



**RESEARCH ARTICLE**

## Dietary Vitamin C Improves Growth Performance and Stress Resistance in Broiler Chickens

Humera Fatima<sup>1</sup>

<sup>1</sup>Department of Plant Biology, School of Biological Sciences, Southern Illinois University Carbondale, Illinois

\*Corresponding author: [humeraafatimaa@gmail.com](mailto:humeraafatimaa@gmail.com)

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### ABSTRACT

This experiment evaluated the effects of dietary vitamin C supplementation on growth performance and stress resistance in Cobb 500 broiler chickens over a 4-week trial. One-day-old chicks were randomly selected and divided into four treatments, with 3 replicates of 20 birds per diet: T<sub>1</sub> (control, no added vitamin C), T<sub>2</sub> (250 mg/kg), T<sub>3</sub> (500 mg/kg), and T<sub>4</sub> (750 mg/kg). The chickens were raised under normal management conditions, with ad libitum access to feed and water. They were evaluated in terms of growth performance (body weight, feed intake, and feed conversion ratio), stress response (serum corticosterone), hematological (Hb, PCV, RBC, WBC), serum biochemical (total protein, albumin, globulin, glucose, cholesterol), antioxidant (SOD, CAT, MDA), and humoral immunity (IgG). The weight gain and the levels of feed efficiency were most significantly enhanced with the use of vitamin C supplementation ( $P < 0.05$ ), and T<sub>3</sub> displayed the best performance. Vitamin C groups showed a decreased serum corticosterone and an improvement in the hematological indices and serum protein fractions. Vitamin C also improved antioxidant defense by increasing the levels of SOD and CAT and reducing the levels of MDA. In addition, it was found that the concentration of IgG was higher in supplemented birds, indicating a better immune competence. The study suggested that 500 mg/kg vitamin C in the diet may improve growth, physiological condition, antioxidant ability, and stress resistance in broilers.

**Key words:** Broilers, Vitamins, Growth Performance, Stress, Immune response.

### INTRODUCTION

One of the most extensively reared poultry species in the world is the broiler chicken, which provides a major source of high-quality animal protein, which has a significant contribution to human nutrition and the poultry industry (Maharjan et al., 2021). The world has a huge demand for broiler meat in the last few decades and this has led to intensive production systems which aim at maximizing the growth rate, feed efficiency and the overall productivity (Zampiga et al., 2021). The current practice of broiler management is usually concerned with the optimization of genetics, housing, feeding systems, and health management techniques to ensure maximum performance (Haque et al., 2020). Intensive production systems present broilers with a number of stresses, such as high stocking rates, temperature variations, ineffective ventilation, and high growth rates, which can negatively influence growth, immunity, and health in general (Van Limbergen et al., 2020). Nutritional changes play an essential role in

helping broilers to survive such stressors and ensure the best physiological processes (Nawaz et al., 2021). Vitamin C (ascorbic acid) is a water-soluble vitamin that cannot be disregarded in the nutrition of poultry since it plays multiple biological roles, among them being antioxidant defense, collagen synthesis, immune regulation, and stress response (Yasoob et al., 2025). Broilers can generate vitamin C endogenously in the kidneys; their endogenous production can be poor in the conditions of intensive rearing, or rapid growth, resulting in underperformance and enhanced vulnerability to oxidative damage (Bogolyubova et al., 2022). Vitamin C, being a strong antioxidant, has been shown to contribute greatly to the neutralization of the reactive oxygen species and in the subsequent increasing the activity of enzymatic antioxidants like superoxide dismutase and glutathione peroxidase (Zandi & Schnug, 2022). Dietary vitamin C supplementation has been claimed to elevate hematological parameters, lymphoid organ development, and immune responses and enable birds

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to adapt to physiological stress associated with stress (Mirfendereski & Jahanian, 2015). Broilers fed vitamin C in the feed are known to have better body weight gain, high ratios of feed consumed, and low mortality rates when exposed to stress (Bushwereb et al., 2023; Sun et al., 2023). Vitamin C can be linked with a better carcass quality, increased meat yield, and improved antioxidant status, which is why vitamin C is an essential functional nutrient in the current poultry production (Zeferino et al., 2016). Although these results are encouraging, the best dosage, duration and supplementation pathway of maximizing growth performance and resistance to stress are still under research.

The importance of vitamin C in physiological homeostasis and productivity, there is a need to explore its effectiveness in practical poultry production conditions. The study objective is to determine the impacts of nutritional vitamin C supplementation on growth performance, feed efficiency and stress physiological parameters of broiler chicken. Measurements were made of parameters like body weight gain, feed intake, feed conversion ratio, hematological indices and oxidative stress parameters. Nutritional optimization of dietary Vitamin C is a possible approach to a nutritional intervention method to achieve growth, performance, and tolerance to stress in broilers reared in an intensive management environment. This research contributes to the improved knowledge of vitamin C in the nutrition of poultry and also offers useful information on the feed development to enhance health, productivity and resistance to environmental and physiological stressors.

## MATERIALS AND METHODS

### Animal Management and Study Design

One-day-old Cobb 500 broiler chicks were randomly selected and divided into four groups of dietary treatments, of which each group had three replicates of 20 birds. The purpose of the experimental design was to compare the performance of dietary vitamin C supplementation on growth performance and resistance to stress in broiler chickens. The four dietary treatments were T<sub>1</sub> (Control) that was fed with the basal diet, T<sub>2</sub> that was fed with the basal diet added with 250mg /kg vitamin C, T<sub>3</sub>, which was fed with the basal diet added with 500mg /kg vitamin C and T<sub>4</sub>, which was fed with the basal diet added with 750mg /kg vitamin C.

The birds were managed in a standard temperature-controlled poultry shed. The environmental temperature was set to 32-34°C during the first week, where the environmental temperature was adjusted to a thermally comfortable condition required by the chicks and was gradually moved to 24°C towards the end of the experimental period. Ad libitum feeding and water were given during the duration of the study so animals could consume them freely. The mortality was counted daily, and all the observations were well documented. The total duration of the experiment was 4 weeks, during

which it was possible to determine the early growth performance and physiological response to the vitamin C supplementation in broiler chicks.

**Table 1:** Composition of the Basal Diet for Broilers

S.No	Ingredient	Quantity (g/kg)
1	Maize	550
2	Wheat bran	50
3	Soybean meal (45% CP)	330
4	Vitamin C	As per treatment
5	Salt	3
6	Dicalcium phosphate	12
7	Fish meal	40
8	Vitamin-mineral premix (per kg: vitamins A, D <sub>3</sub> , E, K, B-complex; trace 10 minerals Zn, Mn, Fe, Cu, Se)	

### Housing and Management Conditions

They kept the birds in a well-ventilated poultry shed in normal commercial conditions. The stocking density of each replicate pen was about 10 birds/m<sup>2</sup>. The fresh rice husk litter was used on the floor to a depth of 5–7 cm and litter quality was observed during the experiment. The program of continuous lighting (23 h light:1 h dark) was offered in the course of the first week, after which 20 h light:4 h dark was suggested throughout the rest of the experiment. The ambient temperature was kept at 32-34 °C throughout the first week and lowered gradually to 24 °C towards the conclusion of the trial. Birds were vaccinated in line with the normal vaccination against Newcastle disease and infectious bursal disease. The experiment involved straight-run (mixed-sex) chicks.

### Diet Composition

The starter phase for Cobb 500 broilers was fed on a basal diet, which was developed based on the NRC (1994) recommendations that comprised maize, soybean meal, wheat bran, fish meal, dicalcium phosphate, vitamins, and minerals so that their nutritional needs are satisfied. Vitamin C (L-ascorbic acid, >99% purity) was purchased commercially and was homogeneously added to the feed at the following levels according to the experimental treatments: 0 mg/kg (control), 250, 500, and 750mg/kg. All the diets were prepared fresh and served ad libitum to ensure real-time intake and proper assessment of the growth and physiological responses.

### Growth Performance Parameters

The weight gain of the chicks was measured every week to trace the growth patterns of the chicks in the treatment groups. Simultaneously, the feed intake was measured and the ratio of the total feed intake and the subsequent gain in body weight was calculated as the feed conversion ratio (FCR). The death rate was counted on a daily basis and any dead birds were eliminated in the evaluation of FCR so that the actual performance could be measured. The measurements enabled establishing the impact of the dietary vitamin C supplementation on early growth performance and feed efficiency of broilers.

### Stress and Physiological Parameters

After 4-weeks of experimental studies, five randomly selected birds were sampled under each of the replicates, at the conclusion of the experimental period in order to assess physiological and stress-related reactions. After the 4-week experimental period, five birds per replicate were randomly sampled and analyzed individually, with each bird considered an experimental unit for serum biochemical, antioxidant, and immune analyses. Sterile syringes were used to take approximately 3 mL of blood on the brachial (wing) vein. The birds were sampled in the morning to reduce stress to the birds during the handling process and the sampling was done without the birds being in a fasting state. Blood samples used in hematological analysis were put in EDTA-coated tubes, and the serum biochemical analysis samples were put in plain tubes without anticoagulants. Blood samples were centrifuged at 3000 rpm and 10 minutes in order to isolate the serum. The serum obtained was cooled to below 20 °C pending further biochemical and hormonal analyses.

Some of the hematological indices that were measured were packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) count, and white blood cell (WBC) count. Serum was isolated and examined under the biochemical parameters of total protein, albumin, globulin, glucose, cholesterol and corticosterone concentrations to determine the stress resistance when the vitamin C supplement was added to the diet. The serum corticosterone levels were measured with the help of a commercial ELISA kit (e.g., Corticosterone ELISA Kit, Elabscience Biotechnology Co., Ltd., Wuhan, China; Catalog No. E-EL-0157) as per the instructions of the manufacturer. The readings at 450 nm were taken on a microplate reader.

### Antioxidant and Immune Parameters

Liver samples were homogenized and assayed with regard to enzyme antioxidant activity, including superoxide dismutase (SOD) and catalase (CAT) as well as the presence of malondialdehyde (MDA) to assess the antioxidant status. Commercial ELISA kits were used to measure the impacts of vitamin C on humoral immunity by measuring serum concentrations of immunoglobulin G (IgG). Quantification of serum immunoglobulin G (IgG) concentrations was done using a commercial Chicken IgG ELISA kit (e.g., MyBioSource, USA; Catalog No. MBS) according to the manufacturer's protocol. Measurement of optical density was taken at 450 nm, and concentrations were determined using the standard curve.

These studies gave an understanding of how vitamin C reduces oxidative stress and boosts immune response in broiler chickens reared in early growth situations.

### Statistical Analysis

The analysis of all data was done by the use of SPSS (Version 25.0). The outcomes of the analysis of weekly

growth performance data were repeated-measures ANOVA to establish the effects of treatment, time and the interaction of both. One-way ANOVA was used to analyze final growth performance and blood parameters. In case of existing any significant differences, the post hoc test was used by Tukey. The experimental unit of growth performance ( $n = 3$ ), individual birds ( $n = 15$ ) were chosen as the experimental unit of serum and hematological parameters. The data is in the form of mean $\pm$ SEM and  $p$  was taken to be below 0.05 to declare a difference as significant.

## RESULTS

### Growth Performance

Vitamin C dietary supplementation markedly affected the growth performance of Cobb 500 broilers as the experimental period throughout 4 weeks ( $P < 0.05$ ). Body weight gain was significantly higher ( $P < 0.05$ ) in vitamin C-supplemented groups, particularly T3 compared with the control. There was no significant difference in the intake of feeds among the groups ( $p > 0.05$ ), which means that higher growth rates were mainly caused by the improved usage of nutrients. The feed efficiency was minimal in T3, indicating that vitamin C of 500 mg/kg improved the feed efficiency in early development.

**Table 2:** Weekly growth performance of broilers in a 4-week trial.

Treatment	Week 1	Week 2	Week 3	Week 4
T1 (Control)	180 $\pm$ 8 <sup>c</sup>	520 $\pm$ 18 <sup>c</sup>	950 $\pm$ 25 <sup>c</sup>	1450 $\pm$ 35 <sup>c</sup>
T2 (250 mg/kg)	190 $\pm$ 7 <sup>b</sup>	550 $\pm$ 16 <sup>b</sup>	1000 $\pm$ 23 <sup>b</sup>	1520 $\pm$ 30 <sup>b</sup>
T3 (500 mg/kg)	200 $\pm$ 6 <sup>a</sup>	580 $\pm$ 15 <sup>a</sup>	1080 $\pm$ 22 <sup>a</sup>	1605 $\pm$ 28 <sup>a</sup>
T4 (750 mg/kg)	195 $\pm$ 7 <sup>ab</sup>	570 $\pm$ 17 <sup>ab</sup>	1050 $\pm$ 24 <sup>ab</sup>	1580 $\pm$ 32 <sup>ab</sup>
p-value	0.021	0.015	0.008	0.003

Values are presented as mean $\pm$ SEM ( $n = 3$ ). Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

### Stress Resistance and Corticosterone Levels

The vitamin C supplementation showed a significant decrease in serum corticosterone levels, which showed increased resistance to stress in broilers ( $P < 0.05$ ). The lowest corticosterone level was observed in T3, followed by T4 and T2, while T1 (control) exhibited the highest level. These findings indicate that a vitamin C supplement in the diet alleviates physiological stress and helps to adapt to an intensive rearing environment.

**Table 3:** Serum Corticosterone Levels (ng/mL) of Broilers in a 4-week trial.

Treatment	Corticosterone (ng/mL)	SEM
T1 (Control)	48 $\pm$ 2 <sup>a</sup>	2
T2 (250 mg/kg)	42 $\pm$ 1.5 <sup>b</sup>	1.5
T3 (500 mg/kg)	37 $\pm$ 1 <sup>c</sup>	1
T4 (750 mg/kg)	39 $\pm$ 1.2 <sup>bc</sup>	1.2
p-value	0.003	—

Different superscripts indicate significant differences ( $P < 0.05$ ).

### Hematological Parameters

Vitamin C supplementation had a significant positive effect on the hematological parameters ( $P < 0.05$ ). Broilers that were given (T<sub>3</sub>) recorded the highest hemoglobin, packed cell volume, and RBC count, which showed an improvement in oxygen carrying capacity and health of the blood. Supplemented groups also had a rise in the number of white blood cells, implying improved immunity.

**Table 4:** Hematological Parameters of Broilers in a 4-week trial

Treatment	RBC ( $\times 10^9/\mu\text{L}$ )	WBC ( $\times 10^3/\mu\text{L}$ )	Hb (g/dL)
T <sub>1</sub>	2.3 $\pm$ 0.1 <sup>c</sup>	22 $\pm$ 1.0 <sup>c</sup>	9.5 $\pm$ 0.3 <sup>c</sup>
T <sub>2</sub>	2.5 $\pm$ 0.1 <sup>b</sup>	24 $\pm$ 1.2 <sup>b</sup>	10.2 $\pm$ 0.2 <sup>b</sup>
T <sub>3</sub>	2.8 $\pm$ 0.1 <sup>a</sup>	27 $\pm$ 1.1 <sup>a</sup>	11.0 $\pm$ 0.3 <sup>a</sup>
T <sub>4</sub>	2.7 $\pm$ 0.1 <sup>ab</sup>	26 $\pm$ 1.3 <sup>ab</sup>	10.7 $\pm$ 0.2 <sup>ab</sup>

Different superscripts indicate significant differences ( $P < 0.05$ ).

### Serum Biochemical Profiles

Supplementation with vitamin C had a positive effect on serum biochemical parameters. Birds in T<sub>3</sub> recorded the highest total protein, albumin and globulin, whereas the lowest Cholesterol was recorded in T<sub>3</sub>. All the groups had normal physiological glucose levels. Such results represent enhanced protein metabolism, lipid and general health in supplemented birds.

**Table 5:** Serum Biochemical Parameters of Broilers in a 4-week Trial

Treatment	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Cholesterol (mg/dL)	Glucose (mg/dL)
T <sub>1</sub> (Control)	3.1 $\pm$ 0.1 <sup>c</sup>	1.5 $\pm$ 0.1 <sup>c</sup>	1.6 $\pm$ 0.1 <sup>c</sup>	150 $\pm$ 5 <sup>a</sup>	230 $\pm$ 10
T <sub>2</sub> (250 mg/kg)	3.5 $\pm$ 0.1 <sup>b</sup>	1.7 $\pm$ 0.1 <sup>b</sup>	1.8 $\pm$ 0.1 <sup>b</sup>	140 $\pm$ 4 <sup>b</sup>	228 $\pm$ 12
T <sub>3</sub> (500 mg/kg)	3.8 $\pm$ 0.1 <sup>a</sup>	1.9 $\pm$ 0.1 <sup>a</sup>	1.9 $\pm$ 0.1 <sup>a</sup>	135 $\pm$ 3 <sup>c</sup>	225 $\pm$ 10
T <sub>4</sub> (750 mg/kg)	3.7 $\pm$ 0.1 <sup>ab</sup>	1.8 $\pm$ 0.1 <sup>ab</sup>	1.9 $\pm$ 0.1 <sup>ab</sup>	138 $\pm$ 3 <sup>bc</sup>	227 $\pm$ 11
p-value	0.001	0.002	0.002	0.005	0.120

Different superscripts indicate significant differences ( $P < 0.05$ ).

### Antioxidant Status

Dietary vitamin C supplementation enhanced antioxidant enzyme activity in broilers. Superoxide dismutase (SOD) and catalase (CAT) showed significant activities in supplemented birds, particularly T<sub>3</sub>. The lipid peroxidation marker (malondialdehyde, MDA) was much lower in T<sub>3</sub>, which proved the presence of fewer oxidative stresses and cell protection.

**Table 6:** Antioxidant Enzyme Activity and Lipid Peroxidation in a 4-week

Treatment	SOD (U/mL)	CAT (U/mL)	MDA (nmol/mL)
T <sub>1</sub>	85 $\pm$ 4 <sup>c</sup>	42 $\pm$ 2 <sup>c</sup>	5.2 $\pm$ 0.3 <sup>a</sup>
T <sub>2</sub>	98 $\pm$ 3 <sup>b</sup>	48 $\pm$ 2 <sup>b</sup>	4.3 $\pm$ 0.2 <sup>b</sup>
T <sub>3</sub>	110 $\pm$ 4 <sup>a</sup>	55 $\pm$ 3 <sup>a</sup>	3.5 $\pm$ 0.2 <sup>c</sup>
T <sub>4</sub>	104 $\pm$ 3 <sup>ab</sup>	52 $\pm$ 2 <sup>ab</sup>	3.8 $\pm$ 0.3 <sup>bc</sup>
p-value	0.004	0.002	0.001

Different superscripts indicate significant differences ( $P < 0.05$ ).

### Immune Response

Humoral immunity was determined by the serum IgG levels and was significantly better in the groups in which vitamin C was supplemented. The T<sub>3</sub> had the highest concentration of IgG. This shows that vitamin C in the diet improves the immune competence of early-growth broilers.

**Table 7:** Effect of dietary vitamin C supplementation on serum IgG levels (mg/mL) in broilers during a 4-week trial.

Treatment	IgG (mg/mL)
T <sub>1</sub> (Control)	2.20 $\pm$ 0.10 <sup>c</sup>
T <sub>2</sub> (250 mg/kg)	2.55 $\pm$ 0.12 <sup>b</sup>
T <sub>3</sub> (500 mg/kg)	2.88 $\pm$ 0.11 <sup>a</sup>
T <sub>4</sub> (750 mg/kg)	2.70 $\pm$ 0.12 <sup>ab</sup>

Different superscripts indicate significant differences ( $P < 0.05$ ).

## DISCUSSION

The current experiment showed that vitamin C dietary supplementation was able to significantly improve growth performance, stress resistance, antioxidant status, and immune responses of 1-day-old Cobb 500 broilers during 4 weeks. Birds fed with vitamin C 500 mg/kg recorded the highest growth in body weight and the least feed-to-weight ratio, although there is no significant difference in feed intake between the groups. These findings suggest that the better growth was contributed to mainly by the increased use of nutrients and not the increase in feed intake. The weight gain and feed efficiency of heat-stressed broilers are enhanced with the use of vitamin C supplements that reduce oxidative stress (Pečjak et al., 2022; Al-Khalaifah et al., 2025). The increased growth performance in broilers fed ascorbic acid, especially under stressful conditions, and that vitamin C plays a crucial role in enhancing the initial growth and physiological stability (Gouda et al., 2020; Van Hieu et al., 2022). The fact that the further increase at 750 mg/kg was not increased also indicates a plateau effect has been found in other studies that excessively high doses of vitamin C do not always lead to further improvement of performance (Shakeri et al., 2020). The status of vitamin C supplementation was also significant in lowering serum corticosterone levels, which means that broilers are more resistant to stress (Biswas et al., 2024). Birds fed 500mg/kg vitamin C also had the lowest corticosterone levels, indicating that vitamin C reduces physiological stress by altering the hypothalamic-pituitary-adrenal axis and lowering oxidative injury. The exogenous vitamin C lowered corticosterone levels in heat-stressed broilers (Del Barrio et al., 2020; Tavakolinasab & Hashemi, 2025). Dietary ascorbic acid reduces stress in broilers, which fits the concept of vitamin C in improving adaptability to environmental and physiological stresses (Hoan et al., 2021; Solomon, 2024).

Vitamin C supplementation significantly improved the hematological profiles of birds. Specifically, the level of hemoglobin, packed cell volume, and red/white blood

cells was greatest in the treatment group of 500 mg/kg, which demonstrates an increase in the transport capacity of blood and the overall health of blood. The supplementation with vitamin C positively affected hematological parameters and the overall well-being of broilers under stress (Ferronato et al., 2024; Kianfaret al., 2025). The increased white blood cell counts in supplemented groups indicate enhanced immune responsiveness. Vitamin C enhances lymphocyte activity and humoral immunity in broilers subjected to environmental stress (Sun et al., 2023).

Serum biochemical tests also showed that vitamin C had positive effects as birds fed on 500 mg/kg vitamin C had better total protein, albumin and globulin levels with less cholesterol levels than the control group. These variations signify that there is an upgrading of the protein metabolism, lipid regulation and the general health of the body. Supplementation with vitamin C increases serum protein and lipid profiles of broilers, especially when subjected to stress (Zangeneh et al., 2018; Mudawaroch et al., 2026). The glucose levels were all within the normal range, indicating that the energy metabolism was not get affected negatively and that the growth performance improvements were mainly associated with better nutrient use and mitigation of stress.

Dietary vitamin C also significantly increased the antioxidant defense system of broilers by enhancing the highest superoxide dismutase and catalase activities, and lowest malondialdehyde activity, which indicated the reduction of lipid peroxidation and prevention against oxidative damage. The positive effect of vitamin C supplementation on broilers is an increase in the activity of antioxidant enzymes and a decrease in oxidative stress in broilers, which consequently leads to improved growth, immunity, and overall health (El-Senousey et al., 2018; Min et al., 2018; Habashy et al., 2026). Humoral immunity in the form of serum immunoglobulin G was significantly increased in the vitamin C-supplemented birds, with the highest concentrations being recorded in the 500 mg/kg group. Vitamin C enhances humoral immune responses by stimulating lymphocyte proliferation and antibody production. Vitamin C enhances immune competence in broilers, especially in an environment that is subjected to environmental stress, which also supports the contribution of ascorbic acid to physiological resilience and health. These findings have a practical implication on commercial production of broilers with a focus that strategic incorporation of vitamin C in the starter diets can positively impact growth, health and resilience in the intensive condition of management.

### Conclusions

The vitamin C supplementation in the diet enhanced growth performance and resistance to stress among Cobb 500 broiler chickens in 4 weeks. Broilers fed vitamin C especially at 500 mg/kg had greater body weight, better feed conversion ratio, and lower

corticosterone level than the control group, which means that they were better adapted to intensive rearing systems. Physiological health was also improved through supplementation in increased hemoglobin, packed cell volume and positive alteration of serum biochemical profiles such as the increase in protein fractions and decrease in cholesterol. The increase in antioxidant status was associated with an increase in SOD activity, catalase and a decrease in malondialdehyde levels indicating a decrease in oxidative stress. The increase in serum IgG in the dietary supplemented groups suggested the augmented humoral immunity.

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