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Effect of dietary BHA supplementation on certain physiological values in broiler chicken

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Abstract

Butylated hydroxyl anisole (BHA) is manufactured from a mixture of two isomeric organic compounds, 2-tertiary-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole, and is widely used in poultry feed as a phenolic free radical scavenger. The current search investigated the impact of different doses of BHA on some hematological and hormonal features of broiler. Fifty-four one-day-old birds were utilized in this study randomly divided into three groups, the control group was given a standard ratio, the 2^{ed} group was given a standard diet filled up with BHA at a rate of 2.5 mg/kg feed, and the 3rd group was given a standard ration fill up with BHA at a rate of 5 mg/kg feed by 18 birds/group, each with three replicates (6 birds/ repeated cycle). The study included three age stages (15, 30, and 45) days. The results showed a significant rise in RBC, WBC, lymphocytes, MCH, SOD, and CAT for the two groups of BHA during 30 and 45 days of treatment compared with the control with a significant improvement in the stress index, as well as a significant decrease in MCV during the three periods of treatment. Supplementation with BHA in both doses caused a significant rise in T3 and T4 during 45 days of the study compared with the control group. We deduce from this research that adding BHA to the poultry diets improved physiological and hormonal characteristics due to its ability to scavenge free radicals. It promotes significant health and growth.

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Introduction

During previous years, different types of food additives were used in poultry rations, including probiotics, organic acids, and others, as (1) indicated that adding the probiotic and emulsifiers to quail diets at a rate of 0.5 mg/kg of feed led to an increase in the level of the FSH and LH, as indicated by (2) that adding organic acids to quail diet led to an elevated in the level of growth hormone. BHA is one of the additives used in poultry diets, but the references to study the physiological importance of BHA. BHA is an industrial antioxidant widely used in animal diets as a free radical scavenger (3). Synthetic antioxidants are manufactured in different ways, and in general, they are phenolic compounds that mainly work to capture free radicals and stop oxidative reactions. Industrial antioxidants are used in various fields, significantly as feed additives in poultry diets, to improve growth and production. BHA is a fat-soluble antioxidant (4), and it is a phenolic preservative similar in composition to vitamin E and is widely used in many industries and feed additives (5,6). It has a vital role in inhibiting the oxidation process of fats by increasing the effectiveness of antioxidants. It was also recorded previously has a remarkable function effect in protecting the liver from oxidative stress when added at a concentration of 0.5 mg/kg to poultry diets (5).

Materials and methods

Animals study

This study was conducted in the College of Veterinary Medicine, Mosul. In this study, 54 rose-bred birds were used

at the age of one day until the end of the experiment to 45 days under appropriate conditions of temperature and light. The birds were fed with two rations, the growth and finisher ration. Ration and water were available freely, according to NRC (7).

Experimental design

Fifty-four birds were utilized in this experiment, randomly partitioned into three groups. Each group included 18 birds/group, and each group was divided into three replicates, six birds/repeat. The birds were divided into three age stages (15, 30, and 45). The study groups included the following; the control group was given a standard diet without the addition. In the second group, a standard diet was given to which BHA was added at a rate of 2.5 mg/kg of feed. In the third group, a standard diet was given to which BHA was added at a rate of 5 mg/kg of feed.

Studied traits

After the end of the experiment, the birds were slaughtered by cutting the jugular vein. The blood samples were collected and divided into two parts. The first part was placed in test tubes containing anticoagulants (heparinized tubes) to conduct blood tests. The blood tests checked for red blood cell count (RBC), white blood cell count (WBC), leukocyte differential count (DLC), stress index (heterophils/lymphocytes), hemoglobin concertation (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concertation (MCHC). According to Campbell (8), the other part was placed in tubes that did not contain anticoagulants and left to clot at room temperature. It was centrifuged at a velocity of 3000 rpm for 15 minutes to impose the serum were kept at -20°C to estimate the level of thyroid hormones using ELISA technique (9), catalase, and superoxide dismutase by using commercial kits.

Statistical analysis

Data was determined using the rate of a one-way analysis of variance (ANOVA). The significance of variations among means was assessed and used the Duncan Multiple Range Test at $P<0.05$ (10).

Results

The results shown in table 1 in the current study indicate a significant rise in the number of WBC in the BHA group supplied with a dose of 5 mg during the 30-45 days of the study. The BHA group with a dose of 2.5 mg during 45 days compared with the control. Treatment with both groups of BHA during the 30-45 days of treatment indicates a significant elevate in the number of lymphocytes compared with the control. There was a significant decrease in the number of heterotrophils of BHA groups during the 45 days of the study compared with the other group. Treatment of the BHA and the control group at a dose of 5 mg during the 45th period of the study and the BHA at the dose of 2.5 mg during the 30 days of treatment decreased the rate of basophils in comparison with the control and the BHA at a dose of 5 mg during the 30 days of the study. As for the results, the number of eosinophil treatments with the BHA and control groups during the periods 30 and 45 days led to a significant rise in their numbers compared to the control during 15 days of the experiment. While results of the BHA and control showed during 30 and 45 days of the experiment indicate a significant improvement in the stress index compared with the control during 15 days of treatment.

Table 1: Effect of BHA on white blood cell count and DLC in broiler after 15, 30 and 45 days of treatments

Groups	Age day	Parameters						
		WBC	Lymphocyte	Heterophil	Monocyte	Basophil	Eosinophil	H/L
Control	15	3.50±0.18b	66.72±3.07c	24.92±0.15a	3.42±0.09a	2.90±0.27b	2.08±0.22a	0.37±0.31a
	30	3.14±0.21b	68.09±2.98c	22.94±0.19^{ab}	3.97±0.16a	3.96±0.92a	1.10±0.09b	0.33±0.27b
	45	3.61±0.20b	72.81±3.10b	20.18±0.24ab	3.89±0.27a	1.59±0.20c	1.55±0.13b	0.27±0.11c
BHA 2.5 mg/kg	15	3.70±0.22ab	67.82±3.10c	24.88±0.17a	3.54±0.21a	2.32±0.20b	1.46±0.11b	0.36±0.29a
	30	3.98±0.27b	71.89±4.01b	22.49±0.17ab	2.68±0.27a	1.82±0.19c	1.25±0.10b	0.31±0.25b
	45	4.12±0.01a	76.21±4.21a	18.72±0.27c	3.30±0.19a	1.42±0.17b	1.01±0.05b	0.24±0.09d
BHA 5 mg/kg	15	3.65±0.13b	66.98±3.11c	25.03±0.29a	2.97±0.10a	3.43±0.90a	1.59±0.13b	0.37±0.31a
	30	4.21±0.07a	73.65±4.74b	21.00±0.20ab	3.12±0.24a	2.01±0.19a	1.27±0.10b	0.28±0.14c
	45	4.35±0.09a	77.82±4.92a	17.89±4.01c	2.99±0.17	1.34±0.15c	1.15±0.09b	0.22±0.04d

Values using various crafts in the pillar are significantly diverse at $P<0.05$.

The data in table 2 shows a significant elevate in the number of RBC for the BHA groups during 45 days of the study compared to the control. The treatment with BHA at a dose of 5 mg in the 30 and 45 days of the study and the BHA group at a dose of 2.5 mg within 45 days to a significant rise in hemoglobin level compared with the control group. The two BHA groups caused a significant elevation in PCV levels during 30 and 45 days compared to the control.

Table 3 referred to a significant reduction in the rate of MCV for the two BHA groups during 15, 30, and 45 days of

the study compared with the control. Treatment with BHA at a dose of 5mg led to a significant increase in the level of MCH during 30 days of the treatment compared with the control and BHA groups at a dose of 2.5 mg, while treatment with BHA at a dose of 5 mg caused a significant rise in the level of MCHC during 30 and 45 days of the study, and the BHA group at a dose of 2.5 mg during 45 days compared with the control.

The data in table 4 denote a significant rise in the level of SOD for the two BHA groups during 30 and 45 days compared with the control. As for the results of CAT, the results indicated a significant increase in the BHA group with a dose of 5 mg within 45 days compared with the control and BHA groups at a rate of 2.5 mg, the BHA group with a dose of 5 mg during the 30 days of the study and the BHA group with a dose of 2.5 mg during the periods of 30 and 45 days indicate a significant elevate in the level of CAT compared with the control. Treatment with the two groups of BHA caused a significant increase in the rate of T3 during 30 and 45 days of the study compared with the control, and the BHA group with a dose of 5 mg illustrate a significant rise in the level of T3 compared to the BHA group at a rate of 2.5 mg during 30 days of the experiment. Treatment with both BHA groups led to a significant rise in T4 levels during the 30 and 45 days of the study compared with the control.

Table 2: Effect of BHA on blood picture in broiler after 15, 30 and 45 days of treatments

Groups	Age /day	Parameters		
		RBCsx10^6/mm^3	Hb mg/100ml	PCV%
Control	15	2.74±0.11b	6.42±0.59b	30.51±0.14b
	30	2.99±0.21b	6.29±0.62b	30.72±0.17b
	45	3.01±0.09b	6.63±0.60b	30.20±0.14b
BHA 2.5mg/kg	15	2.80±0.17b	6.37±0.51b	30.10±0.11b
	30	3.09±0.16b	7.01±0.01ab	32.77±0.22a
	45	3.50±0.19a	7.92±0.09a	32.89±0.21a
BHA 5mg/kg	15	2.97±0.19b	6.77±0.69b	30.81±0.19b
	30	3.11±0.17ab	7.81±0.05a	32.97±0.25a
	45	3.79±0.27a	7.98±0.09a	33.81±0.29a

Values using various crafts in the pillar are significantly diverse at P<0.05.

Table 3: Effect of BHA on some blood parameters in broiler after 15, 30, and 45 days of treatments

Groups	Age / day	Parameters		
		MCV (micron)	MCH (picogram)	MCHC (%)
Control	15	111.34±0.32a	23.45±0.20b	21.09±0.12b
	30	111.37±0.31a	23.40±0.21b	21.07±0.11b
	45	100.45±0.30a	22.11±0.11b	21.99±0.20b
BHA 2.5mg/kg	15	107.61±0.29b	22.81±0.19b	21.19±0.16b
	30	106.11±0.25b	22.70±0.19b	21.41±0.18b
	45	93.99±0.19c	22.60±0.16b	24.01±0.22a
BHA 5mg/kg	15	103.76±0.22b	22.72±0.18b	21.99±0.20b
	30	106.03±0.25b	25.10±0.25a	23.67±0.25a
	45	91.39±0.17c	21.54±0.14b	23.62±0.23a

Values using various crafts in the pillar are significantly diverse at P<0.05.

Discussion

The results obtained noted that BHA led to an improvement in broilers' physiological and blood characteristics. Although it is considered one of the widely used industrial phenolic antioxidants as feed additives in poultry diets (11), scientific research on its essential effects in many physiological traits was not widely recorded. It is noted that the addition of BHA to the broiler diet with different concentrations led to an elevated rate of lymphocytes accompanied by a reduction in the rate of heterophils, eosinophils, and basophils. This effect in the number of blood cells can be attributed to its effect on improving the immune status of the bird, as Lanzhou *et al*. (12) indicated that the addition of BHA to the diets of broilers at the age of 1-21 days at a concentration of 5 and 10 mg/kg of feed led to an improvement in the bird's immune status. Treatment with BHA caused an excess in the number of red and white cells, and this is consistent with what was mentioned by Adeyemi *et al*. (13), where he indicated that adding BHA at a concentration of 0.12 g/kg in broiler diets led to an increase in the numbers of these cells. The effect of

BHA on some hematological characteristics can also be attributed to thyroid hormones. These hormones play an essential role in the progress of lymphoid organs and the arranging of immune assignment in birds and raise the number of lymphocytes, phagocytes, IgM, and IgG (14), as indicated by Keshavarz *et al*. (15) to giving thyroxine at a concentration of 0.3 mg/bird daily to laying hens at the age of 47 weeks for 14 weeks led to an increase in the number of white and red cells, and this was identical to the results of this study. The increase in hemoglobin and packed cell volume is due to a direct relationship with an upraise in the number of RBC. The results also showed a significant reduction in MCV level due to an inverse relationship with an increase in red cells.

Table 4: Influence of BHA on some antioxidant status and thyroid hormone in broiler after 15, 30, and 45 days of treatments

Groups	Age/day	Parameters			
		SOD (nmol/L)	CAT (nmol/L)	T3 (µmol /ml)	T4 (µmol /ml)
Control	15	2.11±1.39c	3.10±1.43c	1.29±1.49c	15.11±2.71d
	30	2.19±1.42c	3.15±1.69c	1.58±1.85c	19.82±1.91c
	45	2.22±1.49c	3.11±1.42c	2.48±2.09b	21.05±2.52b
BHA 2.5mg/kg	15	2.15±1.40c	3.17±1.72c	1.09±1.36c	15.38±2.63d
	30	3.42±1.99b	4.12±2.72b	2.92±2.11b	21.63±2.72b
	45	4.39±2.82a	4.09±2.58b	3.17±2.42a	23.72±3.51a
BHA 5mg/kg	15	2.24±1.52c	3.19±1.89c	1.47±2.05c	16.49±1.70d
	30	4.51±2.87a	4.21±2.63b	3.81±2.64a	22.29±2.80b
	45	4.59±2.98a	5.11±3.01a	3.97±2.82a	25.33±3.72a

Values using various crafts in the pillar are significantly diverse at $P<0.05$.

In contrast, the significant high in the level of MCHC due to using different concentrations of BHA could be attributed to a direct relationship with the observed increase in the level of hemoglobin (16). The data illustrate a significant rise in antioxidants represented by an increase in SOD and CAT in the serum of broilers. This increase can be attributed to its effect on improving the internal environment of the bird, as Kh *et al*. (17) indicated that the gastrointestinal tract is the leading site for taking nutrients from the external environment. It plays an essential role in reducing the oxidative stress produced by food oxidation. This is similar to what Barsha *et al*., Xiaoqing *et al*. (5,18) mentioned. They stated that BHA has a vital role in inhibiting the oxidation process of fats by increasing the effectiveness of antioxidants and has a significant role in protecting the liver from oxidative stress and by increasing the level of CAT, SOD, ALT AST, and glutathione and decreasing the level of malondialdehyde when added at a dose of 0.5 mg/kg to the diet of broilers. The results also noted a significant upraise in the level of thyroid hormones, and no study was recorded on the effect of BHA on the level of these hormones. However, it is believed that its role in this increase can be attributed to improving the health of the bird through increasing the level of SOD and CAT, which is one of the first lines of defense in the body (19), where superoxide dismutase works to break down free radicals and convert them to hydrogen peroxide. In contrast, catalase works to convert hydrogen peroxide into water and oxygen (20). Therefore, it is noted through these results that BHA is one of the essential antioxidants that are used as feed additives in poultry diets. It has physiological effects on some hematological and hormonal parameters.

Conclusion

The results obtained conclude that industrial antioxidants like BHA have significant effects in improving broilers' physiological and hormonal characteristics when used as feed additives in poultry diets.

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Conflict of interest

The researcher indicates that there are no conflicts of benefit related to the publication of this study.

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التأثير الغذائي لإضافة البوتيل هيدروكسي الأنيسول في بعض القيم الفسلجية لأفراخ فروج اللحم

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الخلاصة

يعتبر البوتيل هيدروكسي الأنيسول من مضادات الاكسدة المصنعة من خليط من مركبين عضويين ايزوميريين، ٢-ثالثي-بيوتيل-٤-هيدروكسي انيسول و ٣-ثلاثي-بيوتيل-٤-هيدروكسي انيسول يستخدم بشكل واسع النطاق في اعلاف الدواجن كمواد فينولية كاسحة للجذور الحرة. تناولت الدراسة الحالية تاثير مستويات مختلفة من البوتيل هيدروكسي الأنيسول في بعض الصفات الدموية والهرمونية لفروج اللحم. استخدم في هذه الدراسة ٥٤ طائر بعمر يوم قسمت مجاميع الدراسة بشكل عشوائي الى ثلاث مجاميع وهي مجموعة السيطرة اعطيت عليقة قياسية والمجموعة الثانية اعطيت عليقة قياسية مضاف اليها بوتيل هيدروكسي الأنيسول بتركيز ٢,٥ ملغم/كغم علف والمجموعة الثالثة اعطيت عليقة قياسية مضاف اليها البوتيل هيدروكسي الأنيسول بتركيز٥ملغم/كغم علف وبواقع ١٨ طائر/ لكل مجموعة وقسمت كل مجموعة الى ثلاث مكررات بواقع ٦طائر/ مكرر وشملت الدراسة ثلاث مراحل عمرية عند ١٥، ٣٠ و ٤٥ يوم. اظهرت النتائج زيادة معنوية في خلايا الدم البيض، خلايا الدم الحمر، الخلايا اللمفاوية، معدل هيموكلوبين الكرية، سوبر اوكسيد دسميوتيز وانزيم الكاتاليز مع تحسن معنوي في دليل الكرب اضافة الى انخفاض معنوي في معدل حجم الكرية خلال الفترات الثلاثة من المعاملة. تجهيز البوتيل هيدروكسي الأنيسول بجرعتيه ادى الى ارتفاع معنوي في مستوى هرمون الثايروكسين وثلاثي ايوداید الثايرونين خلال فترة ٤٥ يوم من الدراسة مقارنة مع السيطرة. استنتج من هذه الدراسة ان اضافة البوتيل هيدروكسي الأنيسول في غذاء الدواجن يحسن من بعض الصفات الدموية والهرمونية نتيجة لدوره الكاسح للجذور الحرة مما يؤدي الى صحة ونمو أفضل.