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Research Article

Effect of Betaine Supplementation on Growth Performance, Carcass Characteristics and Antioxidants Status of Broilers

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Abstract:

This study aimed to assess the impacts of betaine supplementation on growth performance, carcass characteristics, and oxidative status of broilers. A total of 240 one-day-old unsexed (Ross 308 classic FF) broiler chicks were randomly distributed into 4 equal groups (60 birds into 3 replicates/group). The 1st group (control) was fed a diet without any betaine, while betaine was added at levels of 1, 2, and 3 g per kg of feed, respectively at the 2nd, 3^{ed} and 4th groups. At the age of 5 wks, the best values of productive performance represented in body weight, weight gain, feed consumption and feed conversion ratio were achieved in groups fed a diet supplemented with 1 and 2 g betaine/kg of diet. No significant ($P>0.05$) differences between all treatments for carcass characteristics, except for the relative weight of both thymus and bursa which were increased by increasing the betaine supplementation level from 1 up to 3 g/kg of feed. A significant ($P\leqslant0.01$) improvement was achieved in antioxidant status; especially in the levels of both TAC and SOD enzymes while, decreasing the amount of MDA in blood plasma. It could be recommended that, supplementation of betaine in broiler diet at the level of 2g/kg diet affected positively productive performance and antioxidant activity during the growing period.

1. Introduction

The poultry industry, especially the broiler chicken sector, plays a vital role in the Arab Republic of Egypt because it provides a very large amount of animal protein in a very short time with high conversion efficiency. According to United Nations forecasts, there will be an exponential increase in human population by the year 2067 (United Nations, 2013, 2017) placing tremendous pressure on the food industry in general and poultry production in particular. It increases competition between humans and animals for food. As a result of this increasing demand, intensive farming methods have been introduced into broilers production to ameliorate productivity. However, this intensive production may bring some negative effects on broilers, such as decreased meat quality, poor welfare and flavor (Petracci et al., 2015). In addition to that, the challenge resulting from the problem of heat stress in the current century comes due to the high ambient temperature, which persists for almost from May to September in most of the agro-ecological zones of developing countries like our region (Saeed, 2017). While, those birds neither have sweat glands, and so avian species use non-evaporative cooling like radiation, convection, panting (Which affects the muscle water-holding, which would change the characteristics of the quality of the carcass) and conduction for heat dissipation. If panting fails to prevent the rising of body temperature, birds become listless, then comatose, and soon die due to respiratory, circulatory, or electrolyte imbalance disorders (Pereira et al. 2010 and Saif et al. 2013).

The corresponding contradiction is that consumers demand better quality and taste meat products with ameliorative living standards. So, a variety of nutritional strategies and various studies have been tried by using different herbal plants and their products to overcome these challenges and improve meat quality of broilers, Among these substances used was betaine which receives considerable attention due to its nutritional and physiological functions (Attia et al., 2009; Attia et al., 2016 and Dong et al., 2020). In terms of nutrition, delivering a well-balanced diet that meets all nutritional requirements is the foundation of successful production (Uniyal et al., 2017). Feed additives may be used to mitigate the effects of various nutritional abnormalities such as accumulation of fat in broiler carcasses, particularly in abdominal and visceral areas, which represents a waste product to consumers who are increasingly concerned about the nutritional and health aspects of their food.

Betaine, as a methyl donor, plays a vital role in regulating the osmotic balance, nutrient metabolism, and antioxidant capacity of broilers (Alirezaei et al., 2012; Eklund et al., 2005 and Attia et al., 2018). Also, betaine (plant extract or a by-product of the agricultural industry) can reduce the negative effects of heat stress (Saeed, 2017). Common sources of betaine are sugar beets and their by-products, such as molasses. Nevertheless, betaine is used as a feed additive in chemically purified form and the most popular forms are anhydrous betaine and hydrochloride betaine. Due to different molecular structures, anhydrous betaine shows higher solubility in water when compared with hydrochloride betaine, thereby increasing its osmotic capacity. Hydrochloride betaine induces the pH decline in the stomach, thereby potentially affecting harmful bacteria and nutrient digestibility in a mode different from anhydrous betaine (Eklund et al., 2005). It had been reported that betaine had positive effects on meat quality by improving muscle water-holding capacity (Matthews et al., 2001; Attia et al., 2009 and Dong et al., 2020). As an important organic osmolyte, betaine can protect the cell against dehydration and increase water retention to maintain water balance (Eklund et al., 2005; Hoffman et al., 2009). Recently, with the economic developments that the whole world is witnessing, especially after the Russian-Ukrainian war and its consequences of the lack of feed raw materials, which negatively affected the development of poultry production in most countries of the world, especially developing countries that import most of their needs of feed raw materials, such as Egypt. It was necessary for these countries, in the wake of these global changes, to search for available alternatives that could be used safely to solve this crisis. So, the goal of the current study was to evaluate the effect of betaine as a feed supplement affected broiler productivity, carcass traits and antioxidant status.

2. Materials and Methods

2.1. Experimental Design

2.1.1. Birds and management

Two hundred and forty one-day-old unsexed (Ross 308 classic FF) broiler chicks were randomly divided into 4 experimental groups with three duplicates of twenty birds. The first group served as control and fed a basal diet without any supplementation, while betaine was added at levels of 1, 2, and 3g per kg of feed in the second, third and fourth, groups, respectively. Throughout the five-week 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 broilers as recommended by a manual of (Ross 308 classic FF) strain, as shown in Table (1).

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 1, 3, and 5 wk of age, as fellow:

$$WG = LBW_2 - LBW_1$$

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

2.2.2. Carcass characteristics:

At the end of the trial, nine birds from each group 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. Spleen, bursa, thymus, liver, heart, and gizzard 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. Antioxidants indices:

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), malondialdehyde (MDA), and glutathione (GSH) using a microplate spectrophotometer with a commercial detection kit (Bio-diagnostic, Egypt), following the manufacturer's instructions.

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).

2.3. Statistical analysis

Data were statistically analyzed by one-way ANOVA, using the general linear model procedure (SAS, 1996). Shapiro-Wilk and Levene tests confirmed variance normality and homogeneity. 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 betaine supplementation levels on broilers performance and physiological status as follows:

$Y_{ij} = U + T_i + e_{ij}$
Where:
Y_{ij} = The observations
U = 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 betaine supplementation level (0, 1, 2 and 3 g/kg feed) are illustrated in Table (2). Betaine supplementation significantly impacted body weight (BW, g), weight gain (WG, g), feed consumption (FC, g/), and feed conversion ratio (FCR, g/g) at the end of the experimental period.

Among all groups, chicks fed diets supplemented with betaine at levels of 1 and 2 g/kg feed showed the highest (P≤0.01) BW and WG compared to the control group. The group fed a diet supplemented with betaine at a level of 1 g/kg recorded the highest (P≤0.01) FC values, while chicks fed the basal diets showed the lowest FC value. Betaine supplementation at the level of 2g/kg of diet promotes (P≤0.05) feed conversion ratio compared to the control.

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

Items	Betaine levels (g/kg feed)				SEM	Significant
	0	1	2	3		
Initial body weight	39.70	40.90	39.80	40.60	±0.58	NS
Body weight at 5 weeks	1858.60^{b}	1997.00^{a}	2017.90^{a}	1922.90^{ab}	±34.21	**
Weight gain at 5 weeks	1818.90^{b}	1956.10^{a}	1978.10^{a}	1882.30^{ab}	±34.22	**
Feed consumption at 5 weeks	2727.66^{d}	2978.94^{a}	2847.66^{c}	2934.60^{b}	±27.50	**
Feed conversion ratio at 5 weeks	1.50^{ab}	1.52^{ab}	1.44^{a}	1.56^{b}	±1.16	*

-Means of each raw 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 -** indicate significance at P<0.01

3.2. Carcass characteristics

Data illustrated in Table (3) shows the effect of betaine supplementation levels on carcass characteristics of broilers. Results indicated that there were no significant (P>0.05) differences between all treatments for carcass characteristics, except for the relative weight of both thymus and bursa. The relative weights of both thymus and bursa were significantly increased by increasing the supplementation level of betaine from 1 up to 3g/kg of diet. Broiler received dietary betaine at the level of 3 g/kg and possessed the highest relative weights of thymus and bursa.

3.3. Antioxidant activity

Data illustrated in Table (4) shows the effect of betaine supplementation levels on antioxidants status of broilers. Results indicated a significant (P≤ 0.01) improvement in the antioxidant 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≤0.01)

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

Items	Betaine levels (g/kg feed)				SEM	Significant
	0	1	2	3		
Liver	2.17	2.40	2.39	2.16	0.26	NS
Gizzard	1.25	1.32	1.25	1.38	0.11	NS
Heart	0.48	0.41	0.45	0.53	0.05	NS
Spleen	0.15	0.14	0.14	0.10	0.01	NS
Thymus	0.30^{c}	0.34^{bc}	0.41^{ab}	0.42^{a}	0.02	*
Bursa	0.12^{b}	0.12^{b}	0.13^{ab}	0.14^{a}	0.01	**
Carcass	76.32	78.84	78.45	78.81	1.82	NS
Giblets	3.91	4.13	4.09	4.07	0.32	NS
Dressing	80.23	82.97	82.54	83.03	1.74	NS

-Means of each raw 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 -** indicate significance at P<0.01

increased with increasing level of betaine from 1 up to3 g/kg of feed. Broilers fed diet supplemented with betaine at the level of 3g/kg of feed possessed the highest amount of TAC followed by those received 2g/kg and then those treated by 1g/kg of feed by 31.05, 20.93 and 12.42% respectively, as compared to the control.

Table (4): Antioxidant status of broilers as affected by betaine supplementation levels.

Items	Betaine levels (g/kg feed)				SEM	Significant
	0	1	2	3		
TAC (mM/L)	14.33^{d}	16.11^{c}	17.33^{b}	18.78^{a}	±0.04	**
SOD (U⁄ml)	1.70^{d}	1.97^{c}	2.15^{b}	2.31^{a}	±0.01	**
MDA (n.mol⁄l)	2.65^{a}	2.64^{a}	2.45^{b}	2.32^{c}	±0.01	**

TAC=Total antioxidants capacity; SOD= Super oxide dismutase MDA= Malondialdehyde

The same direction was observed for the activity of SOD enzyme, broilers fed diet supplemented with betaine at the level of 3g/kg of feed possessed the highest activity of SOD followed by those received 2g/kg and then those treated by 1g/kg of feed by 35.88, 26.47 and 15.88% respectively, as compared to the control. On the other hand, the concentration of MDA was significantly ($P \leqslant 0.01$) decreased by increasing betaine supplementation level from 1 up to 2g/kg of feed. Broilers fed diet supplemented with betaine at the level of 3g/kg of feed possessed the lowest content of MDA followed by those received 2g/kg of feed by 12.45 and 7.55 % respectively, as compared to the control

4. Discussion

The poultry industry has been recognized as a fast-developing sector aiming to produce low-cost and high-nutrient foods for human consumption. This industry is always at risk of infectious and non-infectious agents that cause adverse losses. Many potential feed additives have been investigated as growth and health enhancers, immune stimulants, and antimicrobials (Ferdous et al., 2019 and Rafiq et al., 2022). Among different feed additives, phytogenic feed additives have been widely used to boost immunity and relieve stress (Mehdi et al., 2018). Betaine is a by-product of sugar beet processing and is considered one of phytogenic feed additives with great physiological importance (Ratriyanto et al., 2009).

From our results, the supplementation of a poultry diet with betaine can enhance performance parameters, including feed intake, body weight, FCR, and body weight gain in broiler chickens. Betaine's beneficial effect on performance indicators may be related to its osmotic nature, which protects the intestinal epithelia, promotes intestinal cell proliferation, increases cell activity, and improves intestinal morphology and therefore increases nutrient digestibility. (Honarbakhsh et al., 2007[a,b]). Betaine improves the digestibility of ether extract, crude fibre, crude protein, dry matter, and non-nitrogen fibre extract, which can promote the growth of intestinal mucosa, enhancing the absorption and utilization of nutrients (El-Husseiny et al., 2007). Additionally, betaine may boost feed efficiency and weight gain because it acts as a "methyl group donor." (Hassan et al., 2005 and Sayed and Downing, 2011).

Also, at high temperatures, it conserves the energy needed for the Na+ /K+ pump and uses it to power the development of broiler (Remus, 2021). Furthermore, adding betaine to broiler diets increases the availability of sulphur amino acids (Garcia et al., 2000).

Additionally, dietary betaine has been shown to be a potent stimulator of the development and proliferation of the good microbiota that guards the digestive tract (Ratriyanto et al., 2010). The addition of betaine to the diet may enhance the presence of enterococci in broilers while decreasing the overall crop bacterial count (Kettunen et al., 1999). Betaine can also elevate blood electrolytes and total short-chain fatty acid levels (Park and Park, 2017). Some advantageous intestinal bacteria in the gut of chicken, such *Lactobacillus* and *Bifidobacterium*, depend on the short-chain fatty acid, which includes acetic and propionic acids. As a result, it's possible that certain intestinal colonies of dangerous bacteria will be inhibited (Park and Kim., 2017).

The current results are compatible with that observed by (Ahmed et al., 2021; Wladimir and Barba, 2022 and Chen et al., 2022) who found that, body weight, weight gain and feed conversion ratio of broilers received betaine in their diets (1g/kg) or drinking water (105g/l) were improved. Additionally, (El-Shinnawy et al., 2015) concluded that body weight, weight gain and feed conversion ratio of broilers were significantly improved for group-fed dietary betaine at levels (1.0, 2.0, 2.5 and 1.5g/kg), as compared to the control.

Betaine is a carcass modulator because of its potential to lower carcass fat content while increasing muscle yield. The effectiveness of betaine to boost lean development and decrease fat deposition may differ depending on gender, age, and heredity. An increase in muscle yield following betaine administration may be due to its methyl group donor activity, which is required for the synthesis of methionine, cysteine, lecithin, and glycine for protein synthesis (Eklund et al., 2005; Saunderson et al., 1990; McDevitt et al., 2000 and Yang et al., 2016).

Our results of carcass characteristics are compatible with the observation of (El-Shinnawy et al., 2015; Sun et al., 2008 and Yang et al., 2022) who found that the percentages of gizzard, liver and giblets were not affected by dietary betaine. Moreover, (Sakomura et al., 2013 and Konca et al., 2008) found that adding betaine to broiler chickens' diets induced no significant effect on carcass or breast yield and internal organs

Some studies speculate that the antioxidant mechanism of betaine is due to its ability to scavenge free radicals. The antioxidant activity of betaine has only been confirmed in animal or plant models, which suggests that the antioxidant mechanism of betaine may revolve around its interaction with the organism (Zhang et al., 2016).

Our results are compatible with those observed by Alirezaei et al. (2012) who showed that, broiler fed a diet containing 1g/kg betaine had the highest activities of glutathione peroxidase, superoxide dismutase and catalase than broiler fed basal diet. On the other hand, the concentration of malondialdehyde (MDA) was significantly decreased by using 1 and 2g/kg betaine compared to the control (Nutautaite et al., 2020).

5. Conclusions

In conclusion, it could be recommended that, supplementation of betaine in broiler diet at the level of 2g/kg diet affected positively productive performance and antioxidant activity during the growing period.

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