± 0.03	1.64 ± 0.02	1.66 ± 0.02	0.721
Total weight gain (g)	1762 ± 61	1773 ± 39	1792 ± 41	0.906
ADWG (g)	50.3 ± 1.8	50.7 ± 1.2	51.2 ± 1.2	0.906
Dressing %	70.5 ± 1.1	71.8 ± 0.59	70.8 ± 0.51	0.384
Mortality (Number)	4	1	0	–
Blood parameters				
Total protein (mg/dl)	3.0 ± 0.10	3.20 ± 0.08	3.1 ± 0.08	0.279
Albumin (mg/dl)	1.43 ± 0.06	1.45 ± 0.04	1.41 ± 0.04	0.793
Globulin (mg/dl)	1.56 ± 0.05	1.74 ± 0.05	1.69 ± 0.05	0.053
ALT (U/l)	5.67 ± 0.72	4.34 ± 0.48	4.57 ± 0.52	0.236
γ-GT (U/l)	21.0 ± 1.58	21.1 ± 1.17	20.8 ± 1.11	0.988
Alkaline phosphatase (U/l)	4026^{a} ± 710	2507^{b} ± 368	2398^{b} ± 242	0.036
Creatine kinase (U/l)	15507 ± 2915	12691 ± 2548	11133 ± 1706	0.444
LDH (U/l)	1250 ± 125	1195 ± 164	1069 ± 74	0.588
Glucose (mg/dl)	221 ± 7.6	225 ± 5.8	236 ± 4.5	0.226
Cholesterol (mg/dl)	134 ± 8.8	143 ± 8.2	138 ± 7.0	0.712
Erythrocytes (T/l)	2.25 ± 0.09	2.17 ± 0.51	2.04 ± 0.13	0.381
Leucocytes (G/l)	7.97 ± 1.24	6.48 ± 0.49	5.54 ± 0.53	0.132
Haemoglobin (g/dl)	10.1 ± 0.23	10.0 ± 0.18	10.1 ± 0.20	0.917
Hematocrit (%)	31.3 ± 0.45	30.8 ± 0.55	29.7 ± 0.56	0.220

SEM: Standard error the of mean
[abc]: Means within the same row with different superscript are significantly different (P < 0.05).

Table 4. Effects of EP-F25 and EP-A25 supplementation on performance and blood parameters (experiment 2)
Einfluss der Zulage von EP-F25 und EP-A25 zum Trinkwasser auf die Leistung und auf einige Blutparameter (Versuch 2)

Parameter	Treatments			P value
	Control mean ± SEM	EP-F25 mean ± SEM	EP-F50 mean ± SEM	
Feed consumption (g)	2278 ± 55	2474 ± 47	2390 ± 63	0.551
Final weight (g)	1567 ± 80	1733 ± 53	1695 ± 73	0.258
Total weight gain (g)	1526 ± 80	1691 ± 53	1654 ± 73	0.263
FCR (kg feed/kg gain)	1.72 ± 0.04	1.63 ± 0.03	1.69 ± 0.05	0.841
ADWG (g)	37.2^{b} ± 7.0	48.3^{a} ± 1.5	47.3^{a} ± 2.1	< 0.001
Dressing %	70.6 ± 0.6	71.4 ± 0.5	71.4 ± 0.3	0.370
Mortality (number)	4	1	1	–
Blood parameters				
Total protein (mg/dl)	2.91 ± 0.11	3.06 ± 0.08	2.92 ± 0.12	0.548
Albumin (mg/dl)	1.45 ± 0.09	1.43 ± 0.07	1.35 ± 0.08	0.651
Globulin (mg/dl)	1.46^{b} ± 0.06	1.63^{a} ± 0.04	1.61^{a} ± 0.05	0.041
ALT (U/l)	4.11 ± 0.87	3.58 ± 0.77	4.01 ± 0.62	0.873
Alkaline phosphatase (U/l)	4177 ± 1092	2699 ± 530	2384 ± 467	0.330
Creatine kinase (U/l)	17203 ± 5028	10697 ±2742	13772 ± 3181	0.483
LDH (U/l)	1427 ± 194	1084 ± 116	1370 ± 175	0.295
Erythrocytes (T/l)	2.40 ± 0.14	2.52 ± 0.19	2.40 ± 0.13	0.808
Leucocytes (G/l)	6.25 ± 1.2	6.30 ± 0.84	4.64 ± 0.38	0.269

SEM: Standard error of the mean
[abc]: Means within the same row with different superscript are significantly different (P < 0.05).

Table 5. Effects of EP-F25 and EP-A25 supplementation on performance and blood parameters (experiment 3)
Einfluss der Zulage von EP-F25 und EP-A25 zum Trinkwasser auf die Leistung und auf einige Blutparameter (Versuch 3)

Parameter	Treatments		
	Control mean ± SEM	EP-F25 mean ± SEM	P value
Feed consumption (g)	3367 ± 43	3234 ± 28	0.101
Final weight (g)	1907 ± 52	1851 ± 42	0.424
Total weight gain (g)	1868 ± 52	1814 ± 42	0.438
FCR (kg feed/kg gain)	1.81 ± 0.01	1.74 ± 0.01	0.063
ADWG (g)	53.4 ± 1.5	51.8 ± 1.2	0.438
Dressing %	70.8 ± 0.27	70.8 ± 0.20	0.951
Mortality (number)	0	0	–
Performance parameters			
Total protein (mg/dl)	3.42^{a} ± 0.06	3.20^{b} ± 0.05	0.021
Albumin (mg/dl)	1.93^{a} ± 0.04	1.63^{b} ± 0.08	0.006
Globulin (mg/dl)	1.48 ± 0.06	1.57 ± 0.10	0.488
ALT (U/l)	2.72 ± 0.77	1.60 ± 0.92	0.374
γ-GT (U/l)	25.8 ± 1.64	24.0 ± 1.48	0.427
Alkaline phosphatase (U/l)	1861 ± 527	2285 ± 355	0.520
Creatine kinase (U/l)	41417^{a} ± 6680	24100^{b} ± 2893	0.039
LDH (U/l)	2197 ± 369	1682 ± 92	0.205
Erythrocytes (T/l)	2.26 ± 0.17	2.32 ± 0.38	0.910
Leucocytes (G/l)	10.5^{a} ± 3.9	4.70^{b} ± 1.7	0.013

SEM: Standard error of the mean
^{abc}: Means within the same row with different superscript are significantly different ($P < 0.05$).

Table 6. Comparison of control and EP-F25 on performance and blood parameters
Vergleich der Kontrolle und der Behandlung EP-F25 im Hinblick auf die Leistung und einige Blutparameter

Parameter	Treatments		
	Control mean ± SEM	EP-F25 mean ± SEM	P value
Feed consumption (g)	2577 ± 87	2643 ± 67	0.631
Final weight (g)	1802 ± 43.1	1828 ± 41.1	0.657
Total weight gain (g)	1758 ± 43.1	1785 ± 41.6	0.657
FCR (kg feed/kg gain)	1.67 ± 0.02	1.63 ± 0.02	0.610
ADWG (g)	47.5 ± 1.41	51.0 ± 1.36	0.079
Dressing %	70.6 ± 0.54	71.3 ± 0.48	0.360
Performance parameters			
Total protein (mg/dl)	3.10 ± 0.07	3.21 ± 0.27	0.239
Albumin (mg/dl)	1.58 ± 0.06	1.52 ± 0.03	0.418
Globulin (mg/dl)	1.53^{b} ± 0.04	1.69^{a} ± 0.04	0.006
ALT (U/l)	5.08 ± 0.55	4.00 ± 0.45	0.130
γ-GT (U/l)	22.2 ± 1.3	21.8 ± 0.97	0.800
Alkaline phosphatase (U/l)	3267 ± 504	2416 ± 252	0.137
Creatine kinase (U/l)	23892^{a} ± 3179	15810^{b} ± 2057	0.037
LDH (U/l)	1570 ± 138	1324 ± 116	0.179
Glucose (mg/dl)	216 ± 6.0	222 ± 4.5	0.423
Cholesterol (mg/dl)	137 ± 6.8	140 ± 6.3	0.777
Erythrocytes (T/l)	2.27 ± 0.08	2.29 ± 0.11	0.864
Leucocytes (G/l)	7.58 ± 0.69	6.02 ± 0.42	0.053
Haemoglobin (g/dl)	10.1 ± 0.23	9.95 ± 0.18	0.708
Hematocrit (%)	30.9 ± 0.71	30.5 ± 0.66	0.682

SEM: Standard error of the mean
^{abc}: Means within the same row with different superscript are significantly different ($P < 0.05$).

sorption. The presence of active ingredients and phenolic compounds can reduce numbers of intestinal pathogens, thus minimizing nutrient loss. In present experiments, intermittent application of EP extracts resulted in numerical (non significant) improvement of broiler performance. However in experiment 2, significantly ($P < 0.05$) better ADWG was observed by application of EP-F juices. These results show that intermittent application of EP juices has some beneficial effect on performance in terms of better feed digestion and utilization, which is apparent in terms of better ADWG (experiment 2) and a trend towards improvement of other performance parameters in all experiments. No effect on layer performance was observed by intermittent application of EP juices through feed (Böhmer et al., 2008). Roth-Maier et al. (2005) also observed no significant effect on performance parameters by continuous application of EP cobs through feed to broilers and layers. However, improved weight gain and less intestinal lesions were observed by supplementing EP (ground root preparation) along with combined vaccination against coccidia (Allen, 2003). In present experiments, improvement of feed intake and ADWG may be due to improved digestion of feed by stimulation of digestive enzymes, as phytogenic feed additives are reported to improve performance by increasing the activity of digestive enzymes (Geier and Oster, 2001; Recoquillay, 2006) and better intestinal health. The active ingredients and phenolic compounds present in fermented juice of EP might have positive effect on enzymes and microflora of digestive track, and by this help in improving digestion and utilization of nutrients.

Comparing effects of two EP-juices (experiment 1), no significant treatment effect was observed, however, FCR and dressing percentage of EP-F group was numerically better than in EP-A and control groups. Similarly better results were obtained by Böhmer et al. (2008) by EP-F as compared to EP-A. This shows that activity of both EP juices is not same. Difference in activity of two juice preparations might be due to presence of different amount of active ingredients (especially alkamides) and presence of ethanol in EP-A treated group. Presence of ethanol in EP-A can also affect the activity of active ingredients of EP, and ethanol is also reported to have effect on immune system (Han and Pruett, 1995).

The analysis of serum and blood samples from different treatments revealed interesting results. In general, EP treated birds showed a trend of higher serum globulin level and especially significantly ($P < 0.05$) higher levels of serum globulin (Table 4 and 6) contents in EP-F25 treated birds as compared to control. This indicates that EP-F juice supplementation through drinking water with intervals (3 days treatment followed by 9 days interval) has elevated the level of serum globulin, which acts as an indicator of immune response and source of antibody (Abdel-Fattah et al., 2008) and immunoglobulins production. Therefore, the observed effect might be due to increase in immu-

noglobulin concentration and improved immunity. Similar results were obtained by SCHRANNER et al. (1989) who found that *Echinacea* containing drugs had the ability to induce a rise in serum immunoglobulin concentration, as well as increase in the three classes of antibodies (SCHRANNER et al., 1989). REHMAN et al. (1999) also observed a significant augmentation of primary and secondary IgG in rats. Increase in immunoglobulin level leads to increase in globulin concentration and improvement of immunity and better health status. Intermittent in-feed supplementation of EP-A juices to layers and pigs increased number of lymphocytes and total leucocytes, while this effect was not observed by application of EP-F (BÖHMER et al., 2008).

In humans changes in serum levels of CK and LDH are considered as indicators for clinical diagnosis of circulatory and cardiac disturbances (IMAEDA, 1999). Supplementation of EP extracts have shown a trend towards decreasing CK and LDH activities in general and significantly ($P < 0.05$) less CK activities in EP-F25 supplemented groups as compared to control (Table 5 and 6). Creatine kinase is an ATP dependent enzyme, its elevated levels in blood are an indication of damage to cardiac cells, and increased CK level in blood of heart patients is considered as first indication of heart attack (ADAMS and APPLE, 2004; RAMSBOTTOM et al., 2008). Cardiac disturbances are common among fast growing broilers (GRASHORN, 1994; OLKOWSKI et al., 1997) and sudden death (Sudden Death Syndrome – SDS) may occur following some stressful event (OLKOWSKI, 2007). Increased CK level was observed in stressed birds and it is an indication of pathological changes of cardiac muscle and increased risk of sudden death syndrome in broilers (ITOH et al., 1997). Certain medications used to reduce the risk of heart attack and to alleviate its pathological effects are directed towards maintaining serum CK level to normal (RAMSBOTTOM et al., 2008). Intermittent application of EP juices has shown a trend towards reduced serum CK level and significantly ($P < 0.05$) lower values in EP-F25 treated birds as compared to control (Table 5 and 6). This shows that EP-F juice has beneficial effects on cardiac health and well being of the birds by reducing the chances of SDS. Mortality in EP treated groups was less as compared to control, which might be an indication of improved health and reduced incidence of SDS.

Changes in activities of ALT, γ-GT and ALP are indicators of liver function. In the present experiments levels of ALT and γ-GT did not differ significantly ($P > 0.05$) between treatments. Reduced ALP contents show a trend of better liver health. This indicates that EP extracts do not have any negative effect on liver functions and activity of different enzymes. Similarly, no significant treatment effect on liver enzymes was observed by MAASS et al. (2005) by in-feed application of EP preparations to pigs. Application of EP-F juices upto 2.5 ml/kg $BW^{0.75}$ (5 times of human standard dose) has shown no detrimental effect on different blood parameters (NASIR and GRASHORN, 2007a; NASIR and GRASHORN, 2007b). This shows that EP supplementation has obviously no harmful effect on health of broilers.

Conclusions

Intermittent application of EP juices has shown positive effects as compared to control in terms of ADWG and increased serum globulins. Similarly, reduced level of CK and reduced mortality in EP treated groups, in general, and EP-F treated groups in particular have shown positive effects on health. The beneficial effects of EP can be obtained by intermittent oral administration for 3 days followed by 9 treatment free days. Further research is required to identify effects of EP juices on performance, immune status, and meat quality of broilers. Research is also required to study the mechanism and mode of action of active ingredients of *Echinacea* juices.

Summary

Three experiments were performed to study the effects of intermittent oral application of two different preparations of *Echinacea purpurea* (EP) juices on broiler performance, blood picture, liver and heart associated enzymes. Results of present experiments show that EP juices do not have any harmful effect on broiler health and performance. Numerically better broiler performance was observed in EP treated groups as compared to control, except for ADWG, which was significantly ($P < 0.05$) higher for EP-F treated group in experiment 2. Significant ($P < 0.05$) improvement of serum globulin levels by application of EP-F through drinking water for 3 days followed by 9 treatment free days, showed that EP-F juice has a potential to improve immunity of birds. Reduced creatine kinase levels show that application of EP juices can also have beneficial effect on cardiac vascular system and can reduce the risks of sudden death syndrome in fast growing broilers. No negative effects of EP supplementation on liver function could be observed.

Key words

Broiler, nutrition, *Echinacea purpurea*, growth, serum proteins, creatine kinase

Zusammenfassung

Einfluss des intermittierenden Einsatzes von *Echinacea purpurea* Presssäften auf die Leistung und auf einige Blutparameter von Broilern

Es wurden drei Versuche durchgeführt, um den Effekt einer intermittierenden, oralen Applikation von *Echinacea purpurea* (EP) Presssäften auf die Leistung, das Blutbild und einige Leber sowie Herz-Kreislauf spezifische Enzyme zu untersuchen. Die Ergebnisse der Untersuchung belegen, dass der Einsatz von EP Presssäften keine nachteiligen Effekte auf die Gesundheit und die Leistung von Broilern hat. In der Tendenz war die Leistung in den EP Behandlungsgruppen besser als in der Kontrollgruppe. Nur in Versuch 2 waren die durchschnittlichen täglichen Zunahmen bei der Behandlung EP-F signifikant ($P < 0,05$) besser als bei der Kontrolle. Der Serum-Globulinspiegel war nach der Applikation von EP-F Presssaft über 3 Tage mit anschließender 9-tägiger Applikations-freier Periode signifikant erhöht ($P < 0,05$). Dies bestätigt das immunostimulierende Potential von EP. Der reduzierte Kreatinkinase-Spiegel in den EP-Behandlungsgruppen deutet darauf hin, dass auch ein positiver Effekt auf das Herz-Kreislaufsystem vorzuliegen scheint. Eventuell kann hierdurch das Risiko des Auftretens des Plötzlichen Herztods bei den schnell wachsenden Masthühnerherkünften vermindert werden. Generell konnten keine negativen Effekte der EP-Applikation auf die Leberfunktion festgestellt werden.

Stichworte

Broiler, Fütterung, *Echinacea purpurea*, Wachstum, Serumproteine, Kreatinkinase

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