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Effects of *Echinacea purpurea* and *Nigella sativa* supplementation on broiler performance, carcass and meat quality

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ABSTRACT

Present experiment was performed to study the effects of *Echinacea purpurea* (EP) and *Nigella sativa* (NS) on broiler performance, carcass and meat quality. Four treatment combinations were prepared: C - control group without any feed or water additive; E - drinking water intermittently supplemented with fermented juice of EP; N - feed supplemented with grounded seeds of NS, and EN - drinking water intermittently supplemented with EP and feed supplemented with NS. No significant treatment effect was observed on weight gain, average daily weight gain, feed conversion ratio and abdominal fat percentage. Carcass yield in C and N groups was significantly ($P<0.05$) higher than in E. Significantly ($P<0.05$) higher breast percentage in group N was observed. Crude protein contents were significantly ($P<0.05$) higher in meat samples of C and EN groups. Grill losses were significantly ($P<0.05$) lower in E and cooking losses were significantly ($P<0.05$) higher in N treated birds. No significant treatment effect was observed on meat colour, electrical conductivity and shear force value.

KEY WORDS: broilers, phytogenic feed additives, *Echinacea purpurea*, *Nigella sativa*, performance, meat quality

INTRODUCTION

A number of experiments conducted to find alternative to antibiotics revealed that no single alternative exists with the effects comparable to antibiotics (Nasir and

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Grashorn, 2006). There is need to find more efficient alternatives or combinations of different alternatives for maintaining health and improving performance of poultry and other livestock species. Phytogenic compounds are the groups of feed additives that have been reported to possess a potential for growth enhancement of livestock species due to presence of a number of pharmacologically active substances. They are supposed to enhance feed intake, activate digestive enzymes and stimulate immune function (Lee et al., 2003). *Echinacea purpurea*, family *Asteraceae* (EP) and *Nigella sativa*, family *Ranunculaceae* (NS) belong to the class of phytogenic immunostimulants that help in establishment and strengthening of para-immunity (Akhtar et al., 2003; Nasir and Grashorn, 2006, 2009). *Echinacea* 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.

Seeds of NS 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). NS 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). Application of NS seeds has shown some effects on broilers performance (Al-Homidan et al., 2002; Guler et al., 2006; Durani et al., 2007; Ziad et al., 2008), layer performance and egg quality (Akhtar et al., 2003; Nasir et al., 2005). But, the findings of these studies are inconsistent. In contrast to NS, not enough data is available on application of EP in livestock species, especially in poultry. Some effects of intermittent in-feed EP application on immunity were observed in sows, layers and broilers (Roth-Maier et al., 2005; Koreleski and Świątkiewicz, 2007; Böhmer et al., 2009; Nasir and Grashorn, 2010).

In literature no data is available on a comparative testing of effects of NS and EP, administered singly or combined, on broiler performance, carcass and meat quality. Therefore, present experiment was planned to study the effects of powdered NS seeds, EP fermented juice and their combined application on broiler health, performance and meat quality.

MATERIAL AND METHODS

Animals, housing and management

For this experiment, 800 one-day-old broiler chicks (Ross 308) were used. Chicks were sexed at hatch for speed of feathering and were randomly allocated to 40 pens (20 birds/pen) having equal sex ratio. A continuous 24-h lighting programme was applied; environmental temperature was decreased from 33°C on day 1 to 24°C (±1) on day 21 and maintained thereafter till slaughter. Birds were vaccinated routinely against Newcastle disease (ND), infectious bronchitis (IB) and coccidiosis, but no type of medication was administered during the entire experimental period.

Feeding and watering

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). Contents of nutrients were adjusted to recommendations of GfE (1999). All groups were fed on same basal diets except NS treated groups, which received basal diets supplemented with 10 g NS grounded seeds per kg throughout the rearing period (35 days). Feed was offered daily and residual feed was weighed at the end of each phase. Feed intake per replicate during starter and grower phase was calculated. Daily fresh tap water at room temperature was offered *ad libitum* for drinking. Water in *Echinacea*

Table 1. Nutrient composition of experimental diets, g/kg

Item	Starter diet		Grower diet	
Ingredient				
wheat	564.6		599.6	
soyabean meal	334.7		292.8	
soyabean oil	50.0		34.6	
palm fat	0.0		26.6	
limestone	11.2		10.6	
monocalcium phosphate	21.2		14.6	
trace elements premix[1]	0.8		0.8	
cholin chloride	2.0		2.0	
methionine	2.0		1.7	
L-lysine-HCl	3.5		3.0	
$NaHCO_3$	2.8		2.7	
NaCl	1.0		3.5	
Ca propionate (98.0%)[3]	4.0		5.3	
antioxidant[4]	0.2		0.2	
vitamin premix[1]	2.0		2.0	
Content of nutrients	without NS	with NS	without NS	with NS
crude protein, g/kg (analysed)	22.0	22.3	19.8	20.7
metabolizable energy, MJ/kg[2]	12.5	12.5	12.9	12.9

[1] provided per kg of feed; IU: vit. A 12600, vit. D_3 3150; mg: vit. E 41, vit B_1 3, vit B_2 6, niacin 53, pantothenic acid 13, Fe 81, Mn 96, Zn 64, Cu 14, I 0.58, Se 0.15; μg: vit. B_{12} 32, folic acid 1050, biotin 105; [2] calculations according to WPSA (1984); [3] Luprosil; [4] Loxidan TD 100: containing ethoxyquin, propyl galate and BHT

treated groups was supplemented with EP fermented juice, and thoroughly mixed before offering. Residual water was discarded on the following day.

Treatments

Fermented juice of EP was obtained from Berghof-Kräuter GmbH (Berghof, Heilsbronn, Germany). It was extracted from above ground parts of EP plant and preserved through fermentation (dry matter 9.6%, chichoric acid 2.77 mg/ml, total alkamides 3.95 μg/ml)[1]. NS seeds were obtained from Alfred-Galke GmbH (Gittelde, Germany). Before inclusion in feed, NS seeds were grounded to powder form and analysed according to Naumann and Bassler (1993). NS seeds contained, %: dry matter 94.4, crude protein 21.5, crude fat 43.2, crude ash 4, crude fibre 11.3, Ca 0.54, P 0.51 and metabolizable energy 18.75 MJ/kg (calculated according to WPSA, 1984).

Four treatment combinations (Table 2) were prepared and each treatment was offered to 10 experimental units (200 broilers/treatment). Birds in control groups (C) were fed basal diets and received water without any supplementation. For birds in treatment groups E, drinking water was supplemented with fermented juice of EP at the rate of 0.25 ml/kg $BW^{0.75}$, intermittently for 3 days (on 1-3, 13-15, 25-27) followed by 9 treatment free days. Birds in treatment groups N were fed on feeds supplemented with 1% grounded NS seeds. Birds in treatment groups EN received drinking water supplemented with EP as the group E and feed continuously the diet supplemented with 1 % NS grounded seeds for 35 days.

Table 2. Treatment schedule

Group	Treatment	Application mode	Application level	Application period, days of age
C	Control	-	-	-
E	*Echinacea purpurea*	DW	0.25 ml/$BW^{0.75}$	1-3, 13-15, 25-27
N	*Nigella sativa*	Feed	10 g/kg	1-35
EN	*Echinacea purpurea*	DW	0.25 ml/$BW^{0.75}$	1-3, 13-15, 25-27
	Nigella sativa	Feed	10 g/kg	1-35

DW - drinking water; $BW^{0.75}$ - metabolic body weight

Data collection and analysis

Birds in each experimental unit were weighed pen wise at the start of experiment and at end of starter phase (15th day) and grower phase (35th day) and body weight gain (BWG) was calculated. Based on the average weight on 35th day, two pens per treatment (having near-average body weight) were selected for determination of carcass parameters and meat quality. All birds (n=40), in these two replicates

[2] Results of analysis obtained from B.M. Böhmer, TU München (Germany)

per treatment, were wing-tagged and slaughtered. Mortalities were recorded when they occurred with probable cause of death. Birds having leg abnormalities were culled. Mortality rates given in results section include died and culled birds. Weights of the dead and culled birds were used for calculation of corrected feed conversion ratio (FCR - kg feed consumed per kg of liveweight gain).

Forty birds per treatment were slaughtered automatically and excised manually for determination of carcass yield, abdominal fat and liver weight. The data thus recorded were used to calculate the relative weight (in relation to body weight). After recording of weights the carcasses were stored for over night at 4°C for maturation of meat.

After maturation of carcasses, breast meat colour was measured according to L* (brightness), a* (redness) and b* (yellowness) system perpendicular to the meat surface using photometer Color-Guide 45/0 (BYK-Gardner GmbH, Geretsried, Germany). Electrical conductivity of breast meat was measured by inserting the electrode of Conductometer LF 191 (WTW GmbH, Weilheim, Germany) in breast muscles. Afterwards carcasses were dissected and weights of cut-ups were recorded: thighs (including legs and thighs), breast meat and wings. Eight meat samples from left breast fillets were collected per treatment for determination of dry matter, crude protein, crude fat and crude ash contents by proximate analysis (Naumann and Bassler, 1993).

For determination of cooking losses, grill losses and texture, about 200 g meat samples from right breast portion were cut and stored at -18°C. For determination of cooking losses meat samples, after thawing, were packed in plastic bags and heated in water till the internal temperature of meat samples reached 85°C. Meat samples were weighed after cooling to room temperature for 1 h. Loss in weight by cooking was recorded and percent loss in weight was considered as cooking loss. Similarly, grilling loss was measured as loss in weight of meat samples by grilling. Briefly, thawed meat samples, obtained from the same location in the muscle, were wrapped in aluminium foils and grilled until the internal temperature reached 85°C. Grilled meat samples were weighed after cooling to room temperature for 1 h. After weighing, grilled samples were further used for determination of tenderness by measuring shear force using a Warner-Bratzler shear device (Instron, Model 5565k, Instron Ltd., High Wycombe, Bucks, UK). Cylindrical samples (core) of 2.5 cm diameter were removed from the centre portion of the muscle. Shearing of samples was done rectangular to the direction of the fibre with a head speed of 200 mm/min. The amount of force required to shear these cores was recorded in Newton (N).

Statistical analysis

Data were subjected to one and two way analysis of variance using JMP® 5.0.1 programme (Sall et al., 2005). All data were tested for normal distribution before analysis. Significance of the differences between group means was tested by Tukey-Kramer (HSD) test. Values with different superscripts differ significantly (P<0.05) between treatments.

RESULTS

Mortality and culling was 3.5% in groups C and E, while 2% for N and 1.5 % for EN groups, respectively (Table 3). The live body weight (LBW) of broilers at the end of starter phase and BWG during starter phase has shown non significant treatment effects. However, LBW on 35th day and BWG during grower phase and average daily weight gain (ADWG) were nearly significant (P=0.05, P=0.07 and P=0.05, respectively) in EN treated birds. Feed consumption (FC) during starter phase was significantly (P<0.05) higher in EN treated birds as compared to E, but FCR during starter, grower and whole rearing period were not significantly different among the treatment groups.

Table 3. Effects of supplementation of *Echinacea purpurea* and *Nigella sativa* on broiler performance

Parameters	Treatments				Pooled	P value		
	C	E	N	EN	SEM	E	N	E*N
LBW[1] 0, g	46.9	46.7	46.7	46.6	0.159	0.57	0.78	0.73
LBW 14, g	412	405	412	416	2.378	0.66	0.28	0.28
LBW 35, g	2165	2097	2145	2178	12.67	0.50	0.23	0.05
BWG[2] 0-14, g	366	358	366	369	2.387	0.67	0.26	0.30
BWG 15-35, g	1753	1692	1732	1763	12.36	0.55	0.31	0.07
BWG 0-35, g	2118	2050	2098	2132	12.70	0.50	0.23	0.05
ADWG[3], g	60.5	58.6	59.9	60.9	0.363	0.51	0.23	0.05
Mortality, %[4]	3.5	3.5	2	1.5	-	-	-	-
FC[5] 0-14, g	458^{ab}	453^{b}	456^{ab}	463^{a}	1.587	0.75	0.20	0.049
FC 15-35, g	2982	2903	2966	3002	26.22	0.68	0.43	0.29
FC 0-35, g	3440	3355	3422	3465	26.85	0.70	0.40	0.25
FCR[6] (0-14)	1.26	1.26	1.25	1.26	0.007	0.52	0.56	0.95
FCR (15-35)	1.70	1.72	1.71	1.70	0.011	0.93	0.97	0.56
FCR (0-35)	1.62	1.64	1.63	1.63	0.009	0.84	0.90	0.57

^{a,b,c} means within a same row with different superscripts are significantly different (P<0.05)

[1] LBW - live body weight; [2] BWG - body weight gain; [3] ADWG - average daily weight gain; [4] mortality includes mortality and culling due to leg abnormalities; [5] FC - feed consumption; [6] FCR - feed conversion ratio

Results of slaughter characteristics revealed that carcass yield was significantly (P<0.05) different among treatments (Table 4). Birds supplemented with E have shown significantly (P<0.05) lower carcass yield as compared to control and N treated birds. Relative weight of liver was significantly lower in E group as compared to control. Abdominal fat % was not significantly (P>0.05) different among the treatments. No significant treatment effect was observed on absolute weight of thighs, breast and wing cut-ups; however, breast percentage was significantly (P=0.04) higher in N group as compared to C and E.

Table 4. Effects of supplementation of *Echinacea purpurea* and *Nigella sativa* on slaughter characteristics and meat cut-ups

Parameters	Treatments				Pooled	P value		
	C	E	N	EN	SEM	E	N	E*N
Carcass, % [1]	66.1^{a}	65.0^{b}	66.0^{a}	65.1^{ab}	0.146	0.0012	0.98	0.74
Leaf fat, % [1]	1.01	1.06	1.00	1.01	0.029	0.61	0.60	0.82
Liver, % [1]	2.04^{a}	1.91^{b}	1.99^{ab}	2.00^{ab}	0.018	0.12	0.58	0.047
Thighs, % [2]	29.9	30.4	29.6	29.8	0.166	0.26	0.18	0.67
Breast, % [2]	27.9^{b}	27.9^{b}	29.3^{a}	28.2^{ab}	0.213	0.18	0.04	0.22
Wings, % [2]	10.9	10.9	10.9	10.9	0.042	0.79	0.51	0.92

[a,b,c] means within a same row with different superscripts are significantly different (P<0.05); [1] carcass, leaf fat and liver percentage are relative to live body weight on 35th day; [2] cut up percentages (thighs, breast and wings) are relative to carcass weight

Results of proximate analysis of meat samples (Table 5) indicate that meat samples of birds supplemented with E and N have significantly lower crude protein (P<0.001) and dry matter (P<0.05) as compared to meat

Table 5. Effects of supplementation of *Echinacea purpurea*, *Nigella sativa* and their combined application on physical and chemical parameters of breast meat quality

Parameters	Treatments				Pooled	P value		
	C	E	N	EN	SEM	E	N	E*N
Chemical composition (proximate), %								
dry matter	24.5^{a}	24.1^{b}	24.1^{b}	24.4^{a}	0.031	0.84	0.58	0.035
crude protein	22.4^{a}	21.8^{b}	21.9^{b}	22.5^{a}	0.027	0.89	0.51	<0.001
crude fat	0.78	0.73	0.81	0.70	0.016	0.31	0.99	0.74
crude ash	1.20^{b}	1.20^{b}	1.32^{a}	1.29^{ab}	0.004	0.51	<0.001	0.44
Physical characteristics								
L*	52.3	51.8	51.77	52.6	0.265	0.73	0.89	0.19
a*	-0.28	-0.31	-0.26	-0.56	0.121	0.50	0.64	0.60
b*	6.45	5.73	6.12	6.64	0.154	0.75	0.38	0.05
electrical conductivity, mS/cm	2.46	2.62	2.54	2.52	0.041	0.40	0.93	0.27
grill loss, %	26.0^{a}	24.2^{b}	25.0^{a}	24.8^{a}	0.242	0.045	0.70	0.10
cooking loss, %	23.0^{b}	22.6^{b}	25.0^{a}	24.3^{b}	0.335	0.38	0.007	0.82
shear force (N)	43.0	43.2	40.0	41.2	0.774	0.68	0.12	0.74

[a,b,c] means within a same row with different superscripts are significantly different (P<0.05)

samples of control and EN treated birds. Significantly ($P<0.001$) higher ash percentage was present in meat samples of broilers supplemented with N as compared to C and E treatments. Crude fat percentage in meat samples was not significantly different. Breast meat colour (L*, a* and b*), electrical conductivity and shear force value were not significantly ($P>0.05$) different among the treatments (Table 5). Significantly lower ($P<0.05$) grill losses were observed in E group as compared to other treatments. Cooking losses were significantly ($P<0.05$) higher in N group as compared to birds in E, EN and control groups.

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.

Extracts of EP and NS seeds have a number of pharmacologically active substances (phenolic compounds and alkamides) that are supposed to enhance feed digestion and absorption by stimulating secretion of digestive enzymes leading to better feed utilization and assimilation. Durrani et al. (2007) observed better boiler performance by supplementation of 4% NS seeds, while Guler et al. (2006) and Ziad et al. (2008) observed improved FCR by supplementation of 1 and 1.5 % NS seeds, respectively. In these experiments performance of the birds was less as compared with the performance data obtained during present experiment.

Roth-Maier et al. (2005) observed no significant effect of feeding of EP cobs to broilers for total rearing period, but intermittent application of EP fermented juice through drinking water has shown better performance in terms of ADWG, better health and immunity in broilers (Nasir and Grashorn, 2010). Application of EP through drinking water might be more suitable as compared to in-feed application of EP products, as there are less chances of binding of active sites of pharmacologically active substances and products are easily absorbed after intake.

In present experiment, no significant effect of NS or EP application on BWG, ADWG, and FCR was observed. Combined application of NS and EP has shown a positive effect in terms of better (nearly significant) LBW on 35th day, total WG and ADWG. Obviously, there is a potential of synergism in enhancing broiler performance. The lack of individual effect of the supplements might relate to the composition of basal diets and managemental and husbandry conditions. The diets did not contain high amounts of feed ingredients with low digestibility and were freshly prepared, so that the growth of moulds (aflatoxin) in feed and pathogenic bacteria in intestine may have been limited. Antimicrobial effects of phytogenic compounds may have more impact when the diet contains less digestible ingredients and there is more threat of colonization of pathogenic bacteria in the intestinal tract. Usually it may be expected, that growth promoting effects of feed additives (antibiotic as well as non-antibiotic) become more apparent when birds receive less digestible diets and/or are kept in a less clean environment. It is also known that well-nourished healthy chickens don't respond to antibiotic supplements provided they are housed under clean and disinfected conditions (Lee et al., 2003). In present experiment performance of the birds was at their maximum, leaving no or very little room for improvement. Similarly, no improvement was observed by Botsoglou et al. (2002), by supplementation of phytogenic products to broilers. However, EP fermented juice has been reported to improve health and immunity of the birds by improving serum globulin contents and stabilizing serum creatine kinase activities (reducing the risk of sudden death syndrome) (Nasir and Grashorn, 2010). This shows that phytogenic compounds and their active ingredients have more diverse activities inside the animal body, having effects on different physiological pathways and on immune system.

Reduced mortalities in NS treated groups (N and EN) as compared to control show that NS supplementation has potential to improve health and immunity of the birds and reducing mortality and morbidity. NS supplementation in broiler diets has been reported to strengthen immune system by preventing lipid peroxidation and liver damage (Sogut et al., 2008). In present experiment, reduction in mortality might show that NS seeds possess potential to improve immunity alone as well as in combination with EP. This indicates presence of synergistic effects of active ingredients of two different phytogenic compounds by establishing better intestinal microflora and stimulating non specific immune system by pharmacologically active compounds.

Results of slaughter characteristics show that broilers in treatment group E have significantly ($P<0.05$) lower carcass percentage as compared with control and N, but combined application of EP with NS reduced this effect and maintained carcass percentage. Birds in treatment group N had significantly ($P<0.05$) higher breast percentage, which indicates a probable positive effect of N on protein metabolism.

Meat quality is affected by a number of factors including genetic, environment, feed, slaughtering conditions, processing and storage of meat. Feed additives have been reported to influence physical and chemical characteristics as well as sensory and microbial quality of meat. In present experiment, EN supplementation showed significantly ($P<0.05$) higher crude protein content as compared with either E or N supplementation alone, which might be due to synergistic effects of active ingredients of both compounds leading to better protein metabolism. Gardzielewska et al. (2003) observed no significant effect on broiler meat quality by continuous supplementation of 1% EP herb through feed. Similarly, Koreleski and Świątkiewcz (2007) observed no effect of EP extract (supplemented continuously at the rate of 560 mg/kg feed from 22-42 days of age) on changes in fatty acid composition during frozen storage of broiler meat. NS supplemented group showed significantly higher crude ash contents indicating better availability of minerals by supplementation of NS seeds. Physical parameters of meat quality [colour (L*, a* and b* values), shear force value and electrical conductivity] were not significantly different. Meat samples from treatment group E had significantly ($P<0.05$) lower grill losses, while samples from treatment N showed numerically less (7.62 %) shear force value and significantly higher cooking losses (8.2%) as compared with control. Reduction of shear force value due to inclusion of NS seeds in diets could be due to tenderizing effects of active ingredients of NS. Fat percentage in meat samples of NS treated birds was also numerically higher as compared with control, which might be lost during cooking leading to higher cooking losses as compared with other treatments and control.

CONCLUSIONS

The results of present experiment show that effects of immunostimulating phytogenic compounds like *Echinacea purpurea* (EP) and *Nigella sativa* (NS) are not usually observed, in terms of performance and meat quality, if birds are reared under optimal environmental conditions. But, the combined application of EP and NS has shown some numerically beneficial effects on health and performance of birds.

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