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Research Article

Effect of Piperine as an Alternative Phytogenic Additive to Antibiotic on Broiler Productivity, Carcass Traits and Oxidative Status

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Abstract:

This study aimed to shed more light on the impacts of piperine on growth performance, carcass characteristics and oxidative status of broilers. A total of 300 one-day-old unsexed (Ross 308 classic FF) broiler chicks were randomly distributed into 4 equal groups. The 1st group (control negative) was fed a diet without any supplementation, the second group (control positive) was fed a basal diet supplemented with neomycin at the level of 200 mg/kg of feed, while the third and fourth groups were fed a basal diet supplemented with piperine at the level of 50 and 100 mg/kg diet, respectively. At the age of 5 weeks, the best values of productive performance represented in body weight and weight gain were achieved in groups fed a diet supplemented with 100 mg piperine/kg of diet. Feed consumption and feed conversion ratio of broilers were not affected by piperine supplementation levels up to 100 mg/kg of feed. The relative weights of carcass, dressing, liver, bursa of Fabricius, thymus, and spleen were significantly ($P \leq 0.01$) increased by increasing the piperine supplementation level up to 100 mg/kg of feed. Additionally, dietary piperine at the level of 100 mg/kg improved the oxidative status; this is evident through a significant increase in the levels of both TAC and SOD and decreasing the amount of MDA in blood plasma. It could be recommended that, a supplemented diet with piperine up to 100mg/kg improved the productive performance traits, carcass characteristics and oxidative status of broiler.

1. Introduction

In 2006, the European Union outlawed utilizing antimicrobial feed additives, such as antibiotics and chemotherapeutic medicines, as growth boosters in cattle and poultry diets (Castanon, 2007). Therefore, a global quest was started for equivalently effective alternatives without detrimental effects on consumer health and animal welfare. Possible natural substitutes for antibiotics as growth promoters in broiler diets include herbal extracts, spices, and some of their constituents (phytogenic additives).

Plants play an important role in the identification of novel medicinal medicines. They have garnered a lot of interest lately for the isolation of biologically active compounds for illness treatment. Plant-based medicinal substances have long been utilized as traditional therapy for a wide range of human ailments in various regions of the world. It is still the principal source of medicine in developing-country rural communities. Traditional remedies are used by around 80% of the inhabitants of poor nations to treat health issues. Biologically active chemicals originating from medicinal plants have been used to develop novel chemical leads for the pharmaceutical sector. Evidence suggests that just 1% of the 500,000 plant species found globally have been phytochemically examined, indicating that medicinal plants have a high potential for identifying novel bioactive chemicals. Phytogenic feed additives are plant-derived materials used in animal feeding to increase the performance of agricultural cattle. They may assuage customers' growing concerns because they are safe and effective. Phytogenics may also help to mitigate the severe environmental issue of bacterial resistance, which is produced by the usage of antibiotics as growth promoter chemicals (Perić et al., 2009). Phytogenics can increase food consumption and conversion, as well as digestibility and weight growth in broiler chickens. However, the mechanism of action of these compounds are yet unknown (Scheuermann et al., 2009). Studies on the use of phytogenics in broiler feeding have yielded inconclusive - either good or negative - effects on broiler performance (Botsoglou et al., 2002; Ertas et al., 2005; Cross et al., 2007; Ocak et al., 2008).

In the poultry business, the usage of phytobiotic compounds in their diets became a widespread practice in order to support high performance by chickens. Useful phytochemicals can be categorized into a number of groups, including phenolics and polyphones (including simple phenols, phenolic acids, quinones, flavones, tannins, and coumarins), terpenoids, essential oils, alkaloids, lectins, and polypeptides. The good impact on feed intake, digestive secretions, immunological stimulation, antibacterial and coccidiostatical, antiviral, or anti-inflammatory activity of botanical supplements in poultry may be the source of these benefits (Cowan, 1999). To distinguish between the plant products used in veterinary medicine (prophylaxis and therapy of diagnosed health problems), Windisch and Kroismayr, (2006) redefined phytobiotic as plant-derived compounds added to feed to enhance the performance of agricultural cattle. In addition to their antimicrobial activity (Dorman and Deans, 2000), phytobiotic compounds exhibit antioxidants activities (Botsoglou et al., 2002) and can stimulate animal digestive systems (Ramakrishna et al., 2003) by increasing digestive enzymes secretion and improving the utilization of digestive products through enhanced liver functions (Hernandez et al., 2004).

In this sense, piperine (1-piperoyl-piperidine) is a primary alkaloid component of black (Piper nigrum Linn.) and long (Piper longum Linn.) pepper, responsible for its strong and biting flavor (Dogra et al., 2004). These pepper species have been used as a condiment in cooking as well as a component in traditional medicine to cure a variety of diseases. Piperine has antibacterial (Reddy et al., 2004), anti-inflammatory (Pradeep and Kuttan, 2004), and antioxidant (Mittal and Gupta, 2000) capabilities among its chemical-biological activities. It also improves the absorption of some medications in the body (Karan et al., 1999) and serves as a chemo-preventive factor against cytochrome P-450-activated pro-carcinogens (Reen et al., 1997).

According to Kohlert et al. (2000), the active components of phytogenic additions are absorbed in the gut by enterocytes and promptly digested by the body,. Piperine alters membrane fluidity and permeation properties, as well as protein production related to cytoskeletal function, increasing of the small intestine absorptive surface (Khajuria et al., 2002). Piperine's fast metabolism and short half-life imply a low danger of accumulating in tissue.

So, the goal of the current study was to shed more light on the effect of piperine as an alternative phytogenic additive to antibiotics on broiler productivity, carcass traits and oxidative status.

2. Materials and Methods

2.1. Experimental Design

2.1.1. Birds and management

Three hundred one-day-old unsexed (Ross 308 classic FF) broiler chicks were randomly divided into 4 experimental groups with three duplicates of twenty-five birds. The first group served as control negative and fed a basal diet without any supplementation, the second group served as control positive and fed a basal diet supplemented with neomycin at the level of 200 mg per kg of feed, while the third and fourth groups were fed a basal diet supplemented with piperine at the level of 50 and 100 mg/kg diet, respectively. Throughout the five weeks of the study, all experimental groups were raised in floor pens and reared under similar managerial and hygienic conditions according to the recommendations of the breed guide used in the study.

2.1.2. Experimental diet

The basal diet was a commercial corn-soybean meal diet formulated to meet or exceed the nutritional requirement of growing chicks as recommended by the manual of (Ross 308) strain, as shown in Table (1). A vertical mixer with a 500-kg capacity was used for the homogenization of the feed treatments, and mixing was done 15 min after the last ingredient was added. Micronutrients were premixed, and the different concentrations of piperine were incorporated to the basal diet instead of a fixed amount of the inert material, without altering the composition of the diet.

Table (1): The composition and calculated analysis of basal diet.

Ingredients	Experimental diets	
	Starter	Grower
Yellow corn	50.48	58.64
Soybean meal (44%)	32.55	30.80
Corn gluten meal (62%)	7.10	2.52
Soybean oil	6.00	4.88
Limestone	1.45	1.30
Dicalcium phosphate	1.69	1.16
Salt	0.30	0.30
Premix*	0.30	0.30
Dl-Methionine	0.10	0.10
L. Lysine	0.03	-
Total	100.00	100.00
Calculated analysis**		
Crude protein (%)	23.01	20.05
ME (Kcal/Kg)	3100	3200
Ether extract (%)	2.40	2.50
Crude fiber (%)	3.50	3.50
Calcium (%)	1.03	0.90
Available phosphorus (%)	0.45	0.35
Methionine (%)	0.50	0.43
Lysine (%)	1.11	1.00

* Each 3kg of premix contained: Vit. A 12000IU, Vit. D 2200IU, Vit. E 10mg, Vit. K_3 2000mg, Vit. B_1 1000mg, Vit. B_2 3000mg, Vit. B_6 1300mg, Vit. B_{12} 10mg, Pantothenic acid 10mg, Niacin 30mg, Folic acid 1000mg, Biotin 50mg, Choline chloride 300mg, Manganese 60mg, Zinc 50mg, Copper 10mg, Iron 30mg, Iodine 1000mg, Selenium 100mg, Cobalt 100mg and $CaCo_3$ to 3g.

** Calculated according to NRC (1994).

2.2. Measurements

2.2.1. Performance traits

Live body weight (LBW), weight gain (WG), feed consumption (FC) and feed conversion ratio (FCR) were evaluated at the first and fifth wks of age, as fellow:

WG = Final LBW – Initial LBW

$$FCR = \frac{\text{Feed consumed (g) during a certain period}}{\text{Body weight gain (g) during the same period}}$$

2.2.2. Carcass characteristics:

At the end of the trial, nine birds from each group (3 birds from each replicate) were randomly chosen for the slaughter test, weighed, and then slain by having their jugular veins severed in the morning. The birds were then scalded and defeated following total bleeding. Liver, heart, gizzard, spleen, bursa, and thymus were individually weighed after the carcasses had been carefully dissected and eviscerated. All organ weights were converted to an arcsine expression and represented as a percentage of body weight.

2.2.3. Oxidative status:

At the end of the trial, nine birds from each treatment were randomly chosen to collect the blood samples into heparinized tubes and centrifuged for 15 min at 5000 rpm to obtain plasma. Plasma samples were examined for levels of superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) using a microplate spectrophotometer at the wave length of (275, 405, and 534 nm), respectively with a commercial detection kit (Bio-diagnostic, Egypt), following the manufacturer's instructions.

2.3. Statistical analysis

Data were statistically analyzed by one-way ANOVA, using the general linear model procedure (SAS, 1996). Tests of significance for differences among treatments were done according to Duncan (1955). The statistical model was used for the analysis of variance to estimate the effect of piperine supplementation levels on broiler performance and physiological status as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = The observations.

μ = Overall mean.

T_i = Effect treatments (i = 1, 2, 3and 4).

e_{ij} = Residual effects (Random error).

3. Results

3.1. Productive performance traits

Data of broiler chick's body weight, weight gain, feed consumption, and feed conversion ratio as influenced by piperine supplementation level are illustrated in Table (2). Piperine supplementation significantly ($P \leq 0.01$) impacted body weight (BW, g) and weight gain (WG, g) at 5 weeks of the experimental period. Chicks fed a diet supplemented with piperine at the level of 100 mg/kg of feed showed the highest BW and WG at 5 weeks of the experimental period followed by those received a diet supplemented with piperine at levels of 50 mg/kg, as compared to both control negative and control positive.

Feed consumption (FC, g) and feed conversion ratio (FCR, g/g) of broilers were not affected by piperine supplementation levels up to 100 mg/kg of feed.

3.2. Carcass characteristics

Data illustrated in Table (3) shows the effect of piperine supplementation levels on the carcass characteristics of broilers. Results indicated that all carcass characteristics were positively ($P \leq 0.01$) affected by piperine supplementation except the relative weights of heart and gizzard. The relative weights of carcass, dressing, liver, bursa of Fabricius, thymus, and spleen were significantly ($P \leq 0.01$) increased by increasing piperine supplementation level up to 100 mg/kg of feed, as compared to the control group. The improvement in the relative weight of lymphoid organs (bursa of Fabricius, thymus, and spleen) indicated that there was an improvement in the immune-response as a result of using piperine in broiler diets up to 100 mg/kg.

3.3. Oxidative status

Data illustrated in Table (4) shows the effect of piperine supplementation levels on the oxidative status of broilers. Results indicated a significant ($P \leqslant 0.01$) improvement in the oxidative status; this is evident through a significant increase in the levels of both TAC and SOD and decreasing the amount of MDA in blood plasma. The amount of TAC was significantly ($P \leqslant 0.01$) increased with increasing levels of piperine from 50 up to 100 mg/kg of feed. Broilers fed a diet supplemented with piperine at the level of 100 mg/kg of feed possessed the highest amount of TAC followed by those received 50 mg/kg by 37.96 and 22.86% respectively, as compared to the control negative group. The same direction was observed for the activity of SOD enzyme; broilers fed a diet supplemented with piperine at the level of 100 mg/kg of feed possessed the highest activity of SOD followed by those received 50 mg/kg by 76.09 and 21.54% respectively, as compared to the control negative group.

Table (2): Productive performance of broilers affected by piperine supplementation levels.

Treatments	Initial BW	BW at 5 weeks	WG at 5 weeks	FC at 5 weeks	FCR at 5 weeks
T_1	41.76	1863.31^{b}	1821.55^{c}	3275.43	1.97
T_2	41.80	1912.15^{ab}	1870.35^{bc}	3362.07	1.79
T_3	41.58	1969.67^{a}	1928.09^{ab}	3452.83	1.79
T_4	41.80	1995.45^{a}	1953.65^{a}	3394.83	1.73
SEM	±0.36	±28.56	±17.08	±38.68	±0.03
significant	NS	**	**	NS	NS

-Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

-NS indicate not significant -**indicates significance at P<0.01 - SEM indicate standard error of the mean

- BW= Body weight - WG= weight gain - FC= Feed consumption - FCR= Feed conversion ratio

Table (3): Carcass characteristics of broilers affected by piperine supplementation levels.

Treatments	% Carcass	% Total giblets			%	% Lymphoid organs		
		Heart	Liver	Gizzard	Dressing	Bursa	Thymus	spleen
T_1	78.43[bc]	0.51	2.68[b]	1.32	82.95[bc]	0.044[d]	0.26[b]	0.14[b]
T_2	76.56[c]	0.52	2.49[ab]	1.34	80.91[c]	0.049[c]	0.26[b]	0.16[ab]
T_3	80.12[ab]	0.54	2.74[ab]	1.36	84.76[ab]	0.052[b]	0.34[a]	0.17[ab]
T_4	82.01[a]	0.55	2.90[a]	1.38	86.93[a]	0.055[a]	0.36[a]	0.18[a]
SEM	±0.81	±0.02	±0.08	±0.05	±1.18	±0.01	±0.02	±0.01
significant	**	NS	**	NS	**	**	**	**

-Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

-NS indicate not significant -**indicates significance at P<0.01 - SEM indicate standard error of the mean

Table (4): Oxidative status of broilers affected by piperine supplementation levels

Treatments	TAC (mM/L)	SOD (U/ml)	MDA (n.mol / l)
T_1	7.35[ab]	17.36[c]	20.81[a]
T_2	5.93[b]	17.05[c]	22.65[a]
T_3	9.03[a]	21.10[b]	11.64[b]
T_4	10.14[a]	30.57[a]	10.95[b]
SEM	±0.87	±28.56	±3.12
significant	**	**	**

- Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

- NS indicate not significant -* indicate significance at P<0.05 - SEM indicate standard error of the mean

- TAC= Total antioxidants capacity - SOD=super oxide dismutase - MDA=Malonaldehyde

4. Discussion

Piperine is known to improve the bioavailability of a wide range of structurally and therapeutically varied medicines, nutrients and phytochemicals (Zutshi et al., 1985 and Bano et al., 1991). Also, it is absorbed relatively fast through the intestinal barrier, according to research. It has non-saturable absorption kinetics, a quick absorption clearance, and a high permeability coefficient (Khajuria et al., 1998). Because piperine is a polar molecule, it is fair to assume that it may affect membrane dynamics due to its facile partitioning in the hydrophobic core and hence aid in solute permeability. Improvement of broiler's body weight and weight gain as a result of piperine treatment was observed by (Ghazalah et al., 2007; Hosseini, 2011; Cardoso et al., 2012; Ghaedi et al., 2014 and Moradi et al., 2016). Additionally, Hosseini, (2011) showed that the most active component in black pepper, piperine, had the ability to increase digestion through arousing digestive liquids in the stomach and eradicating infectious bacteria. Furthermore, piperine affects the absorption power, decreases material transit velocity, increases digestive enzymes, and promotes pancreatic digestive enzymes such as lipase, amylase, and proteases, which play important roles in the digestion process and result in higher body weight and weight gain (Platel, and Srinivasan, 2000 and Al-Kassie et al., 2011).

Also, piperine has the ability to modify the morphology of the small intestine by reducing the inflammatory reactions at the intestinal mucosa which leads to the increase of the villus area and the functions of secretion, digestion, and absorption of nutrients by the mucosa (Miles et al., 2006). Piperine significantly stimulated γ-glutamyl transpeptidase activity, enhanced the uptake of radiolabelled amino acids so the absorptive function of the intestine has been improved. Piperine may interact with the lipid environment to produce effects leading to increased permeability of the intestinal cells (Johri et al., 1992). All of these factors play together to improve productive performance as well as improve relative weights of carcass and dressing.

The improvement in carcass characteristics (carcass and dressing percentage) may be attributed to the improvement in the growth rate that occurred as a result of the use of piperine in broiler feeding. Our results of carcass traits are compatible with that observed by (Ghazalah et al., 2007; Hosseini, 2011; Al-Kassie et al., 2011; Cardoso et al., 2012; Ghaedi et al., 2014 and Moradi et al., 2016).

Free radicals are created in the body as by-products of regular metabolism or as a result of radiation and some environmental contaminants exposure. The body's sensitivity to free radical-mediated damage is related to the balance between pro-oxidant load and antioxidant defense sufficiency (Young and Woodside, 2001). It may be possible to reduce the damage by raising the antioxidant content in tissues (Beckman and Ames, 1998). Many studies have indicated that various spice principles form an important group as antioxidants. Piperine has been demonstrated in in vitro experiments to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species and inhibit lipid peroxidation. Piperine was found to act as a hydroxyl radical scavenger at low concentrations, but at higher concentrations, it activated the Fenton reaction resulting in increased generation of hydroxyl radicals (Mittal and Gupta, 2000; Krishnakantha and Lokesh, 1993). Piperine suppresses the accumulation of lipid peroxidation products, enhances the activity of antioxidant enzymes and eliminates the accumulation and activation of polymorphonuclear cell. Selvendiran et al. (2004) investigated the impact of piperine on alterations of mitochondrial antioxidant system and lipid peroxidation. Oral supplementation of piperine (50 mg/kg body weight) effectively decreases the extent of mitochondrial lipid peroxidation and concomitant increase in the activities of enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidant (reduced glutathione, vitamin E, and vitamin C) levels. This suggests that piperine may extend its chemopreventive effect by modulating lipid peroxidation and augmenting the antioxidant defense system.

5. Conclusions

In conclusion, it could be recommended that, supplemented diet with piperine up to the level of 100 mg/kg of feed is used to improve productive performance traits and oxidative status broiler.

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