

Effects of Dicalcium Phosphate (DCP) and Vitamin E on Growth Performance and Hemato-Biochemical Parameters in Broilers

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Abstract

This study was conducted to assess the effect of DCP and vitamin E supplementation on body weight, hematological- (TEC, Hb content, PCV and ESR) and biochemical (AST and ALT) parameters of “Cobb 500” broiler. A total of 20 chicks (14 days old) were reared and randomly divided into four equal groups (n=5). Body weight was measured at each 7 days interval up to the end of the 35 days of experimental period. Group A was considered as control, fed with commercial ration. Group B, C and D were treated with 1 gm DCP per kg feed, 1 ml vitamin E per litre of drinking water, and 1 gm DCP per kg feed plus 1 ml vitamin E per litre of drinking water with commercial ration, respectively from day 14 to day 35. It was observed that DCP and vitamin E supplementation significantly ($p < 0.01$) increased body weight. Moreover, TEC and Hb content increased significantly ($p < 0.01$) in the treated groups as compared to control. Besides, ESR, AST and ALT values decreased significantly ($p < 0.01$) in the treated groups. Therefore, it may be concluded that DCP and vitamin E could be used to improve body weight gain and blood profiles without any detrimental effect to liver and muscle on broilers.

Keywords: broiler, body weight, dicalcium phosphate, vitamin E, hematological parameters, biochemical parameters

1. Introduction

As a part of commercial poultry enterprise, broiler production is growing rapidly in Bangladesh. Broiler production reveals the fact of maximum return from minimum cost. About 6 million people directly or indirectly engaged with poultry production and its other support services (Ansarey, 2012). Poultry can significantly contribute to food security because this is one of the vital sources of high-quality protein, minerals, vitamins and micronutrients. Poultry meat alone contributes approximately 37% of the total meat production and 22-27% animal protein supplied in Bangladesh (Hamid *et al.*, 2017). Therefore, it provides a large part of increasing demand for protein, cash income and employment opportunities through the production and marketing of broiler in Bangladesh (Hamid *et al.*, 2017). Despite these advantages, broiler sector continuously faces the challenge of nutritional deficiencies, which causes mortality and reduction of body growth and body resistance level etc. (Rashid *et al.*, 2015). Nutritional deficiencies are concerned with vitamin and mineral which occur in ordinary ration. The optimum vitamin-mineral premix supplementations are required for poultry and formulating premixes are to be necessary (McDowell, 2000). Vitamin and mineral play an essential role in efficient broiler production. Among the vitamins, vitamin E is essential for the integrity and optimum function of the reproductive, muscular, circulatory, nervous and immune system (Sheffy and Williams, 1981 and Mac Dwell *et al.*, 1996). It is well established that some functions of vitamin E, however, can be fulfilled in part or entirely by traces of selenium or by certain synthetic antioxidants. It also plays a vital role in improving health, by enhancing both cell-mediated and humoral immune functions (Rizvi *et al.*, 2014). Moreover, the antioxidant properties of vitamin E have been investigated regarding its vital role in the prevention of diseases, which occur as a result of protein oxidation and lipid peroxidation via a free radical mechanism (Colombo, 2010; Rizvi *et al.*, 2014). Vitamin E is broadly recognized for its positive effects on the meat quality and immune response of broiler chickens (Gao *et al.*, 2010).

Furthermore, in recent years the importance of certain trace minerals in immune function has become increasingly evident. Selenium, copper, zinc, cobalt and iron have been shown to alter various components of the immune system (Suttle and Jones, 1989). It has been observed that mineralization and body growth were reduced in the

absence of Ca, P, Mg and vitamin D (Hart *et al.*, 1930). Calcium and phosphorus are the principal components of skeleton of broiler. Calcium is concerned in blood clotting and in the control of metabolic rate at which the vital processes are carried out and in nervous control. Phosphorus is needed for the muscular energy, digestion of oils and fats, and making new body cells for growth and reproduction. Calcium and phosphorus are closely concerned with metabolic diseases. The low level of calcium and phosphorus in the body may be associated with either deficiency or impaired ability to mobilize reserved within the body. Besides, their roles as an electrolyte are essential in tissue metabolism clinically as a reference to a pathological condition. These may be involved in osteodystrophy, rickets, pseudo-rickets, osteoporosis, infertility, and other conditions (Driver *et al.*, 2006). These components of the mineral should be checked carefully in the broiler ration. The concentration of calcium and phosphorus might be influenced by many factors like age, sex, status, seasons, and breed.

Many studies have done on the effect of vitamin E on broiler ration (Al-Gamal *et al.*, 2013; Gao *et al.*, 2010; Khan *et al.*, 2011). Watson *et al.*, (2006) determined the effects of phytase on growth performance and intestinal transit time in chicks fed nutritionally adequate diets and diets deficient in Ca and nonphytase. They reported that reduction in dietary Ca and P reduced ($p < 0.01$) average daily gain, average daily feed intake in diets deficient in Ca and P and in the nutritionally adequate diets. They also suggested that chicks fed the nutritionally adequate diet may be due to a faster transit time of feed through the digestive tract, resulting in a higher feed intake and body weight gain. Toss *et al.*, (2003) showed that addition of vitamin E, vitamin C alone or the combination of vitamin E and vitamin C corrected the adverse effects of ochratoxicosis on body weight gain and hematological parameters and during values to about that of the control. Ochratoxin increased serum levels of alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and uric acid, creatinine, triglyceride, very-low-density lipoproteins (VLDL) and free fatty acid concentration in the exposed chickens. They found ration with vitamin E alone or combination with vitamin C improved the case except for serum AP and ALT. However, due to the controversial results regarding vitamin E effects on broiler growth performance (Pompeu *et al.*, 2018; Lu *et al.*, 2014), there is few reliable experimental evidence about the performance of the selected vitamin and mineral on the broiler chickens. Considering all of these issues the present study was carried out to investigate the effect of dicalcium phosphate (DCP) and vitamin E supplementation on growth, hematological parameters (RBC, Hb, PCV and ESR), and on serum biochemical values (calcium, phosphorus, aspartate aminotransferase and alanine aminotransferase) of broiler.

2. Materials and Methods

2.1 Experimental Design

The experiment was conducted to study the effect of DCP and vitamin E on “Cobb 500” broilers from 14 to 35 days of age. A total of twenty broiler chicks ageing 14 days were used. These broilers were randomly divided into four equal groups ($n=5$). The initial body weight of each bird was recorded and caged group wise. One out of four experimental groups was considered as a control group (group A) and was fed commercial ration. Group B was fed with supplementation of DCP (DCP plus) at 1 gm/kg feed; group C was fed with vitamin E supplementation at 1 ml/litre drinking water and group D was supplemented with both DCP at 1 gm/kg feed plus vitamin E at 1 ml/litre drinking water for next 21 days.

2.2 Parameter Measured

The growth performance data (body weight of individual bird) was recorded at 7 days interval at the age of 14, 21, 28 and 35 days old. The physical appearances were observed during the experimental period and at the end of the experimental period the broilers were sacrificed to collect blood samples for hematological (TEC, Hb, ESR and PCV) and biochemical (calcium, phosphorus, AST and ALT) studies. For this purpose, about 3 ml of blood was collected from the wing vein of chicken into sterile glass test tubes and then were centrifuged at 1000 rpm for 15 minutes to have a more clear serum from the blood. The serum was then collected in the screw cap serum vial and stored at -20°C until time of chemical analysis.

2.2.1 Determination of Total Erythrocyte Count (TEC)

The counting and calculation of erythrocytes were performed. For erythrocyte count, dry clean red pipette was dipped into the blood and exactly 0.5 mark blood was drawn into the pipette. Then the tip of the pipette was cleaned by cotton and immediately placed into Hayem's solution and was filled up to 101 marks. The pipette was mixed thoroughly by using an electric shaker for 5 minutes. The cells were counted from the recognized 80 small squares under high power objective (45x) in microscope and was calculated accordingly. The unit was expressed in millions per mm^3 of blood.

2.2.2 Estimation of Haemoglobin (Hb)

0.1N Hydrochloric acid (HCl) was taken in the graduated diluting tube up to 2 gm mark with the help of a dropper. Exactly 0.02 ml of sample blood was added directly into the diluting fluid by Sahli pipette. Distilled water was added drop-by-drop and stirred continuously until the color of the content matches to the standard color of the comparator. The haemoglobin (Hb) was recorded within 10 minutes.

2.2.3 Determination of Packed Cell Volume (PCV)

For calculating packed cell volume, wintrobe's tubes were placed in the centrifuge machine and centrifuged @ 3000 rpm for 30 minutes. Then the hematocrit of PCV was recorded. The percent volume occupied by the hematocrit was calculated by using the following formula:

$$\text{PCV (\%)} = \frac{\text{Height of the red cell column in cm}}{\text{Height of total blood in cm}} \times 100$$

2.2.4 Determination of Erythrocyte Sedimentation Rate (ESR)

The citrated blood was placed into the wintrobe's hematocrit tube by using wintrobe's pipette exactly up to the 0 (zero) mark. Then the filled tubes were placed vertically in the wooden rack. After elapsing one hour the erythrocytes sedimentation rate was recorded from the top of the pipette. The result was expressed in mm in first hour.

2.2.5 Determination of Biochemical Parameters

The biochemical characteristics of blood were determined by using spectrophotometer. A rapid colorimetric method as described by Tsao (1952) was used for the determination of serum calcium, which is based on a colour reaction of the calcium ion. Serum phosphate was determined based on the molybdivanadate method for orthophosphate as described by Kitson and Mellon (1944). The method is based upon the yellow colour formed when an excess of molybdate is added to an acidified solution of orthophosphate and vanadate. This method is less sensitive than the molybdenum blue methods, but is adequate for most biological applications, and has a great advantage of providing a stable solution for colorimetry (Simonsen *et al.*, 1946). Spectrophotometric detection of AST and ALT was used in this study as it is the widely adopted clinical standard method in the serum concentration of AST and ALT determination (Hsueh *et al.*, 2011).

2.3 Statistical Analysis

All data were compiled and tabulated for statistical analysis which was done with the help of MSTAT, statistical analysis software. The comparisons of means among the treatments were made by using Duncan's Multiple Range Test (Steel and Torrie, 1984). The result was presented by mean±SD and P-value of <0.01 considered as significant.

3. Result and Discussions

3.1 Effect of DCP and vitamin E on Body Weight

The body weight of each bird was measured with the help of balance on the age of 14 day (0 day experiment) and sequentially 7 days interval up to the end of the experiment. Body weight of different groups of birds is presented in Figure 1. Body weight on 14 days of age was more or less similar and ranged from 528 gm to 554 gm in four different groups. Data catalogued on days 21, 28 and 35 shows that the body weight increased significantly ($p < 0.01$) in all groups with advancement of age. On the day of 21, the highest body weight recorded in group D (993.6 ± 7.16 gm) and lowest body weight recorded in controlled group A (878 ± 10.72 gm). Similarly, the highest body weights were recorded in treated group D on both 28 days (14th day of the experiment) and 35 days (21th day of experiment). The body weight increased slowly in the control group A (65.28%) in respective day of experiment but increased at a higher rate in the treated groups - B (68.65%), C (67.64%), and D (70.40%) - than control group A. The significant body weight gain in the treated groups might be due to increased digestibility of dry matter, crude protein nitrogen. Gao *et al.*, (2010) and Selim *et al.*, (2013) identified that biological antioxidant, vitamin E, could contribute to improve growth, physiological, and immunological performance in broiler chickens as it has the ability to neutralize free radicals and reduce lipid peroxidation in both the plasma and skeletal muscle. Rashid *et al.*, (2015) shown that vitamin and mineral supplementation increased body weight gain in broiler. Lauzon *et al.*, (2008) determined the effect of vitamin E on growth performance and liver vitamin E concentrations of broilers. They found that increased concentration of vitamin E increases the liver alpha-tocopherol concentrations.

The present experiment result concluded that the highest body weight found in group D indicates the synergistic effect of DCP and vitamin E. This finding contradicts with the reported of Preston *et al.*, (2000) who found that DCP inclusion did not improve performance. This work also differs from the earlier report that weight gain and

feed efficacy were not affected by the excess supplementation of vitamin E or lysine for broiler that fed a corn-soybean diet.

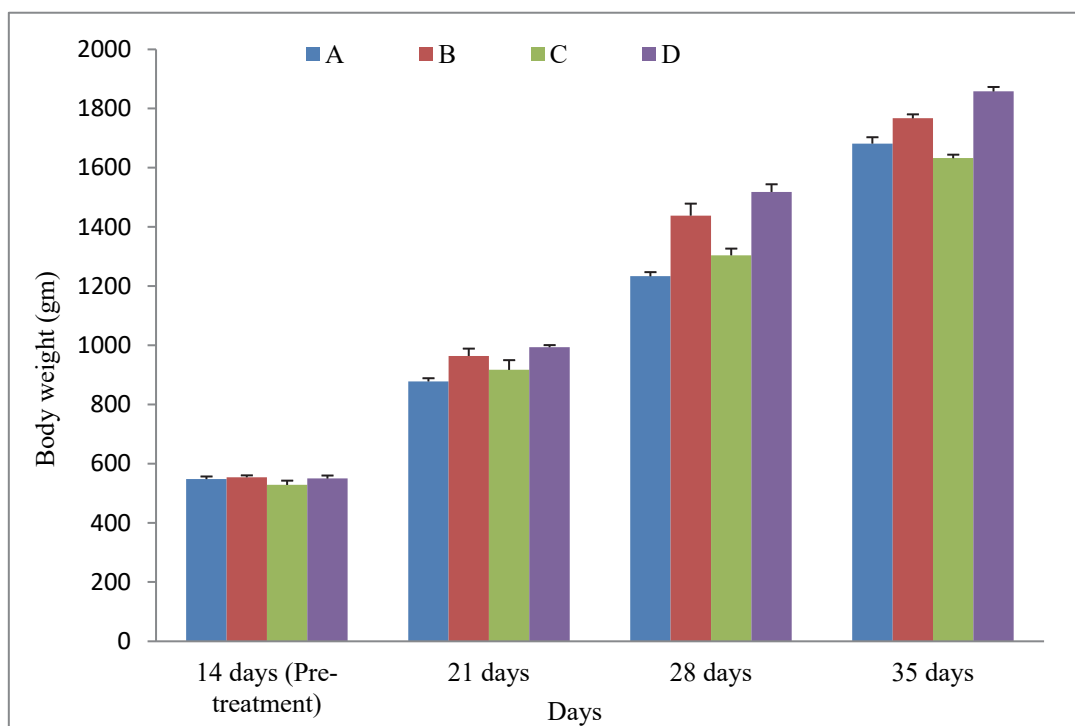


Figure 1. Body weight on different days of treatment with commercial feed (group A), fed with DCP (group B), fed with vitamin E (group C) and fed with both DCP and vitamin E (group D) in broilers (n= 5 in each group). Feed with DCP supplementation at 1 gm/kg, vitamin E at 1 ml/litre of drinking water, DCP and vitamin E at 1 gm/kg feed and 1 ml/litre of drinking water, respectively. Data are expressed as mean \pm SD.

3.2 Effects of DCP and Vitamin E on Hematological Parameters

The effects of DCP and vitamin E on hematological parameters are presented in the table 1. The TEC in control group A was 2.93 ± 0.04 million/mm³, and in the treated group B was 3.54 ± 0.05 million/mm³, group C was 3.44 ± 0.03 million/mm³ and group D was 3.64 ± 0.03 million/mm³. The highest TEC was found in the broiler group of D (3.64 ± 0.03 million/mm³) and lowest in the control group A (2.93 ± 0.04 million/mm³). All the values of treated groups were significantly ($p < 0.01$) higher than the control group A. Similar to TEC, maximum values of HB content and packed cell volume (PCV) value found in group D. In contrast lowest values found in control group A. All the values of treated groups were significantly ($p < 0.01$) higher than the control group A. At final day of experiment ESR value of groups A, B, C, and D were 6.17 ± 0.04 mm, 5.87 ± 0.07 mm, 5.7 ± 0.12 mm and 3.59 ± 0.18 mm in 1st hour, respectively. All the values of treated groups were significantly ($p < 0.01$) decreased than the control group A.

The hematological parameters remained within normal range but differ significantly with the control group. The hematological parameters are unchanged by treatment groups of dietary protein level. The increased level of total erythrocyte count, haemoglobin content and packed cell volume might be due to effects on hematopoietic organs. The hematological parameters in present findings reported that the number of erythrocyte and other components of blood varied due to sex, environment, exercise, nutritional status and climate. Similar results were reported by Abida *et al.*, (2017). However, the present finding differs from the earlier study of (Trans *et al.*, 2000), who observed no significant effect of vitamin E supplementation on any of the hematological parameters (TEC, Hb, PCV and ESR). Besides, decreased ESR value might be due to improved colloidal state by increased level of amino acid supplementation.

Table 1. Hematological parameters in broilers (n=5 in each group) after treating with DCP and vitamin E

Groups	RBC (million/mm ³) (Mean±SD)	Haemoglobin content (gm/dl) (Mean±SD)	Packed Cell Volume (%) (Mean±SD)	ESR mm in 1 st hour (Mean±SD)
A (fed commercial ration)	2.93±0.04 ^d	7.29±0.17 ^d	2.15±0.65 ^c	6.17±0.04 ^d
B (fed DCP @ 1gm/kg feed)	3.54±0.05 ^c	8.81±0.25 ^c	25.49±0.20 ^c	5.87±0.07 ^b
C (fed vitamin E @ 1 ml/L drinking water)	3.44±0.03 ^b	8.6±0.20 ^b	25.3±0.52 ^b	5.7±0.12 ^c
D (fed DCP @ 1gm/kg feed) plus vitamin E @ 1 ml/L drinking water)	3.64±0.03 ^a	8.87±0.31 ^a	26.27±0.57 ^a	3.59±0.18 ^a

Note: ^{a-d} Means with different letters in the same column differ significantly (P<0.01)

3.3 Effect of DCP and Vitamin E on Biochemical Parameters

The effects of DCP and vitamin E on biochemical parameters are presented in Table 2, Figure 2 and Figure 3 which indicate that at final day of experiment (day 35) the serum calcium level was 8.05±0.05 mg/dl in group A (control group). However, in the treated groups the values were 9.70±0.06 mg/dl, 9.82±0.05 mg/dl, 10.43±0.13 mg/dl in group B, group C, and group D, respectively. The highest serum calcium level was found in group D and lowest serum calcium level found in group A. Data available in the treated group were more or less similar but higher than group A. Moreover, at the final day of the experiment (day 35), the serum phosphorus level was found 4.93±0.06 mg/dl in control group A whereas in treated groups the values were 5.75±0.05 mg/dl, 7.05±0.05 mg/dl, 7.34±0.07 mg/dl. The highest serum phosphorus level was found in group D and lowest serum calcium level was found in group A.

Table 2. Biochemical parameters in broilers (n=5 in each group) after treating with DCP & vitamin E

Groups	Calcium(mg/dl) (Mean±SE)	Phosphorus(mg/dl) (Mean±SE)	AST(U/L) (Mean±SE)	ALT(U/L) (Mean±SE)
A (fed commercial ration)	8.05±0.05 ^d	4.93±0.06 ^d	334.494±0.2 ^c	6.28±0.36 ^a
B (fed DCP @ 1gm/kg feed)	9.70±0.06 ^c	5.75±0.05 ^c	311.69±0.3 ^c	4.25±0.20 ^b
C (fed vitamin E @ 1 ml/L drinking water)	9.82±0.05 ^b	7.05±0.05 ^b	305.388±0.5 ^b	4.588±0.09 ^c
D (fed DCP @ 1gm/kg feed) plus vitamin E @ 1 ml/L drinking water)	10.43±0.13 ^a	7.34±0.07 ^a	283.388±0.7 ^c	4.28±0.12 ^d

Note: ^{a-d} Means with different letters in the same column differ significantly (P<0.01)

The present work resembled Hemme *et al.*, (2005) worked on inorganic phosphorus sources monocalcium phosphate (DCP) and defluorinated phosphate diets contained up to 9 gm calcium, 6 gm phosphorus per kg, vitamin E, comparable energy and nutrient contents. In both trials, body weight gain, feed consumption and feed conversion were proved as well as the calcium and phosphorus levels in serum in the balance trial the retention of calcium and phosphorus were determined by calculation as well as by analysis of body composition. On a high-performance level, the addition of DCP and vitamin E resulted in significantly reduced phosphorus availability. The significantly reduced phosphorus level in serum indicates the lower phosphorus retention in broilers. It was interesting that the phosphorus level in the serum markedly reflected the different concentrations of available phosphorus in the diet.

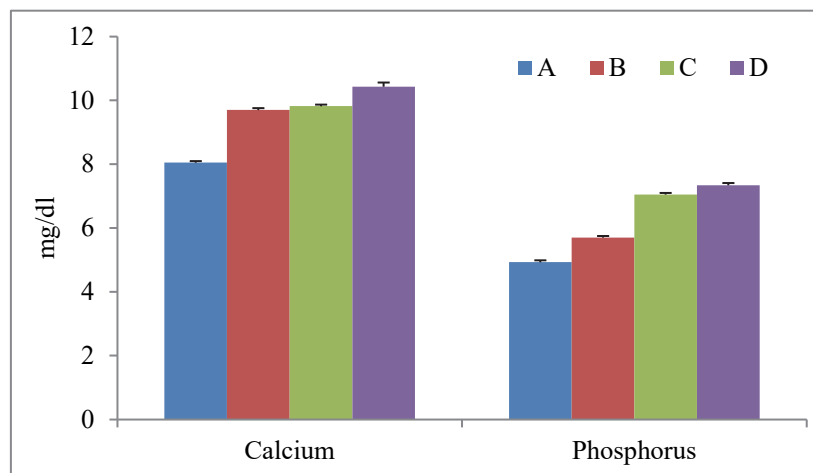


Figure 2. Effects of DCP and vitamin E on serum calcium and phosphorus levels.

Note: Group A was fed commercial ration. Group B was fed with supplementation of DCP (DCP plus) at 1 gm/kg feed; group C was fed with vitamin E supplementation at 1 ml/litre drinking water and group D was supplemented with both DCP at 1 gm/kg feed plus vitamin E at 1 ml/litre drinking water. Data are expressed as mean \pm SD.

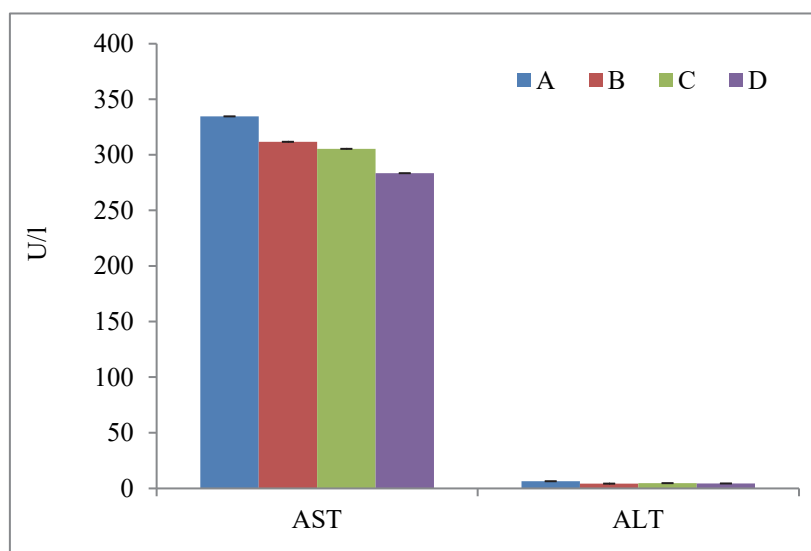


Figure 3. Effects of DCP and vitamin E on AST and ALT concentration levels.

Note: Group A was fed commercial ration. Group B was fed with supplementation of DCP (DCP plus) at 1 gm/kg feed; group C was fed with vitamin E supplementation at 1 ml/litre drinking water and group D was supplemented with both DCP at 1 gm/kg feed plus vitamin E at 1 ml/litre drinking water. Data are expressed as mean \pm SD.

Aspartate aminotransferase (AST) concentration

The AST concentration in different groups of birds was presented in Table 2 and Figure 3. At final day of experiment (35 days of age), the AST concentration level was 334.494 ± 0.2 U/L in control group A and in treatment groups the values were 311.69 ± 0.3 U/L in group B, 305.388 ± 0.5 U/L in group C, and 283.388 ± 0.7 U/L in group D respectively. The highest values was found in group A and lowest found in treated group D. It has concluded from the experiment that SGOT/AST concentration was decreased in all treatment groups in comparison to the control group (group A). However, the value of treated group B was not significantly decreased than the control group A. All the values of AST in group C and D were decreased significantly ($p < 0.01$) than the control group A.

Alanine aminotransferase (ALT) concentration

The ALT concentration in different groups of birds is presented in Table 2 and Figure 2. At the final day of the experiment (35 days of age), the concentration level of ALT in control group A was 6.28 ± 0.36 U/L, and in the treatment group B was 4.25 ± 0.20 U/L, group C was 4.588 ± 0.09 U/L and group D was 4.28 ± 0.12 U/L (Table 2 and Figure 3). The highest ALT was in group A, and lowest in group D. All the values of treated groups were significantly ($p < 0.01$) decreased than the control group, A. Among the treated groups B, C and D the ALT values were more or less similar and comparisons among them were found insignificant.

The significantly reduced AST, ALT concentration level, as observed in the present study, implies good health conditions with less muscle damage by increase supplementation of protein.

4. Conclusion

The results of this study demonstrated the effect of DCP and vitamin E supplementation on growth performance, hematological parameters, and serum biochemical values of broilers. It was observed that supplementation enhanced the growth of broilers and the highest growth (body weight) was observed in group D (where feed was supplemented with 1g DCP/kg feed plus 1g vitamin E/L drinking water). Blood parameters like TEC, HB concentration and PCV values increased significantly ($p < 0.01$) in the treated groups (B, C, and D) as compared to the control group A. However, ESR values and biochemical parameters like AST and ALT values were decreased significantly ($p < 0.01$) in the treated groups in comparison to that of the control group. Therefore, it can be concluded that DCP and vitamin E supplementation are essential for rapid muscle development, synthesis of amino acids, proper digestion of feedstuffs, more use of low-quality feed, expected body growth.

Competing interests

The authors declare that they have no competing interests.

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