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RESEARCH ARTICLE

The effect of phytogenic feed additives to substitute in-feed antibiotics on growth traits and blood biochemical parameters in broiler chicks challenged with *Salmonella typhimurium*

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Abstract There is a growing concern over the use of antibiotics due to the increased resistance of pathogens in broiler. The present study was designed to find the comparative effect of an antibiotic, and some phytogenic on performance traits, blood biochemical parameters, and antioxidant status during starter phase exposed to *Salmonella typhimurium* challenge. A total of 560-day-old broiler chicks (Ross 308) were randomly allocated to seven treatments (eight replicates). Control (basal diet); T1, infected with *Salmonella enterica* subsp. *typhimurium*; T2, infected + avilamycin at the rate of 0.2 g/kg; T3, infected + essential oil of thymol; T4, infected + phytogenic; T5, infected + anti-Salmonella organic acid; and T6, infected + essential oils of thyme, anise, and other components. Body weight gain and feed conversion ratio (FCR) were significantly ($P < 0.05$) high in the T2 and T5 at the end of the first and the second week and similar to T4. During the second week, European production efficiency factor (EPEF) was also significantly ($P < 0.05$) high in T2, T4 and T5. Blood albumin increased significantly ($P < 0.05$) in birds of T2 in the first week, while during the second week, blood glucose and triglyceride concentration decreased significantly ($P < 0.05$) in T5. Blood ALT concentration decreased significantly ($P < 0.05$) in T6 compared to other treatments in the second week. Total antioxidant capacity (at the end of the second week) and thiobarbituric acid reactive substances (TBARS) did not change significantly. From the results of the present study, it was concluded that different feed additives could be substituted with antibiotics in the feed of broiler exposed to *S. typhimurium* challenge.

Keywords Antibiotic · Broiler · Salmonella · Phytogenic · Essential oil

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Introduction

Salmonellosis is one of the most expensive diseases in broiler (Calenge et al. 2010). Salmonella is also a human food borne pathogen which is transferred from the environment into the chicks and easily carried into the meat in the processing unit. Chicks during the starter phase may be exposed more frequently to the significant level of Salmonella from a number of sources, including hatchery and the broiler house (Wilson et al. 2016). Salmonella colonizes the intestinal tract of chicks can be frequently found during the initial stage of life and there is typically a gradual decline in later stages (Wilson et al. 2016). This disease is transferred to human by the consumption of contaminated meat and causes food poisoning (Calenge et al. 2010; Bajpai et al. 2012). In poultry, several steps have been taken to control the disease, including prophylactic measures, vaccination, and antibiotics (Calenge et al. 2010; Bajpai et al. 2012; Abudabos et al. 2016).

For the last few decades, antimicrobial growth promoters (AGPs) have been successfully used to improve feed efficiency and reduce mortality in broiler. The medication of AGPs has been very useful in controlling Salmonella; however, acquired resistance and meat residues of these antimicrobial agents are one of the major growing concerns (Bajpai et al. 2012; Khan et al. 2016a, b). Therefore, in many countries of the world, the use of AGPs has been banned by the European Union (Kabploy et al. 2016). Consequently, the poultry researchers are searching for the alternative to antibiotics to improve broiler performance and optimize gut health (Wu et al. 2011; Chand et al. 2016). Among possible alternatives, phytogenetic feed additives (PFA) have shown positive effects on health and productivity in broiler (Amad et al. 2011; Raza et al. 2016). It has been reported that the essential oils extracted from herbs and spices can play a significant role in health and performance of birds by stimulation of feed intake, secretion of endogenous enzymes, antioxidant status, and antibacterial effect (Amad et al. 2011; Lee et al. 2015; Kim et al. 2016). It has been suggested that PFA modulate the fluidity and permeability of the cell membrane, resulting in enhanced absorption of nutrients from the gut (Amad et al. 2011). Recently, several studies have documented inconsistent results of PFA in broiler production (Lee et al. 2003; Amad et al. 2011; Abudabos et al. 2013; Lee et al. 2015). Therefore, the objective of the present study was to assess the effect of different phytogenics in comparison with avilamycin in broilers during the starter phase exposed to experimentally induced *Salmonella typhimurium*.

Materials and methods

Experimental design and management of birds

A total of 560-day-old broiler chicks (Ross 308) having similar weight were randomly allocated to seven treatments. Each treatment was further divided into eight replicates having 10 birds per replicate (80 birds/treatment). Upon arrival, the chicks were confirmed for the absence of *Salmonella* spp. infection. The experiment was conducted under controlled conditions where the temperature was gradually reduced from 35 ± 0.5 °C during the first week to 25 ± 0.3 °C in the following week. A standard starter diet (1–14 days) was offered in the mash form as suggested in the recommendation of National Research Council (NRC 1994) given in Table 1. The chicks were randomly distributed to one of the seven treatments as follows: control; T1, infected with *Salmonella enterica* subsp. *typhimurium*; T2, infected + avilamycin at the rate of 0.2 g/kg (Maxus, Viena, Austria); T3, infected + essential oil containing 29 % active components of thymol (CRINA Poultry, Akzo Nobel, Crina S.A., Gland, Switzerland) at the rate of 0.45 g/kg; T4, infected + Sangrovit Extra at the rate of 5 g/kg (Sangrovit®Extra, Phytobiotics, Eltville, Germany), consisting of various nutritional acids and four different alkaloids that are obtained from special plants; T5, infected + organic acid (4 g/kg) acting through four different barriers that prevent *Salmonella* colonization of the intestinal tract (Fysal 4 fit, Netherland); and T6, infected + essential oil (5 g/kg) consisted of essential oils with thyme and anise as leading active ingredients and other include oregano, carvacol, yucca extract, cinnamaldehyde, and some minor herb mixture (Biostrong 510, Delacon, Steyregg, Austria).

Table 1 Composition and calculated nutrient of the experimental diet (%)

Ingredients	Starter
Yellow corn	57.62
Soybean meal	35.24
Corn oil	2.37
Di-calcium phosphate	2.30
Ground limestone	0.83
Choline chloride	0.05
DL-Methionine	0.20
L-Lysine	0.20
Salt	0.46
Threonine	0.11
Vitamins and minerals premix[a]	0.50
Chemical analysis	
ME (kcal/kg)	3000
Crude protein (%)	22.0
Non-phytate P (%)	0.50
Calcium (%)	1.05
Lysine (%)	1.30
Methionine (%)	0.55
Sulfur amino acids (%)	0.90
Threonine (%)	0.95

[a] Vitamin-mineral premix contains in the following per kg: vitamin A, 2,400,000 IU; vitamin D, 1,000,000 IU; vitamin E, 16,000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18,000 mg; selenium, 60 mg; and zinc, 14,000 mg

Challenge inoculum

On day 1, the chicks were infected with *S. enterica* subsp. *typhimurium* (MicroBiologics, St. Cloud, MN, USA) at a dose rate of 3×10^9 CFU/ml orally as described by Abudabos and Al-Mufarrej (2014) confirming the viability of the bacteria before and after the inoculation. Briefly, stored at −80 °C, the bacteria were retrieved and plated twice on a tryptone soy agar for 24 h at 37 °C. A single colony of the bacteria was transferred into sterile prewarmed tryptone soy broth and incubated at 37 °C for 18 h. The challenge inocula were diluted to the adjusted dose.

Performance measurements

Feed intake was calculated for each treatment at the end of the first and second week. Mortality was recorded at the occurrence. Live body weight of the birds was recorded weekly on replicate basis and body weight gain (BWG) was computed. Feed conversion ratio (FCR) was computed for each treatment by using the following formula, FCR = feed intake (g)/weight gain (g).

European production efficiency factor (EPEF) as suggested by Griffin (1979) was determined as follows,

$$\text{EPEF} = \Big(\text{Livability} \times \text{Live weight(kg)} \div (\text{Age in days} \times \text{FCR}) \times 100$$

Biochemical measurements of blood

At the end of the first and second week, two blood samples (3 ml) per replicate were obtained from the wing vein (brachial vein) of the bird and centrifuged at 3000 rpm for 10 min. Serum was transferred into clean plastic tubes and stored at −20 °C. Serum thiobarbituric acid reactive substances (TBARS) and total antioxidant capacity (TAC) were measured by using ELISA kits (Cayman Chemical Company, MI, USA). Glucose, alanine transaminase (ALT), aspartate aminotransferase (AST), total protein, and albumin were measured by enzymatic calorimeteric kits (M di Europa GmbH Wittekamp 30. D-30163 Hannover, Germany).

Statistical analysis

All statistical analysis was performed using the Statistical Analysis System (SAS 2003). The overall level of statistical significance was set at $P < 0.05$. All values were expressed as statistical means ± standard error of the mean (SEM).

Results

Performance traits

Tables 2 and 3 show the results of feed intake, body weight gain, FCR, and EPEF in the first and second week. Body weight gain and FCR were significantly ($P < 0.05$) high in the T2 and T5 in the first and second week but not significantly diffrent from T4. In the second week, the EPEF was also significantly ($P < 0.05$) high in T2, T4 and T5.

Blood biochemical parameters

Table 4 presents the results of treatments during the first week on the blood biochemical parameters in broiler. The result revealed that blood albumin increased significantly ($P < 0.05$) in birds of T2. Blood protein, globulin, glucose, triglyceride, and ALT concentration was not affected significantly ($P > 0.05$) by the treatments. Similarly, in the second week, blood glucose and triglyceride concentration decreased significantly ($P < 0.05$) in T5 as shown in Table 5. Blood ALT concentration decreased significantly ($P < 0.05$) in T6 compared to the other treatments. However, no significant changes ($P > 0.05$) were observed in glucose, total protein, globulin, AST, and ALT concentration between the control and treated birds.

Serum antioxidant status

The antioxidant status in the form of TBARS and TAC is shown in Fig. 1. No significant change ($P > 0.05$) was observed in TBARS (first and second week) and TAC (first week) between the control and experimental treatments.

Table 2 The effect of treatments on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), and European performance efficiency factor (EPEF) of broiler chickens at the end of the first week

Treatments	FI (g)	BWG (g)	FCR	EPEF
Control	141.5	94.8^{ab}	1.492^{ab}	122.60
T1	136.8	89.0^{b}	1.537^{a}	118.64
T2	135.3	101.2^{a}	1.339^{c}	145.49
T3	137.3	90.0^{b}	1.535^{a}	120.64
T4	135.0	95.3^{ab}	1.420^{bc}	135.22
T5	142.8	101.5^{a}	1.410^{bc}	139.22
T6	139.5	92.9^{ab}	1.506^{ab}	125.65
SEM	3.00	3.08	0.036	6.66

Mean values in a column bearing different superscripts differ significantly ($P < 0.05$)

Table 3 The effect of treatments on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), and European performance efficiency factor (EPEF) of broiler chickens at the end of the second week

Treatments	FI (g)	BWG (g)	FCR	EPEF
Control	382.5	269.6^{ab}	1.421^{bc}	201.1^{ab}
T1	389.3	249.5^{c}	1.562^{a}	175.5^{bc}
T2	372.4	274.2^{a}	1.358^{c}	210.8^{a}
T3	397.2	251.9^{bc}	1.577^{a}	171.3^{c}
T4	367.5	264.8^{abc}	1.390^{bc}	206.6^{a}
T5	386.2	273.4^{a}	1.418^{bc}	211.1^{a}
T6	379.6	250.9^{c}	1.513^{ab}	176.8^{bc}
SEM	10.6	6.2	0.046	8.88

Mean values in a column bearing different superscripts differ significantly ($P < 0.05$)

Table 4 Effect of treatments on blood biochemical parameters and liver enzymes of broiler chickens at the end of the first week

Treatment	Glucose (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Triglyceride (mg/dl)	ALT (U/l)	AST (U/l)
Control	239.4	3.09	1.60^{b}	1.5	99.4	7.22	180.8
T1	252.2	2.92	1.62^{b}	1.3	106.2	8.48	160.5
T2	262.6	2.78	2.03^{a}	0.8	110.5	8.00	177.2
T3	273.4	3.02	1.58^{b}	1.4	103.4	8.00	209.5
T4	259.8	2.46	1.81^{ab}	0.7	111.4	6.04	164.8
T5	247.8	3.06	1.66^{b}	1.4	74.5	8.20	224.0
T6	225.4	3.60	1.76^{ab}	1.8	106.6	7.12	204.2
SEM	13.39	0.37	0.098	0.38	15.87	0.96	29.88

Different superscripts in a column differ significantly ($P < 0.05$)

Mortality

There was no significant ($P > 0.05$) effect among all studied treatments regarding the mortality. However, a mortality rate about 3 % was registered in infected treatment (T1) in the entire experimental period (Fig. 2).

Discussion

Due to the ban of antibiotics (as growth promotors) in poultry feed, non-antibiotic feed additives are gaining increasing importance to improve the growth and feed utilization (Abudabos et al. 2015). In the present study, we found that that body weight gain and FCR were similar when birds were treated with antibiotic organic acid (T5) and phytogenic (T4). In addition, the essential oils and phytogenic treatments showed similar performance like in the control treatment, although birds were infected with Salmonella in both treatments. The addition of essential oils and other phytogenics were as effective as an antibiotic in terms of growth performance and feed utilization, suggesting their possible role as potential alternatives. An improved growth performance and feed utilization with essential oils and phytogenics without any bacterial challenge (Buchanan et al. 2008; Amad et al. 2011; Lee et al. 2015; Kim et al. 2016) and with a bacterial challenge (Abudabos et al. 2013) has been reported previously. The improved performance in birds treated with essential oils and phytogenics has been attributed to the mechanism of action through which they exert their positive effect (Kim et al. 2016). A considerable number of studies have documented that herbs, spices, and various plant extracts have digestion-stimulating and antimicrobial effects (Amad et al. 2011), cause stimulation of digestive enzymes, and improve nutrient utilization and absorption process in the intestines (Buchanan et al. 2008; Khan et al. 2012). Sanguinarine is a potent phytogenic with well established biological activities (Lee et al. 2015). Sanguinarine is well known for its anti-inflammatory, antimicrobial, and immune-modulating effects (Zdunczyk et al. 2010). In addition, it influences the gastrointestinal functions such as gut architecture, fermentation process, and motility (Jankowski et al. 2009). An improved growth performance was noticed in the Sanguinarine-treated birds compared to the infected birds and this improvement may be due to the multiple beneficial effects of this compound (Vieira et al. 2008).

Table 5 Effect of treatments on blood parameters and liver enzymes of broiler chickens at the end of the second week

Treatment	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Triglyceride (mg/dl)	ALT (U/l)	AST (U/l)
Control	275.6^{ab}	2.93	1.56	1.37	117.9^{bc}	9.84^{a}	231.1
T1	282.0^{a}	2.52	1.31	1.22	127.4^{b}	8.92^{ab}	185.6
T2	294.4^{a}	2.74	1.67	1.07	146.2^{a}	9.10^{ab}	186.6
T3	255.4^{ab}	2.75	1.51	1.23	126.4^{b}	8.44^{ab}	177.1
T4	237.8^{bc}	2.74	1.43	1.31	115.4^{bcd}	7.06^{bc}	247.1
T5	208.2^{c}	2.64	1.41	1.23	102.5^{d}	8.34^{ab}	254.4
T6	255.8^{ab}	2.75	1.42	1.32	110.6^{cd}	5.50^{c}	164.8
SEM	14.17	0.14	0.086	0.139	5.07	0.88	28.19

Different superscripts in a column differ significantly ($P < 0.05$)

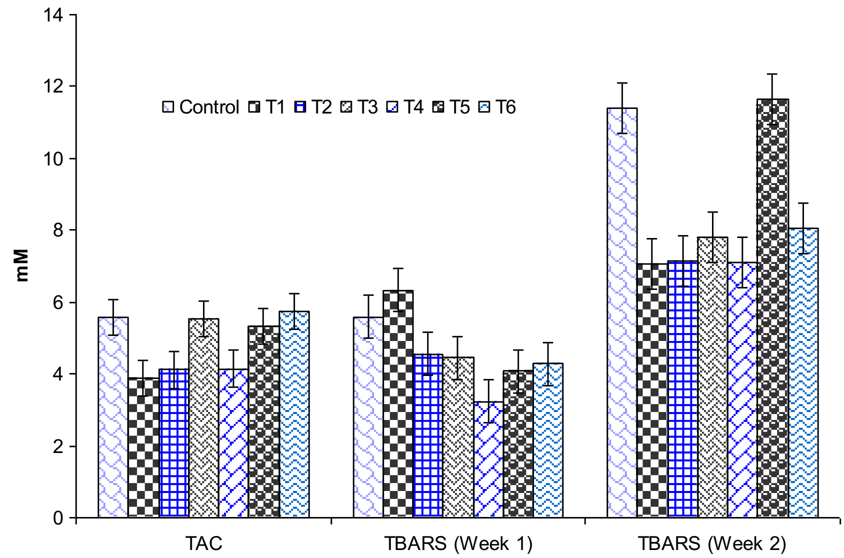

Fig. 1 Effect of treatments on blood total antioxidant capacity (TAC) and thiobarbituric acid reactive substances (TBARS) of broiler chickens at the end of the second week

In the present study, blood albumin concentration increased significantly in T2 at the end of the first week with no significant difference in the other metabolites. A slight increase in protein and albumin level was observed when thyme powder was compared with an antibiotic in broiler feed (Toghyani et al. 2010). Similarly, Amad et al. (2013) also reported significantly increased blood albumin and protein concentration in broilers in response to Biostrong feed additive with no significant change in the blood glucose. In the present study, serum ALT decreased significantly in T6 compared to the other treatments. Serum AST and ALT are a specific indicator of liver damage and dysfunction (Alhidary et al. 2016). During any pathological manifestation, serum AST and ALT are released from the liver to the bloodstream (Toghyani et al. 2011). The reduction of ALT in serum at the end of the second week in response to Biostrong indicates the positive effect on the liver health. No significant effect on AST and ALT level was observed when broilers were treated with Biostrong in a previous study (Kim et al. 2016). In the present study in the second week, blood glucose and triglyceride concentration decreased significantly in T5. Reduced cholesterol and increased high density lipoprotein were reported in broiler challenged with *Clostridium perfringens* in response to Biostrong feed additive (Cho et al. 2014). Triglyceride in broiler fed Biostrong did not change in comparison to the control in the study of Amad et al. (2013). There is much discrepancy in the biochemical parameters in broilers in response to different feed additives in the published literature (Toghyani et al. 2010; Amad et al. 2013; Cho et al. 2014; Kim et al. 2016); the plausible reasons may be due to the differences in the genetic, nutrition, age, and experimental design.

In the current study, TAC and TBARS did not differ between the control and the treated groups. Improved antioxidant status was reported by Giannenas et al. (2014) in response to combined supplementation of benzoic acid and essential oil of thymol in turkey poults. Similarly, Lee et al. (2015) reported reduction in malandialdehyde (MDA) in response to dietary sanguinarine at the level of 20 and 50 ppm. The TAC increased significantly when broiler was supplemented with 0.8 % sweet orange peel (Alzawqari et al.

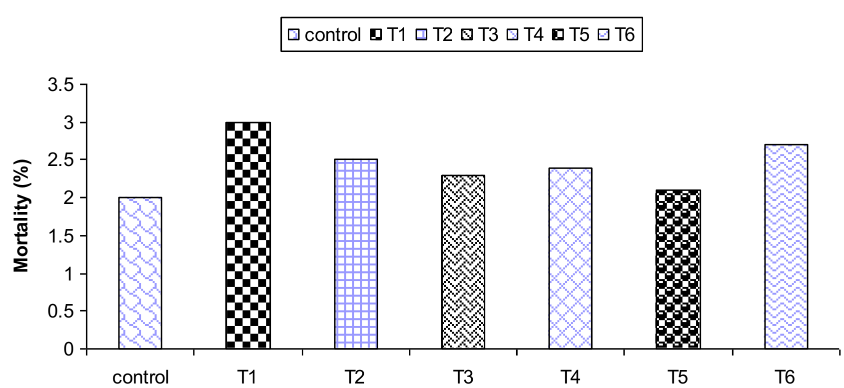

Fig. 2 Effect of treatments on mortality percentage of broiler chickens at the end of the second week

2016). At different concentrations (3.75 and 7.5 mg/kg) of sanguinarine in broiler diet, Bavarsadi et al. (2016) found improvement in antioxidant status in laying hens.

The authors concluded that organic acid and phytogenic could be used successfully in comparison of an antibiotic in maintaining the growth and biochemical profile of broiler challenged with *S. typhimurium*.

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