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Full Length Research Paper

The effects of *Nigella sativa* powder (black seed) and *Echinacea purpurea* (L.) Moench extract on performance, some blood biochemical and hematological parameters in broiler chickens

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This experiment was conducted to evaluate the effects of *Nigella sativa* (NS) powder and *Echinacea purpurea* (L.) Moench extract (EP) on performance and some blood biochemical and haematological parameters in broiler chickens. A total of 144, one day-old broiler chickens (Ross 308) were divided into three treatment groups, with four replicates per treatment and 12 chicks per replicate. Chicks in control groups were fed basal diets and received water without any supplementation. Birds in treatment groups two were fed on feeds supplemented with 1% NS. For birds in treatment groups three, drinking water was supplemented with EP at the rate of 0.25 ml/kg body weight (BW). Body weights of broilers were measured at one, 21 and 42 days, feed intake was measured at the same periods and feed conversion was calculated, accordingly. Blood samples were taken weekly until 6th weeks, for biochemical and hematological analysis. Result of this experiment show that, supplementing 1% NS improved body weight gain (BWG) and feed conversion ratio (FCR) of broilers at starter and grower periods ($P<0.05$), and also increased serum albumin levels ($P<0.05$). Supplementation of EP increased globulin levels and white blood count (WBC) count significantly ($P<0.05$). Total protein and glucose concentrations were not statistically ($P>0.05$) influenced. Supplementing 1% *N. sativa* decreased serum triglyceride and total cholesterol of broilers in overall ($P<0.05$). In conclusion, results of this study show that addition of 1% NS powder seem to have a positive influence on growth performance and it could be considered as a antibiotic growth promoter substitution for broiler chicks.

Key words: *Nigella sativa*, *Echinacea purpurea* (L.) Moench, performance, serum biochemistry, blood hematology, broiler.

INTRODUCTION

Usage of phytogenic additives in animal nutrition is increasing globally. Since the ban on use of antibiotic additives in animal feeds in Europe in 2006, phytogenic additives are considered as one viable alternative due to their activity in different physiological systems. Phytogenic substances, extracts which have wide range

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of activities in animals, on digestive, immune, endocrine systems etc., show physio-pathological (anti-inflammatory, anti-oxidative) and anti-microbial activities (Nasir and Grashorn, 2010a) in addition.

One of the alternatives used as feed additives is *Nigella sativa* (NS) (black seed), family *Ranunculaceae*. The seed of *N. sativa* has been used for centuries in the Middle East, Northern Africa, Far East, and Asia for medicinal affairs (El-Daly, 1998). NS has been reported to have many biological properties including antiparasitic (Mahmoud et al., 2002), antidiabetic (Al-Hader et al., 1993), and diuretic effects (Zaoui et al., 2000). A few studies showed that black cumin also has antibacterial activity (Mouhajir et al., 1999; Nair et al., 2005). *N. sativa* contain alkaloids, fixed and volatile oils and a variety of pharmacologically active substances like thymoquinone, dithymoquinone, thymol, carvacrol, nigellicine-N-oxide, nigellidine and alpha-hedrin (Al-Homidan et al., 2002; Nasir et al., 2005). *N. sativa* and its various extracts were found to possess antibacterial activity against gram positive and gram-negative bacteria, and they caused inhibition of aflatoxin production (Nasir and Grashorn, 2006). There are some studies conducted on the effects of dietary NS or oils on the performance of poultry (Al-Homidan et al., 2002; Tollbaand and Hassan, 2003; AL-Beitawi et al., 2009; Nasir and Grashorn, 2010a; Toghyani et al., 2010). Another phytogenic additive is *Echinacea purpurea* (L.) Moench, family *Asteraceae*. *E. purpurea* (L.) (EP) is a popular herbal immune-stimulator in North America and Europe, while NS has been used traditionally in Mediterranean and South Asian countries for its beneficial effects on immune system and as natural remedy, both in humans and animals (Nasir and Grashorn, 2010a). Landy et al. (2011) reported positive effects of EP on growth performance and humoral immune responses in broiler chicks. The current study was conducted to evaluate the potential of applying *E. purpurea* (L.) Moench extract and *N. sativa* powder on the performance of some blood biochemical and haematological parameters in broiler chickens.

MATERIALS AND METHODS

A completely randomized experimental design was used, and chicks (Ross 308) were divided into three treatment groups, with four replicates per treatment and 12 chicks per replicate. A total of 144, one day-old broiler, chicks (a mix of both sexes) (Ross 308) were raised in floor pens with *ad libitum* access to feed and water and controlled ventilation. Chicks of a uniform body weight were placed in individual pens and average initial body weight was 48 g. Temperature was maintained at 32°C for the first four days and then gradually reduced according to normal management practices until a temperature of 22°C was achieved at day 28. The light regimen was 23 h of light and 1 h of dark. The diets were formulated to meet the nutrients requirements of broilers as recommended by the National Research Council (1994). Table 1 presents the ingredients and the composition of the basal diets. During the experiment, no antibiotics were offered to broilers via either feed or water. The duration of study was six weeks.

Chicks in control groups were fed basal diets and received water without any supplementation. Birds in treatment groups two were fed on feeds supplemented with 1% grounded NS seeds. For birds in treatment groups three, drinking water was supplemented with EP extract at the rate of 0.25 ml/kg body weight (BW). In this study, crude powder of EP root was used. A 10% aqueous extract was prepared by infusion with 70°C water for 30 min. This extract was filtered, vacuum concentrated and kept in dark bottles at 4°C until used. NS seeds were obtained from local area in North West of Iran and used in the diets after grinding. Feed consumption was recorded on a replicate basis at weekly intervals. Feed conversion ratio (FCR) was calculated on a weekly basis for every group in the study. The FCR was expressed as kilograms of feed consumed per kilogram of live weight gain. Blood samples of five birds per replicate (selected randomly) were collected weekly until 6^{th} weeks from wing vein. Blood samples were collected in ethylene diamine tetra-acetic acid (EDTA) containing tubes for determination of blood profile, while whole blood samples for determination of serum proteins were collected without anticoagulant. Serum was separated after centrifugation of clotted whole blood at 3,500 rpm for 20 min. 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 cholesterol, triglyceride, total protein, albumin, globulin, glucose, and numbers of white blood cells (WBCs) and red blood cells (RBCs). Quantitative determination of blood cells from un-coagulated blood (in EDTA tubes) was carried out by using the Coulter counter.

Statistical analysis

Data were evaluated with analysis of variance (ANOVA) for a complete randomized design, using the general linear models procedure of statistical analysis software [SAS (SAS Institute, 2002)]. The treatment means with significant differences were compared by using Duncan's new multiple range tests. All statements of differences were based on significance at $P<0.05$.

RESULTS

Growth performance

Data on performance parameters are summarized in Table 2. Result of this experiment show that, supplementing 1% NS improved body weight gain and feed conversion ratio of broilers at starter and grower periods ($P<0.05$). Supplementation of *E. purpurea* increased body weight gain (BWG) in grower period but it was not significant ($P>0.05$). Feed intake of broilers was not markedly affected by treatments ($P>0.05$).

Blood biochemical parameter

Results show that supplementation of *N. sativa* decreased ($P<0.05$) serum total cholesterol and

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

Ingredients (g/kg)	Starter (1 - 21 days)	Grower (21 - 42 days)
Wheat	30	30
Corn	24.64	25.08
Corn gluten	10	7.37
Wheat bran	0	7.21
Soybean	24.97	19.17
Soybean oil	5.64	7.00
Oyster shell	1.77	1.79
Ca phosphate	1.75	1.30
Salt	0.41	0.28
Mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.25	0.25
Lys	0.23	0.28
Met	0.09	0.02
Nutrient composition calculated		
ME, kcal/kg	3200	3200
Crude protein (%)	23	20
Ca (%)	1.0	0.9
Available P (%)	0.45	0.35
P (Total) (%)	0.702	0.41
Na (%)	0.20	0.150
Lys (%)	1.1	0.91
Met (%)	0.50	0.380
Met + Cys (%)	0.90	0.62

[1]Per kilogram of diet provided: Cu ($CuSO4.5H_2O$), 4.0 mg; I, potassium iodate (1.0 mg); Fe, ($FeSO_4.7H_2O$), 60 mg; Mn ($MnSO_4.H_2O$), 60 mg; Se (Na_2SeO_3), 0.1 mg; Zn ($ZnSO_4.7H_2O$), 44 mg; and Ca ($CaCO_3$), 723 mg. [2]Per kilogram of diet: vitamin A (vitamin A palmitate), 4,500 IU; vitamin D3, 450 IU; vitamin E (vitamin E acetate), 50 IU; menadione (menadione sodium bisulfite), 2.4 mg; vitamin B12, 0.02 mg; biotin (D-biotin), 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; Ca-pantothenate, 20 mg; pyridoxine (pyridoxine_HCl), 6.4 mg; riboflavin, 15 mg; and thiamin (thiamin_HCl), 15.2 mg.

Table 2. The effects of *Nigella sativa* powder (NS) and *Echinacea purpurea* (*L.*) Moench extract (EP) on the performance of broilers.

Item	Treatment		
	Control	NS	EP
Starter (0 to 21days)			
Feed Intake (g)	937.23 ± 34	954.08 ± 41	940.66 ± 26
Body weight gain (g)	618.65^{a} ± 18	655.73^{b} ± 35	624.55^{a} ± 52
Feed/gain (g/g)	1.514^{a} ± 0.03	1.455^{b} ± 0.02	1.506^{a} ± 0.03
Grower (22 to 42 days)			
Feed intake (g)	2947 ± 67	3084.50 ± 85	2926.47 ± 79
Body weight gain (g)	1322.50^{b} ± 58	1667^{a} ± 74	1422^{b} ± 88
Feed/gain (g/g)	2.066^{a} ± 0.04	1.850^{b} ± 0.02	2.058^{ab} ± 0.03

Values in the same row not sharing a common superscript differ significantly ($P < 0.05$).

Table 3. The effects of *Nigella sativa* powder (NS) and *Echinacea purpurea* (*L.*) Moench extract (EP) on some blood biochemical and haematological parameters in broiler chickens.

Treatment	Factor		
	Control	NS	EP
Cholesterol (mg /dl)	$136^{a} \pm 32$	$122^{b} \pm 25$	$141^{a} \pm 26$
Triglycerides (mg /dl)	$47^{a} \pm 1.5$	$41^{b} \pm 1.2$	$48^{a} \pm 2.4$
Total protein (g/dl)	3.1 ± 0.45	3.2 ± 0.53	3.2 ± 0.25
Albumin (g/dl)	$1.37^{b} \pm 0.04$	$2.21^{a} \pm 0.08$	$1.43^{b} \pm 0.02$
Globulin (mg/dl)	$1.38^{b} \pm 0.02$	$1.42^{b} \pm 0.03$	$1.72^{a} \pm 0.02$
Glucose (mg /dl)	203.34 ± 6.6	208.52 ± 8.4	207.46 ± 5.9
RBCs (10^{6})	1.37 ± 0.02	1.54 ± 0.03	1.44 ± 0.03
WBCs (10^{6})	$23.34^{b} \pm 1.2$	$22.53^{b} \pm 0.9$	$31.44^{a} \pm 2.1$

Values in the same row not sharing a common superscript differ significantly ($P < 0.05$). RBCs, Red blood cells; WBCs, white blood cells.

triglycerides levels and increased ($P<0.05$) albumin levels. No significant effects were noticed in serum glucose, globulin, WBCs and RBCs level. On the other hand, supplementation of *E. purpurea* increased ($P<0.05$) globulin and WBCs levels. *E. purpurea* supplementation did not affect other mentioned biochemical parameters (Table 3).

DISCUSSION

The beneficial effect of growth promoting feed additives on animals arises from stabilizing feed hygiene and beneficially modulating the gut ecosystem by controlling potential pathogens. Phytogenic compounds have a number of active ingredients and pharmacologically active substances that are beneficial for maintaining health and improving performance of poultry and other livestock species. They are reported to stimulate secretion of digestive enzymes (lipase and amylase) and intestinal mucous in broilers, to stimulate feed digestion, to impair adhesion of pathogens and to stabilize microbial balance in the gut (Lee et al., 2003). However, effects of phytogenic compounds and their active ingredients are not always observed in terms of performance parameters, as they also affect different metabolic pathways and activity of different body systems (Nasir and Grashorn, 2010a). Phytogenic substances are supposed to improve performance of the broilers by stimulating secretion of digestive enzymes leading to improved nutrient digestion and absorption. The presence of active ingredients and phenolic compounds can reduce numbers of intestinal pathogens, thus minimizing nutrient loss (Nasir and Grashorn, 2010a). In present experiments, supplementation of NP resulted in improved broiler performance. However, EP supplementation led to non significant improvement of performance. These results show that supplementation of NS has beneficial effect on performance in terms of better feed digestion and utilization, which is apparent in terms of better BWG and a trend towards improvement of other performance parameters in all experiments (Nasir and Grashorn, 2010a).

Roth-Maier et al. (2005) also observed no significant effect on performance parameters by continuous application of *E. purpurea* 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 BWG 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 (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 (Nasir and Grashorn, 2010a). According to results of analysis of blood samples from different treatments, *E. purpurea* treated chicks showed higher serum globulin level and WBCs count as compared to control. This indicates that *E. purpurea* extract supplementation 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 immunoglobulin 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 signify-

cantaugmentation 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. Supplementation of EP to layers and pigs increased number of lymphocytes and total leucocytes, while this effect was not observed when fermented EP extract was applied (Bohmer et al., 2008). *N. sativa* supplementation had no significant effect on serum glucose level when compared to control group. Results agree with Toghyani et al. (2010) who recorded non significant effect for *N. sativa* on glucose level, but disagree with El-Dakhakhny et al. (2002), Zaoui et al. (2002) and Meral et al. (2004). They recorded hypo-glycemic effect for *N. sativa* supplementation. Addition of *N. sativa* in the diet led to decreasing serum cholesterol level when compared to control. Results agree with those obtained by Tollbaand and Hassan (2003), Hassan and Abo Taleb (2007) and AL-Beitawi et al. (2009) who recorded that *N. sativa* significantly decreased serum levels of total cholesterol and triglyceride, while Toghyani et al. (2010) found that serum triglyceride and total cholesterol concentrations were not significantly affected by supplementation of *N. sativa*. The decrease in plasma cholesterol level may be attributed to the high content of *N. sativa* from unsaturated fatty acids which may stimulate the cholesterol excretion into the intestine and the oxidation (Khodary et al., 1996). Higher serum albumin level was recorded in group that received 1% NS/Kg diet. The same results were recorded by Hassan and Abotaleb (2007); they found increase in serum albumin. Albumin levels are taken as indicators of the synthetic activity of the liver (Black, 1996). A common mechanism seems to affect albumin leading to response which could be an enzyme induction by the *N. sativa* (Al-Jishi and Abuo, 2003). RBCs and WBCs count are not varied between control and NS supplemented group. In conclusion, results of present study show positive effects of phytogenic additives like *E. purpurea* and *N. sativa* on performance and blood biochemical parameters as an index of general metabolism of chicks' body. However, more research is required to study the mechanism and mode of action of active ingredients of *E. purpurea* and *N. sativa*.

Abbreviations

NS, *Nigella sativa*; **EP**, *Echinacea purpurea* (*L.*) Moench extract; **BWG**, body weight gain; **WBC**, white blood cell; **FCR**, feed conversion ratio; **EDTA**, ethylene diamine tetra-acetic acid.

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