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Original Article

Performance, Lymphoid Organ, and Carcass Characteristics of Broiler Chicken as Affected by Feed Supplementation with Vitamin C and Vitamin E

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ABSTRACT

The purpose of this study was to look at the potential impacts of vitamin C, vitamin E, or a combination of the two on growth performance, carcass traits, and lymphoid organs. 300 Cobb male broiler chicks were used in the experiment; they were divided into 4 treatments, each with 5 subgroups of 15 birds. The following are the four treatment diets: Diet 1 (control): 8% sunflower oil (SO); Diet 2: 8% SO + 250 mg/kg vitamin C; Diet 3: 8% SO + 200 mg/kg vitamin E; Diet 4: 8% SO + mix (250 mg/kg vitamin C + 200 mg/kg vitamin E). The addition of vitamins C and E had no impact on the growth's efficacy. Over 42 days, neither vitamin C nor vitamin E significantly affected the body weights (BW) of broilers. There were no differences in body weight gain (BWG) between birds given vitamin C or vitamin E, but the two together boosted BWG significantly (P>0.05). Broilers on vitamin-supplemented diets consumed more feed than the control group did. Feed conversion rates (FCR) were significantly (P>0.05) higher in the vitamin experimental groups than in the control group (2.8, 2.9 versus 1.95). Therefore, the feed consumed was not efficiently utilized by the broiler chickens. However, introducing a blend of vitamins C and E to the meal significantly

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(P>0.05) improved the birds' FCR at a ratio of 1.95 when compared to the vitamin C or E groups. All vitamin supplementation significantly (P>0.05) increased carcass yields in comparison to the control diet. There were no appreciable weight changes between the spleen, liver, and heart (P>0.05). There were no weight differences across the dietary treatments for the spleen, liver, and heart (P>0.05) compared to the control group. When compared to the control diet and other dietary treatments for the C or E vitamins, the weights of the spleen, liver, and heart were larger (P 0.05) in broilers fed a diet enriched with a combination of vitamin C and vitamin E. When compared to the control diet, vitamin dietary interventions had an impact on Fabricius bursa weight (P>0.05). The trial's findings recommend adding vitamins C and E to sunflower oil to maintain animal health and boost their capacity for development. The negative effects of lipid peroxidation and environmental stress factors may be lessened by taking vitamin C and vitamin E supplements together. To identify and quantify the essential antioxidant vitamin for chicken productivity, more research is required.

Keywords: Broiler, Vitamin E, Vitamin C, Growth Performance, Lymphoid Organ, Bursa of Fabricius, Carcass Yield.

INTRODUCTION

Heat stress can cause harmful effects in birds increased lipid peroxidation, decreased vitamin concentrations, and consequently lower growth performance; therefore, production diets containing antioxidant vitamins can decrease the amounts of free radicals, and prevent lipid peroxidation in poultry (Horváth and Babinszky 2018). McDowell LR (2007) reviewed four different mechanisms of antioxidant function, including 1) preventive antioxidants, 2) free radical scavengers, 3) sequestration of elements by chelation, and 4) quenching active oxygen species. It has been demonstrated that unsaturated fats (oils) induce less overall fat deposition in chicken than saturated fats do (Abdulla *et al.*, 2016, Wongsuthavas *et al.*, 2018). Because fat is considered to be a superior source of energy and improves nutritional value by supplying the necessary fatty acids, adding fat to chicken feed also increases productivity. According to research by Fouad *et al.,* (2014), too much-saturated fat causes chickens to gain more belly fat, which may make it more difficult for them to get enough energy and impair their performance and productivity. However, because they are more soluble and digested than saturated fats, unsaturated fats give poultry more affordable energy. A reaction between polyunsaturated fatty acids and oxygen free radical’s damages cell membrane structure. Both in vitro and in vivo studies showed that these antioxidant vitamins generally enhance different aspects of cellular and noncellular immunity (McDowell LR. 2000a). Particularly when added to broiler, unsaturated fat increases the amount of metabolizable energy. As a result, when there is more energy available, the chicken will function better and produce more (Abdulla *et al.*, 2016, Thacker and Petri, 2011). Yegani (2008) asserts that extreme heat increases the risk of death, lowers feed intake, slows body weight gain, and results in inefficient feed usage. A membrane antioxidant known as vitamin E is thought to aid intracellular defense mechanisms. Breaking apart antioxidant chains is one of vitamin E's most crucial functions in protecting the lipids that make up cellular membranes (Halliwell and Gutteridge, 1999). Furthermore, it reduces the fat peroxidation caused by polyunsaturated fatty acids (PUFA) in the membranes (Zhang *et al.*, 2020.). As a result, vitamin E has an important biological function in cell and subcellular organ membranes, first as a lipid antioxidant and secondly as a free radical neutralizer. In addition to its anti-lipid peroxidation function, vitamin E has well-known immune-regulatory effects on humeral and cell-mediated immunity (Niu *et al.,* 2009). Thus, vitamin E decreases the harmful effects of free radicals and stops the oxidation of phospholipids (Zhang *et al.*, 2020.). According to reports (Goni *et al.*, 2007), adding vitamin E to the feed increases the number of muscles, which increases the oxidative stability of the chicken meat while being preserved. Ascorbic acid, often known as vitamin C, is reasonably necessary nutrition for animals. However, it is not a necessary diet for hens since they can synthesize vitamin C from glucose through the biosynthetic pathway (Whitehead *et al.*, 2003; Sahin *et al.*, 2003). Vitamin C has well-known anti-inflammatory and antioxidant effects (El-Senousey *et al.*, 2018). Numerous studies (Rouhier *et al.*, 2008; Verma *et al.*, 2007) have shown that vitamin C has the essential virtue of being a strong, water-soluble antioxidant that decreases oxidative stress and neutralizes reactive oxygen species (ROS). It protects biomembranes from lipid peroxidation by removing peroxyl radicals in the aqueous phase before oxidation (Truong) (Van Hieu *et al.*, 2021). It is therefore beneficial for poultry during times of inflammation, especially when the amount of vitamin generated is below the daily minimum requirement (El-Senousey *et al.*, 2018). In addition, birds may suffer from severe heat stress (National Center for Biotechnology Information, 2021). The active involvement of vitamins C and E in metabolic processes is well known. Vitamin C enhances the antioxidant activity of vitamin E by converting tocopheroxyl radicals to the vitamin's active form (Niki, 1987; Jacob, 1995). In chickens, the immune system is strengthened by vitamins C and E through humoral immunity, antibody production, and macrophage activity (Shojadoost *et al.*, 2021). Vitamin C acts as an antioxidant or oxidant when vitamin E is present, helping to maintain vitamin E levels by reducing vitamin E degradation and so increasing vitamin E's antioxidant effectiveness. Because they interrupt the series of events that lead to lipid oxidation in cell membranes, vitamins C and E are crucial antioxidants in biological systems (McDowell LR, 2000b). Numerous studies have demonstrated the synergistic effects of vitamins C and E (Niki, 1987; Sahin *et al.*, 2002b). The link between vitamins C and E is strong because vitamin C replaces vitamin E to counteract its loss, necessitating the requirement for vitamin E in birds. Van Hieu and colleagues discovered that vitamin C improved poultry performance, particularly in

heat stress conditions. The immune system was also improved with the addition of ascorbic acid to the diet. Vitamin C, in particular, improves immune and inflammatory responses. Numerous studies have shown that supplementing chickens with vitamin E increases their feed intake (FI), body weight (BW), and FCR. (Sahin *et al.*, 2001b; Harsini *et al.*, 2012; Hashizawa *et al.*, 2013; Habibian *et al.*, 2016). Numerous studies have shown that adding more vitamin C to the feed (150–500 mg/kg) enhances broilers' performance under a variety of high-temperature circumstances (Attia *et al.*, 2011; Farooqi *et al.*, 2005; Kutluand Forbes, 1993; Mckee *et al.*, 1997; Sahin *et al.*, 2001a; Sahin *et al.*, 2002a). Vitamin C and vitamin E supplementation enhanced the humoral immune response and performance indices in broiler chicks, according to Shakeri *et al.* (2020). The addition of vitamin E increased the performance and altered the expression of cytokines that may positively influence immune function in broiler chickens (Khatun *et al.*, 2020).
The current study aimed to establish a knowledge base for the chicken sector in Libya by examining the effects of a progressive diet supplemented with vitamins C and E, either separately or jointly as feed additives, on overall performance, carcass attributes, and lymphatic organs (liver, heart, spleen, and Fabricius bursa).

MATERIAL AND METHODS

Management, experimental meals, and birds 300 male Cobb broiler chicks were sold by a commercial hatchery in Tripoli at one day old. The birds were then marked, weighed, and randomly divided into four nutritional groups (treatments). There were five copies of each dietary treatment, each with fifteen birds. The basal meal diet (Table 1) was prepared according to the NRC (1994). The four treatment groups are as follows: Diets 1 (control): 8% SO; Diets 2: 8% SO + 250 mg/kg vitamin C; Diets 3: 8% SO + 200 mg/kg vitamin E; Diets 4: 8% SO + mix (250 mg/kg vitamin C + 200 mg/kg vitamin E). The nutrients DL-a-tocopherol acetate and ascorbic acid were used to supply the vitamins E and C, respectively. Diets were formulated to satisfy the nutritional needs of broiler chickens (NRC 1994). The broilers were raised for six weeks. At seven and fourteen days for infectious bronchitis (IB) and Newcastle disease (ND), respectively, and at twenty-one days for infectious bursal illnesses, they received vaccinations (IBD).

Sampling and Data Collection

Four experimental diets were prepared following the NRC's recommended specifications (2001). The various treatment diets are displayed in Table 1. The birds were given a basal diet ad libitum for the first week after hatching, then treatment diets from days 8 to 42. Weekly records of feed intake (FI) and body weight (BW) were used to compute the birds' body weight gain (BWG) and feed conversion rate (FCR). After an overnight fast on the 43rd day of the experiment, 2 birds were randomly chosen from each replicate and killed to sample the carcass and internal organs (liver, spleen, heart, and bursa of Fabricius). The carcass and various organ weights, including those of the heart, liver, spleen, and Fabricius bursa, were noted. Data from the experiment was calculated and expressed as the mean plus the standard error of measurement for all parameters. When a significant F value for the treatment effect was observed, the findings were statistically analyzed using the general linear model (GLM) process of MINITAB (2015), and the means were compared using the least significant difference method (LSD). The comparison of treatment means was done using the tests at the 0.05 significant levels.

Table 1: Feed composition

Item	%
Corn	60
Soybean	27
Fish meal	6
Vegetable Oil	2
Methionine	0.04
Dicalcium phosphate	2
Salt	1.65
Limestone	1
Premix*	0.3
Determined Analysis	
Moisture	9.5
Crude protein	23.57
Ash	9.77
Ether extract	3.23
Crude fiber	2.67
Nitrogen free extract	50.73
Calcium	1.00
Phosphorus	0.40

*Lohmann Animal Health GmbH& Co. Cuxhaven/ Germany: Each 1 Kg contains: Vitamin A, 4000000 IU; Vitamin D3, 833333IU; Vitamin E,6.666 g; Vitamin K3, 3666 mg; Vitamin B1,666 mg; Vitamin B2, 1666mg; Vitamin B5, 10mg;Vitamin B6, 1000mg; VitaminB12, 5 micro. g.; Vitamin Biotin, 33333 micro. g.; Vitamin Folic acid, 333 mg; Choline chloride, 166 mg; Methionine, 3331333mg; Calcium, 3333 mg; Manganese 33333 mg; Coper, 2500 mg; Cobalt, 33 mg; Iodine, 166 mg; Selenium, 33 mg; Iron, 10mg

RESULTS and DISCUSION

Broiler Performance

Table 2 displays the results of broilers given sunflower oil with vitamin C, vitamin E, or a combination of vitamin C and vitamin E supplements. When compared to broilers fed the control diet during the finisher period, broilers fed sunflower oil supplemented with vitamin C or vitamin E did not significantly differ in body weight or weight gain. The group that got sunflower oil together with a mixture of vitamins C and E experienced significantly greater weight gain (P>0.05) than the control group. The results showed that Broiler chickens fed a diet fortified with the three vitamins C, E, and C + E consumed significantly more feed per day (P>0.05) than those fed the control diet. Furthermore, when compared to broilers fed a control diet, those fed vitamin C and E-enriched diets converted feed at a higher rate (P>0.05). The feed conversion rate between the broilers fed the control diet and the diet enhanced with a combination of vitamins C and E were not substantially different (P>0.05) and was improved over the group fed either vitamin alone.

Table 2: Growth performance of broilers fed diets supplemented with vitamin C, E and a blend of C and E (throughout the experiment; 0-42 days)

Treatments	Final Growth weight (g/d)	Body weight gain (g/d)	Feed Intake (g/d)	Feed/GW
(T1)	1665.23± 73.57^{a}	37.43± 1.84^{a}	73.04± 3.61^{a}	1.95± 0.09^{a}
(T2)	1669.29± 93.12^{a}	38.41± 2.39^{a}	80.12± 4.99b**	2.08± 0.12^{b}
(T3)	1670.32± 118.8^{a}	38.84± 3.09^{a}	81.52± 6.48b**	2.09± 0.16^{b}
(T4)	1678.51± 99.24^{a}	42.52± 2.81^{b**}	83.07± 5.49^{b**}	1.95± 0.13^{a**}

T1 control basal+ sunflower oil, T2 Basal+ sunflower oil +Vit E, T3 basal + sunflower oil +Vit. C, T4 basal+ sunflower oil + (vit. E+ vit C)

Carcass and Lymphoid Characteristics

Review the findings in table 3 to see how the weight of the lymphoid organs (liver, spleen, heart, and carcass) in broiler chickens was affected by nutritional supplements of sunflower oil with the addition of vitamin C, vitamin E, or a combination of vitamins C and E. According to the results, the carcass weight was significantly (P>0.05) higher in the vitamin-added group than in the control group.

Table 3: Effect of feeding broilers diets supplemented with vitamin C, E, and a blend of C and E on Carcass yield and lymphoid organs (throughout the experiment; 0-42 days)

Diets	Spleen	Liver	Heart	Bursa	Carcass
(T1)	1.15± 0.05^{a}	17.50± 0.05^{a}	3.15± 0.05^{a}	2.16 ±0.05^{a}	1293.6± 0.09^{a}
(T2)	1.20± 0.12^{a}	17.43± 0.12^{a}	3.17± 0.12^{a}	3.17 ±0.12^{b}	1339.7± 0.13^{b}
(T3)	1.22± 0.23^{a}	17.45± 0.23^{a}	3.22± 0.23^{a}	3.17 ± 0.24^{b}	1341.7± 0.17^{b}
(T4)	1.30± 0.26^{c**}	20.21± 0.26^{b**}	4.27± 0.26^{b**}	3.18 ± 0.26^{b**}	1467.4± 0.13^{c**}

T1 control basal+ sunflower oil, T2 Basal+ sunflower oil +Vit E, T3 basal + sunflower oil +Vit. C, T4 basal+ sunflower oil + (vit. E+ vit C).

There was a significant difference in carcass yield between the groups that received vitamin C and vitamin E together and the groups that received only vitamin C or vitamin E. Compared to the broiler fed the control group, the weights of the other internal organs (liver, spleen, and heart) did not alter substantially (P>0.05) after adding vitamins. However, the treatment group, which received the vitamin C and E combination, had considerably (P>0.05) larger weights of the spleen, liver, and heart compared to the control diet. There were no differences between the treatment groups that were statistically significant; however, the weight of the Fabricius follicles was significantly (P>0.05) higher in the vitamin-treated diet group.

Vitamin C and E combination showed no appreciable benefit, based on the information that is currently available. However, numerous evaluations have indicated that the current growth performance is erratic (Choct *et al.*, 2004). The information supports Coetzee and Hoffman (2001), which showed that dietary vitamin supplementation did not affect BWG. The results are consistent with those of Coetzee and Hoffman (2001), who observed that the addition of dietary vitamins did not affect BWG. However, when a combination of dietary vitamins C and E was added, BWG considerably increased by 10.03 g, or 13.7%, in comparison to the control group (83.07 and 73.04 g, respectively). When vitamins C and E were combined, weight increased significantly (P>0.05). over 42 days; neither vitamin alone, however, resulted in a statistically significant rise in weight. Due to the vitamin C and E feed supplements and their combination, the daily feed intake increased significantly (p>0.05) during the trial period. This latter discovery is validated by Elgogari *et al.* (2015). Several studies show that adding vitamins to the meal increased the birds' voluntary consumption and appetite (Kutlu and Forbes, 2000; Najafi *et al.*, 2015). Contrarily, Konca *et al.,* (2009) observed that adding vitamin C to broiler feed dramatically decreased daily feed intake. With the addition of vitamins C and E, Lagana *et al.* (2007) also noted a decrease in feed intake. According to several studies, increasing the amount of vitamin E in chicken diets beyond 100 mg/kg can improve feed conversion (Tengerdy and Nockels, 1975; Jackson *et al.*, 1978; Colnago *et al.*, 1984). Despite an increase in feed intake, body weight gain did not decrease. AinBaziz *et al.* (1996) and Geraert *et al.* (1996) speculate that this may be because nutritional supplements have a stronger impact on feed utilization, broiler physiology, and metabolism. With vitamin E supplementation, feed consumption was not significantly impacted (Niu *et al.*, 2009). The cost per kilogram of feed increased after adding vitamins C and E because they increased consumption by 0.13 and 0.14 kg, respectively. This had an impact on the efficiency of the feed conversion rate. The feed conversion rate of broilers fed a diet containing a combination of vitamins C and E, however, was significantly improved (p>0.05) when compared to broilers fed the vitamins separately. When vitamin C and vitamin E were combined and administered to chicken broilers, there was a 12.1%–12.0% increase in feed intake and a corresponding improvement in body weight gain, which led to a decreased feed conversion rate of 1.95. This was in agreement with the study of Khatun *et al.*, (2020). Birds that have a low FCR are considered efficient feeders. The improvement in nutrient digestibility and utilization efficiency may be the cause of vitamin C and E supplementation's beneficial effects on feed conversion rate (Lagana *et al.*, 2007; Sahin *et al.*, 2009). The current investigation generally demonstrated that adding antioxidant vitamins E and C to chicken diets did not enhance growth performance.

This is consistent with the research conducted by Franchini *et al.*, 1988, who stated that no appreciable improvement in the performance of broilers fed a vitamin E-supplemented diet. Although little to no change in growth performance should be anticipated, with poorer performance, vitamin C and vitamin E supplementation may have a greater impact. However, the additive containing only 200 mg of alpha-tocopheryl acetate presented high antioxidant activity (Papageorgiou *et al.*, 2003), and increased immune efficiency (Tengerdy and Nockels, 1975; Jackson *et al.*, 1978; Colnago *et al.*, 1984). However, the results of the current research showed no increase in body weight while feed intake increased when birds received supplementation of vitamins C, E, or a combination of C and E in the diet. The addition of vitamins C and E did not affect body weight gain. Because vitamins C and E protect tissues from oxidation, they may aid in the secretion of digestive enzymes, thereby improving nutrient digestion and, as a result, overall performance. In the same direction, dietary supplementation with vitamin C (Sahin *et al.*, 2003; Adebiyi and Makanjuola, 2012) or vitamin E (Guo *et al.*, 2003; Lohakare *et al.*, 2005; Niu *et al.*, 2009) revealed efficient weight gain. However, in contrast, different studies testing the effects of supplementation of vitamin E (Niu *et al.*, 2013) and vitamin C (Marron *et al.*, 2001; Konka *et al.*, 2009) did not reveal any significant effects. It is generally accepted that chickens in good environmental condition -well-fed and kept in clean and disinfected conditions-would not be expected to respond to supplementation or nutritional supplementation to promote growth, such as vitamins. Long chains of polyunsaturated fatty acids are present in used oils like soybean and sunflower oil, and it has been discovered that increased oxidation lowers fatty acid production, preventing the accumulation of belly fat in chicken flesh. Vitamin supplementation lowers the expression of hepatic FASmRNA and ACCmRNA, which reduces fatty acid synthesis and prevents the buildup of belly fat, according to several studies (Goñi, *et al.*, 2007). According to the study's findings, adding vitamins to broiler feed had a significantly better outcome than the control group ($P>0.05$). Comparing the experimental group to the control group, the addition of vitamin C, vitamin E, or a combination of vitamin C and vitamin E resulted in the following increases in carcass weight: 3, 7, and 13.5 percent, respectively. When compared to the group of chickens that only received individual supplements of vitamin C or vitamin E, the increase was noticeably higher in the group fed a combination of vitamin C and vitamin E. An increase in the quantity of fat accumulated in the abdomen region is the likely cause of the carcass' rising weight. The vitamin supplement in the feed may promote the production of proteins and hydroxyproline, which are essential for the development of bone tissue, breast meat, and tissues (Jiao *et al.*, 2010). Chen JM *et al.* (2011) outlined how the rate of fat accumulation and muscle building corresponds to the increase in chicken weight. Because of this, broiler body fat content is determined by the amount of fat in the belly region. Long chains of polyunsaturated fatty acids are present in used oils like soybean and sunflower oil, and it has been discovered that increased oxidation lowers fatty acid production, preventing the accumulation of belly fat in chicken flesh. Vitamin supplementation lowers the expression of hepatic FASmRNA and ACCmRNA, which reduces fatty acid synthesis and prevents the buildup of belly fat, according to several studies (Goñi, *et al.*, 2007) it has been discovered that increased oxidation lowers fatty acid production, preventing the accumulation of belly fat in chicken flesh. Vitamin supplementation lowers the expression of hepatic FASmRNA and ACCmRNA, which reduces fatty acid synthesis and prevents the buildup of belly fat, according to several studies (Goi *et al.*, 2007). The weight of Fabricius follicles increased significantly by 47% in the experiment when vitamins C and E were added, as well as when they were combined, compared to the control group. This observation corroborates Biswas *et al.*'s (2011); Nimse and Pal's (2015) findings that the vitamin was added to a suspension of chicken meat, which changed the size of Fabricius' cyst. However, Rostami *et al.* (2018) discovered that the extra vitamin had no impact on Fabricius follicle size. The study's findings on the lymphoid organs revealed that adding vitamin C or E alone had no significant impact on the weight of the spleen, liver, or heart; however, adding a combination of vitamin C and E had a significant impact ($p>0.05$). El-Gogari *et al.*, (2015) came to the conclusion that vitamin addition to feeding did not affect the weight of the lymphoid organs examined. This observation supports the findings of Abdukalykova and Ruiz (2006), who found that broiler chicken spleen weight was unaffected by Vitamin E supplementation. We observed such conflicting findings regarding the effectiveness of vitamin supplements as growth performance enhancers in all investigations in this field. But dietary oils containing antioxidant vitamins have proven to be essential for preventing the oxidation of fatty acids when added to the feed for chicken broilers. This method enhances the health of the animal. Numerous studies have also shown that antioxidant vitamins should be added to the feed when hens are kept in heated environments during the care and production phases, a condition known as "heat stress."

CONCLUSION

Chicken productivity will often suffer significantly under stressful circumstances. It is simple to include vitamins C and E in poultry diets. There are numerous advantages. It has various advantages, including boosting the immune system and promoting general bird growth. The species of poultry and the goal of poultry production determine the amount of vitamin C or E. The use of vitamins C and E as antibiotic substitutes should be the subject of greater research.

CONFLICT OF INTERESTS

The authors declared no competing interests exist.

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