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EFFICACY OF HERBAL EXTRACTS ON GROWTH PERFORMANCE, SERUM BIOCHEMISTRY AND INTESTINAL SELECTED BACTERIAL POPULATION IN BROILERS

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Abstract

This study was conducted to evaluate the effects of two herbal extracts (Emerald and Gundelia tournefortii L. Seed) on growth performance, serum biochemistry and intestinal bacterial population in broilers. A total of 280 Ross 308 1-day-old male broiler chicks were distributed into 5 groups of 7 in each consisting 8 replicates per treatment for 42 days. Five treatments were used; control group received basal diet (without any herbal extract supplementation), the Emerald at 100 mg/kg diet and G. tournefortii L. seed extracts (GTE) at 2, 4 and 8 g/kg diet were added to basal diet. The addition of 4 and 8 g GTE to the diet resulted in significantly higher body weight compared with control group (P<0.05). Moreover, supplementing the highest level of GTE (8 g/kg diet) significantly increased intestinal lactic acid bacteria counts (P<0.05).However, no differences were observed among treatments for feed intake and feed conversion rate at the end of the study (P>0.05). In addition, serum uric acid (UA) and glucose (Glu) concentrations and aspartate amino transferase (AST) and alkaline phosphatase (ALP) activities were not affected by any treatment (P>0.05). In conclusion, our results showed that different dietary levels of GTE may improve the growth performance by increasing intestinal lactic acid bacteria counts.

Key words*: Gundelia tournefortii L., growth performance, serum biochemistry, bacterial population, broiler.*

INTRODUCTION

Various types of antibiotics have been widely used as growth promoters in animal production for a large number of years worldwide. Following the banning of the antibiotics using as growth promoters in animal feeds many researchers started to research novel approach for alternative feed additives in poultry production (Denli et al., 2016). Different types of probiotics, prebiotics, plant extracts, aromatic herbs, spices, essential oils have been applied as feed additives to improve growth performance of livestock animals (Fulton et al., 2002). Many types of them have been tested within varying levels and receiving great interest as replacement of antibiotics in animal production (Akyildiz and Denli, 2016). During the last decade, various herbs, spices, plant extracts, essential oils and phytogenic compounds have been used in animal nutrition due to their potential beneficial effects or active compounds. Many of them have been approved in poultry as growth promoters (Alloui et al., 2011; Hafeez et al., 2016) and due to their antimicrobial effects in poultry by stimulating appetite and digestion (Kamel, 2001; William and Losa, 2001).

In this study we aimed to evaluate the effects of different levels of *Gundelia tournefortii L. seed extracts* (GTE) and a commercial plant extract (Emerald) on growth performance, serum biochemistry and intestinal bacterial population in broilers.

MATERIALS AND METHODS

This study was performed at the Dicle University, Animal Research Center according to the guidelines for animal experimentation of Dicle University and approved by the Ethical Committee (DUHADEK- No: 01.12.2016-36).A total of 280 Ross 308 1-day-old male broiler chicks were distributed into 5 groups of 7 in each consisting 8 replicates per treatment for 42 days. Five treatments were used; chicks

were fed by basal diet as control group, basal diet plus 100 mg Emerald/kg diet, three levels of GTE (2, 4 and 8 g GTE/kg diet).

Two basal diets; grower (1 to 21 day) and finisher (22 to 42 days) were formulated according to the NRC (1994) recommendations to meet the nutrient requirements of broilers. The composition of the basal diets is presented in Table 1. Chickens received the feed and water as *ad libitum* throughout the experiment. Emerald is a commercial product which has developed by Igusol Advance SA, Spain. Emeraldis a combination of essential oils designed to be gradually released into the digestive tract improving animal's well-being. *Gundelia tournefortii* L. seeds were collected from Bismil town in Diyarbakir in Turkey. The *Gundelia tournefortii* L. seeds was dried then ground through 1.0 mm mesh and incorporated into the experimental diets.

Table 1: Composition of experimental diets (%)

Ingredients	Starter (1-22 day)	Finisher (23-42 day)
Maize	57.0	58.0
Soybean meal (46 % CP)	25.5	22.3
Full fat soybean	13.7	13.0
Sunflower oil	-	2.9
Dicalcium phosphate[a]	2.00	2.0
Limestone	0.90	1.0
NaCl	0.30	0.35
Vitamin premix[b]	0.10	0.10
Mineral premix[c]	0.15	0.15
L-Lysine HCl	0.20	-
Dl-Methionine	0.15	-
Calculated composition		
Crude Protein	22.0	20.4
ME (kcal/kg)	3,010	3,197
Calcium	0.97	0.99
Available phosphorus	0.47	0.43
L-lysine	1.36	1.26
Methionine+cystine	0.90	0.76

[a] Contains 240 g Ca and 17.5 g P/kg;[b]Provided (per kg of diet): vitamin A, 8,000 IU; vitamin D3, 1,200 IU; vitamin E, 10 IU; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 5 mg; pyroxidine, 0.2 mg; vitamin B12, 0.03 mg; pantothenic acid, 10 mg; niacin, 50 mg; biotin, 0.1 mg; folic acid, 0.5 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg

[c]Provided (per kg of diet): Iron, 80 mg; zinc 40 mg; manganese 60 mg; iodine 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg.

Chickens were weighed individually and feed intake determined by pen from 7 to 42 d (n=8). Mortality was checked daily and recorded throughout the experimental period. Body weight gain and feed intake were determined weekly then feed conversion rate (FCR) (g:g) was calculated and recorded.

At the end of the experiment, blood samples (2 mL per bird) were collected from 10 chickens per treatment for serum biochemical determination. Within 1 h, the serum was obtained by centrifugation (2,500 × g for 15 min) and stored at −80°C until further analysis. Serum biochemical parameters were measured by using Architect System Reagents and an automatic clinical chemistry analyzer. The concentration of total protein (TP) was measured by following the Biuret method; uric acid (UA) by following the uricase method; cholesterol by following the cholesterol esterase-peroxidase method; respectively; triglyceride by following the glycerol phosphate oxidase method; and the enzymatic activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) by using the recommended International Federation of Clinical Chemistry and Laboratory Medicine reference methods. After taking blood samples, chickens were euthanized with an intravenous injection of sodium pentobarbital and immediately intestinal tract, liver, gizzard and abdominal fat pad were removed and weighed (data expressed as relative organ weight; grams of organ per 100 g of BW). Small intestine was immediately removed and digesta contents (from final part of small intestine) from 50 chickens (10 chickens per treatment) were collected separately, cooled at once used for microbial assays *(Escherichia coli, Enterococcus, Colostridium* and *Lactobacillus).*

The data were analyzed by using the one-way ANOVA with the General Linear Model (GLM) procedure of SPSS 16.0 (2011). Treatment means were also partitioned into linear, quadratic, and cubic effects of dietary supplementation of GTE level with orthogonal polynomial contrasts. Statistical significance was considered at $P < 0.05$. Differences among means were evaluated using Tukey's test.

RESULTS AND DISCUSSIONS

The effects of dietary Emerald and different levels of GTE supplementation on growth performance and internal organ weights are shown in Table 2. In the present study, body weight gain was correlated with the level of GTE (P=0.009) and maximized by the supplementation level of 4 g/kg of diet (Table 2). Besides, body weight gain and feed conversion rate were showed a linear response

to GTE (P<0.05). Our study clearly indicates the positive effects of the tested GTE on body weight gain and feed conversion rate in broilers.

In our previous study, dietary supplementation of Emerald resulted in useful effects as enhancer of growth performance by reducing by reducing the number of *Escherichia coli* count in the intestines of broiler chickens (Akyildiz et al., 2016).This study confirmed the useful effects of Emerald in broilers. These results are in agreement with those reported by Ocak et al. (2008) in that dietary supplementation of peppermint and thyme significantly improved body weight gain in broilers. Similarly, Halle et al. (2004) reported that graded oregano extracts and essential oil collected from oregano significantly improved feed conversion rate in broilers. Our results contrast with those of Calıslar et al. (2009) who found that phytogenic additives containing extracts from *Origanum vulgare ssp. hirtum* had no effects on the body weight gain in broilers.

All groups had similar feed intake and feed conversion rate at 42 day. However, chickens were fed diets supplemented 2 and 8 g of GTE/kg diet had an improved feed conversion rate compared with control chickens at 42 day.

The supplementation of Emerald and GTE had no significant effect on the weights of liver, gizzard, or spleens and abdominal fat pad (P>0.05). However, the intestine weight of chickens supplemented with GTE at 8 g had a higher (P<0.05) compared with the control groups and linear effects of GTE on intestine weight were observed. Differences between study results may be attributable to different composition of the plant extracts, used levels and the active substances and their biological activity, respectively (Amad et al., 2011).

Table 2. Effects of Emerald and *Gundelia tournefortii* L. seed extracts (GTE) on growth performance and internal organ weights in broilers at 42 d of age

Measurements	Control	Emerald (100 mg/kg)	(GTE) (g/kg)			SEM	P	Contrasts		
			2	4	8			L	Q	C
BWG, g	2152.9[b]	2232.3[ab]	2265.4[ab]	2297.6[a]	2282.2[a]	14.07	0.009	**	*	NS
FI, g	3778.1	3682.9	3713.9	3886.7	3669.6	35.50	0.291	NS	NS	NS
FCR	1.75	1.71	1.69	1.70	1.67	0.009	0.148	*	NS	NS
Intestine weight[2]	5.71[b]	5.85	5.92[ab]	5.97[ab]	6.37[a]	0.088	0.160	**	NS	NS
Liver weight[2]	2.10	2.07	2.08	2.18	2.26	0.03	0.213	*	NS	NS
Gizzard weight[2]	2.87	2.92	2.88	2.77	3.01	0.06	0.852	NS	NS	NS
Abdominal fat pad[2]	1.56	1.25	1.17	1.23	1.47	0.05	0.092	NS	*	NS

SEM: Pooled standard error of mean- L: Linear, Q: Quadratic, C: Cubic effects.
[1]Each value represents the least square mean from 8 pens per each treatment: [2](g/100 g body weight)
[a-d]Means within a column without a common superscripts differ statistically (P < 0.05).
NS: No significant (P>0.05), *: P<0.05, **: P<0.01

In our study, ileo-cecal microflora populations and serum biochemistry were determined to explore a possible mechanism for the improvement of performance in broilers with GTE supplementation. Effects of Emerald and GTE on serum biochemistry in broilers are shown in Table 3. Liver enzymes are indicators for many diseases and they are a marker of damage to live cells. ALP and AST activities and serum glucose and uric acid concentrations were not affected by treatments (P>0.05). Albumin and globulin are main component of total amount of protein in serum help growth and healing. Low total protein concentration in serum can imply a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. Serum cholesterol concentrations responded quadratically (P < 0.01) with increasing levels of dietary GTE. These results are consistent with those of Mohan et al. (1996) and Jin et al. (1998) who conducted that intestinal microbial population affect the serum cholesterol concentration in broilers. In present study, decreases of serum cholesterol concentration may be in resulting of the effects of dietary GTE on intestinal microbial population in broilers.

Serum triglyceride concentrations were decreased in chickens were fed the diet supplemented with 4 and 8 g GTE/kg feed (P<0.05).

Table 3. Effects of Emerald and *Gundelia tournefortii* L.seed extracts (GTE) on serum biochemistryin broilers at 42 d of age

Measurements	Control	Emerald (100 mg/kg)	(GTE) (g/kg)			SEM	P	Contrasts		
			2	4	8			L	Q	C
ALP (U/L)	1391.4	1500.2	1270.6	1247.5	1156.7	50.71	0.235	NS	NS	NS
AST (U/L)	250.7	297.1	252.5	250.2	239.7	8.70	0.263	NS	NS	NS
CHOL (mg/dL)	109.5^{b}	112.1^{ab}	124.6^{a}	104.1^{a}	112.6^{ab}	1.82	0.004	NS	NS	**
Glucose (mg/dL)	227.2	258.2	244.2	225.12	227.9	4.64	0.098	NS	NS	NS
TP (g/dL)	2.78^{ab}	3.25^{a}	2.83^{ab}	2.57^{b}	2.88^{ab}	0.07	0.036	NS	NS	NS
TRG (mg/dL)	60.87^{b}	80.62^{a}	65.12^{ab}	50.5^{b}	57.37^{b}	2.51	0.001	NS	NS	*
UA (mg/dL)	8.65	9.96	9.71	8.56	10.41	0.35	0.189	NS	NS	NS

SEM: Pooled standard error of mean,ALP:Alkalinephosphatise, AST: Aspartate amino transferase, CHOL:Cholesterol, UA: Uric acid, TP: Total protein,TRG: Trigliseride- L: Linear, Q: Quadratic, C: Cubic effects.
$^{a-d}$Means within a column without a common superscripts differ statistically (P < 0.05).
NS: No significant (P>0.05), *: P<0.05, **: P<0.01

In our study, the colony numbers of *Lactobacillus* was increased in the ileal gut contents of chickens fed diet supplemented Emerald and GTE levels at the 42 day (P<0.05). Our results are supported by the findings of Franciosini et al. (2016), who showed that *Lactobacillus* count increased in ileo-cecal content in broilers fed diet supplemented with oregano *(Origanum vulgare* L.*)* and rosemary *(Rosmarinus officinalis* L.*)* aqueous extracts. On the other hand the *Escherichia coli* counts was decreased in chickens fed diet Emerald and GTE supplemented with compared to control. Both types of *Clostridium perfringens* (A and C) may cause necrotic enteritis in many species of birds (Crespo et al., 2007). Kaldhusdal and Hofshagen (1992) reported that increasing of numbers of *Clostridium perfringens* in the gut of broilers may lead to lower growth rate and feed efficiency.

Table 4. Effects of Emerald and *Gundelia tournefortii* L. seed extracts (GTE) on serum biochemistry in broilers at 42 d of age

Measurements	Control	Emerald (100 mg/kg)	(GTE) (g/kg)			SEM	P	Contrasts		
			2	4	8			L	Q	C
Escherichia coli (log CFU g-1)	4.84^{a}	3.63^{b}	4.35^{ab}	4.30^{ab}	4.29^{ab}	0.13	0.086	NS	NS	NS
Enterococcus (log CFU g-1)	1.00	3.41	1.05	2.04	1.55	0.34	0.148	NS	NS	NS
Colostridium spp. (log CFU g-1)	3.74^{a}	3.50^{ab}	3.46^{ab}	3.23^{b}	3.44^{ab}	0.05	0.070	*	*	NS
Lactobacillus spp. (log CFU g-1)	2.97^{b}	4.61^{a}	4.31^{ab}	4.20^{ab}	4.69^{a}	0.18	0.014	NS	NS	NS

SEM: Pooled standard error of mean- L: Linear, Q: Quadratic, C: Cubic effects.
$^{a-d}$Means within a column without a common superscripts differ statistically (P < 0.05).
NS: No significant (P>0.05), *: P<0.05, **: P<0.01

CONCLUSIONS

In conclusion, results of this study showed that different dietary levels of GTE may improve the growth performance by increasing intestinal lactic acid bacteria counts. However, more experiments are needed to explain whether GTE may affect antimicrobials or antioxidants in poultry diets.

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