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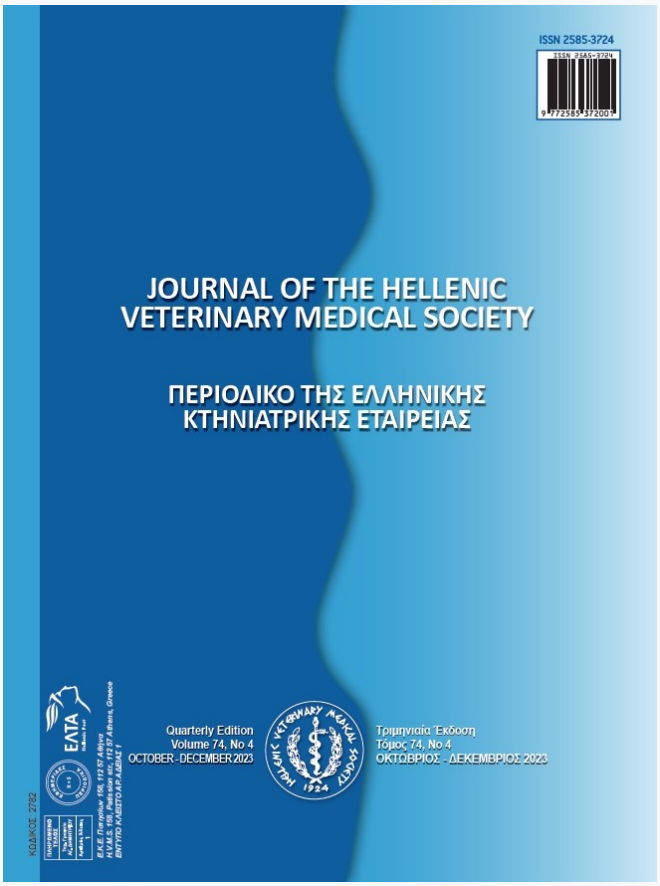

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The effect of different levels of vitamin C on performance, carcass characteristics, digestive organs, immunity, blood parameters, liver enzymes, cecal microflora, evaluation of meat taste and fatty acid profile of breast meat of broilers

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ABSTRACT: The objective of this experiment was to investigate vitamin C on performance, carcass characteristics, digestive organs, immunity, blood parameters, liver enzymes, cecal microflora, evaluation of meat taste and fatty acid profile of Arbor Acres commercial strain broilers. The study was based on a completely randomized design 120 one-day-old male broilers with three various levels of vitamin C (0, 250 and 500 mg/kg diet) and four replicates of10 chicks in each repetition for 42 days. Data were analyzed with SPSS statistical software, and to determine the effective of different levels of vitamin C, polynomial contrast test was performed. In the whole period, no statistical difference was observed between the treatment groups in feed consumption, weight gain and feed conversion ratio (P>0.05). Carcass and intestinal characteristics was not affected by vitamin C (P>0.05), except for the relative weight of breast by adding 250 mg/kg vitamin C (P<0.05). Vitamin C was not affected on blood parameters, except HDL and total cholesterol were increased at 250 mg/kg level. Creatine phosphokinase and lactate dehydrogenase in broilers receiving experimental treatments decreased by higher levels of vitamin C in the diet (P<0.05). The results showed that the comparison of the average amount of white and red blood cells, hemoglobin, HTC, MCV, MCH and MCHC were not affected by different levels of vitamin C in the diet (P>0.05). The percentage of lymphocytes and heterophils were affected by different levels of vitamin C (P<0.05). The amount of antibody titer against Newcastle virus, influenza, SRBC and organs related to the immune system were not affected by experimental treatments (P>0.05), however relative weight of bursa was increased in broilers fed vitamin C (P<0.05). Also, color, general acceptance, elasticity and chewing ability of breast meat were different in all three treatment groups (P<0.05). The effect of vitamin C on the fatty acid profile of breast meat showed C12:0 was unchanged in the 3 treatment groups, but C14:0, C16:0, C18:0, C18:3 decreased with increasing consumption of vitamin C. According to these results, using250 mg/kg of vitamin C inbroilers diet of the Arbor Acresstrain may have potential economic benefits.

***Keywords*:** vitamin C, blood parameters, liver enzymes, carcass components and breast meat profile.

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INTRODUCTION

In recent years, chicken meat has been widely used as a source of protein and has replaced red meat in many countries. Chicken meat is more salable than red meat due to its protein value and favorable ratio of amino acids (Hosseini et al., 2013). Also, the rapid growth of commercial broilers to meet human needs due to genetic improvements and increased nutritional knowledge nutrition has led to an increase in the production of broilers (Higgins et al., 2008). Since perceived health giving properties are the most important factors influencing consumer food choice, some compounds can be used as antioxidants to increase meat production and improve meat quality in the poultry industry. Vitamin C is one of the compounds that have beneficial antioxidant properties (Kucuk et al., 2003). Ascorbic acid, also known as vitamin C, is involved in many essential functions in the body, including the synthesis of collagen and carnitine as well as the metabolism of vitamin D3 (Wang et al., 2018). Vitamin C is a strong reducing agent that is easily oxidized to dehydroascorbic acid in a reversible reaction (Padayatty and Levine, 2016). Also, ascorbic acid is an antioxidant compound with unique chemical formula and properties (National Center for Biotechnology Information, 2021), and its addition to poultry diet has beneficial effects on reducing inflammation, oxidative stress and infection (El-Senousey et al., 2018). Since poultry are able to synthesize it through the biosynthetic pathway, it is not considered an essential nutrient. However, in cases where birds do not synthesize enough ascorbic acid in normal synthesis (El-Senousey et al., 2018) or when they are in hot or stressful weather conditions, it is essential to add ascorbic acid in poultry diets (National Center for Biotechnology Information, 2021). Initially, researchers added ascorbic acid to poultry diets to improve performance by improving body weight and reducing death loss. Then, the use of ascorbic acid was used with the aim of improving the immune response and antioxidant capacity of birds. Specifically, in a heat stress condition, ascorbic acid participates with corticosterone biosynthesis in poultry energy supply, in this way, vitamin C inhibits the activity of 21-hydroxylase and 11-β-hydroxylase (El-Senouseyet al., 2018; Barrio et al., 2020). In addition, the use of ascorbic acid also plays an important role in the treatment and prevention of Salmonella enteritidis (Hernandez-Patlanet al., 2019). Modulation of physiological functions caused by ascorbic acid has also been reported in some studies (El-Senouseyet al., 2018). According to Gan et al., (2018(, ascorbic acid supplementation increases the level of ascorbic acid in the spleen and the level of serum immunoglobulin G (IgG). Also, in other studies (Madamanchiet al., 2005) it has been shown that the consumption of ascorbic acid, aspirin, vitamin E and selenium supplements significantly reduced the number of white blood cells and they stated that the increase of free radicals causes oxidation with resultant cellular destruction,therefore, it can cause many disorders in the intestinal tissue. Antioxidants, with their ability to effectively inhibit free radicals, may solve problems related to intestinal disorders and improve functional characteristics (Wang et al., 2012).

The use of ascorbic acid in poultry feed improves health and, as a result, increases the growth performance of birds (Hieuet al., 2022). In this way, the concentration of blood glucose determines the consumption of feed in poultry, also the increase in the concentration of blood glucose leads to the suppression of appetite and the reduction of the stimulus concentration in the hunger center of the hypothalamus, resulting in the reduction of feed consumption. Due to their genetic characteristics and the ability to synthesize large amounts of triglycerides and lipoproteins in the liver, broilers have this mechanism that leads to the storage of large amounts of fat in the body. These fatty acids are transformed into triglycerides in the liver cells, enter very low density lipoproteins (VLDL) and are transported to other tissues through the blood stream, which ultimately leads to a reduction in body fat (Ferreira et al., 2015).

Therefore, the effect of different levels of ascorbic acid with antioxidant properties on growth performance, carcass and digestive tract characteristics, blood parameters, immune system, cecal microbial flora and breast meat fatty acid profile can be various in different physiological conditions. Considering the contradictory results regarding the effect of antioxidant compounds on the a forementioned factors, and since, because there are limited results regarding the use of ascorbic acid in reducing the serum level of cholesterol and blood lipoproteins and reducing the abdominal fat of broilers in order to marketability in the diet of broilers, Therefore, this study was conducted with the aim of investigating the effect of vitamin C on growth performance, carcass characteristics and digestive organs, blood parameters, immunity, and ceccal microbial flora of broilers. Finally, it investigates the effects of vitamin C consumption on blood and carcass fats and investigates and reports their ef-

fects on meat taste evaluation and fatty acid profile of breast meat.

MATERIALS AND METHODS

This study was conducted in a broiler farm in Rasht, Iran. In order to investigate the effect of different levels of organic vitamin C on performance, carcass characteristics, digestive organs, immunity, blood parameters, liver enzymes, cecal microbial flora, evaluation of meat taste and fatty acid profile of breast meat of broilers, 120 one-day-old male chicks of the commercial strain Arbor Acres were completely randomized including 3 treatments, 4 replications and 10 chicks per replication in a broiler breeding farm. One-day-old chicks from the Arbor Acres commercial strain had an average weight of 45±2 grams. The three treatments included (1) control treatment, no vitamin C (VC0 mg/kg diet), (2) 250 mg/kg diet vitamin C (VC 250 mg/kg diet) and (3) 500 mg/kg diet vitamin C (VC500 mg/kg diet). The vitamin C supplement was reported by the manufacturer to be ionically bound to molecules of the amino acid methionine and was delivered in a concentrated carrier consisting of corn, soybean, barley and wheat bran. The vitamin C was obtained from Science Laboratories (Science Laboratories, Qazvin, Iran).

All three groups were fed with the same basic diet containing the recommended minimum nutrients from Arbor Acres commercial broiler feed guide (2021 guide) (Table 1). Chickens were reared in 1 x 1 mcages on a bed of cellulose rolls and fed with experimental diets for 42 days. Each cage contained a cylindrical feeder and a manual drinker. The temperature of the pens was 33°C during the first week. It was gradually decreased to 23°C until the 18th day after which it remained constant at 23°C. The humidity of the room was adjusted to 65-70% during the study period and the chicks were exposed to light for 23 hours and dark for one hour. Feed and water were

Table 1. Ingredients, chemical composition and energy of diets (from the 1st-42nd days of

Experimental diet	Starter diet(1st-10th days of age)	Grower diet (11st -24th days of age)	Finisher diet (25th-42nd days of age)
Corn	47.03	59.60	65.99
Wheat	5.58	5.00	5.00
Soybean meal(44% crude protein)	29.02	16.15	10.28
Corngluten	10.00	11.48	11.50
Soy Oil	3.50	3.40	3.09
Limestone	1.45	1.23	1.00
Di-calcium phosphate	1.95	1.80	1.83
Salt	0.20	0.20	0.20
Vitamin and mineral supplements[1]	0.50	0.50	0.50
DL-Methionine	0.52	0.58	0.57
L-Lysine hydrochloride	0.25	0.06	0.04
Calculated analysis			
Metabolizable energy (kcal/kg)	2950	3000	3050
Crude protein	22	20	19
Lysine (%)	1.3	1.2	1.1
Methionine (%)	0.56	0.54	0.52
Met+Cys (%)	0.92	0.90	0.88
Threonine	0.861	0.804	0.722
Tryptophan	0.2	0.185	0.169
Arginine	1.39	1.284	1.177
Isoleucine	0.861	0.800	0.743
Leucine	1.41	1.302	1.187
Valine	1.014	0.936	0.869
Available.phosphor	0.52	0.47	0.41
Calcium(%)	1.04	0.95	0.92

1. The amount of vitamins and minerals per kg of the final diet: vitamin A, 9000 IU; vitamin D3, 3000 IU; vitamin E, 18 IU; vitamin K3, 3 mg; vitamin B1 (Thiamine), 1.8 mg; vitamin B2 (Riboflavin), 6 mg; vitamin B6 (Pyridoxine), 3 mg; vitamin B12 (Cyanocobalamin), 0.012 mg; vitamin B3 (Niacin), 30 mg; vitamin B9 (Folic acid), 1 mg; vitamin H3 (Biotin), 0.24mg; vitamin B5 (Pantothenic acid), 10 mg; Choline, 100 mg; Mn, 100 mg; Zinc, 80 mg; Iron, 10 mg; Cu, 1 mg; I, 0.2 mg.

offered ad libitum throughout the study. All birds were vaccinated against infectious bronchitis (1st and 12nd days of age), Newcastle disease (1st, 7th, 21st and 35th days of age), infectious bursal disease (16th days old) and avian influenza (1st day of age). All vaccines were obtained from Razi Vaccine and Serum Institute (Karaj, Iran). The use and care of birds in this study were approved by the Rasht Branch, Islamic Azad University from the point of Ethical issues (11748259376765013981423 45).

Growth performance and economic efficiency

Chickens were weighed on day 1, 10, 24 and 42 using a digital scale with an accuracy of ±0.1g (A and D GF-300, A and D Weighing Design and Manufacture, San Jose, California). Weight gain of chickens was determined in 3 periods: day 1-10; day 11-24; and day 25-42. At the end of each period the amount of feed remaining in each feed container was weighed. The amount of feed consumed was calculated by subtracting feed remaining from the amount of feed fed in each period. The feed conversion ratio was calculated by dividing the feed intake by the weight gain for each period and for the whole experiment (Sigolo et al., 2019). The European Production Index (EPI) was calculated using the following formula:

EPI= mean live weight (g) × survival percentage/ feed conversion ratio × number of breeding days × 10

The cost of consumed feed per kilogram of live chicken (C/kgLW) was calculated as follows: C/ kgLW= (Price of feed consumed during 42 days for each chicken in Iranian Rials/ Weight of a chicken per kilogram at 42 days of age).

The price of vitamin C at the time was calculated separately for each diet and included in the formula.

Carcasses characteristics digestive organs and intestinal components

At the end of the experiment (day 42), the feed was removed for two hours and two birds with a weight close to the average weight of the pen from each replicate were slaughtered, and the weights were measured using the same digital scale. (The measured weight parameters include: live body weight, defeathered body weight, empty abdominal carcass weight, eviscerated carcass, relative weights of bursa of Fabricius, breast, drumsticks, thigh, wing, digestive organs (spleen, pancreas, gizzard, heart, liver, fat) abdomen, duodenum, jejunum and ileum, and also the length of duodenum, jejunum and ileum.

Parameters of blood plasma component and liver enzymes

For the final tests of parameters of blood plasma components and liver enzymes on the 42nd day, 3 birds with a weight close to the average were randomly selected from each replicate and 5 ml of blood was taken from the wing vein. The samples were pooled and clear serum was extracted using a centrifuge (Sorvall super T21, Du Point Co. USA); the samples were transferred to microtubes and kept at room temperature for 12 hours; further they were centrifuged (Sorvall super T21, Du Point Co., USA) for 3 minutes (before serum separation) at a speed of 5000 rpm. The serum was stored at -20°C in a freezer (Emersun freezer, NRF3292D Emersun Co., Tehran, Iran) until further analysis. Analyzes were performed using Pars Azmoun commercial kits and by an autoanalyzer (Hitachi 917, Hitachi) (Tokyo, Japan; reagents from Roche Diagnostics, Mannheim, Germany), according to the instructions of the commercial kits (RANDOX Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY) was determined and calculated (Golrokh et al., 2016).

The measured metabolites include the number of red blood cells, the number of white blood cells, hemoglobin, hematocrit, the average volume of red blood cells (MCV), the average hemoglobin in one unit of red blood cells (MCH), the average concentration of hemoglobin. per erythrocyte (MCHC), heterophil, lymphocyte, monocyte, eosinophil, triglyceride, cholesterol, total protein, albumin, VLDL (very low density lipoprotein), HDL (high density lipoprotein), LDL (low density lipoprotein), creatine phosphokinase and lactate dehydrogenase.

Immune responses

To investigate humoral immunity, broilers were vaccinated against SRBC (sheep red blood cells) by Lerner's method (Lerner et al., 1971). To prepare the suspension, blood samples were taken from three sheep and poured into a glass container containing EDTA. Then a 2% SRBC suspension was prepared in PBS. The SRBC injections were done to three birds in each replication using one hundred microliters of 2% SRBC solution intravenously through the wing vein at 28 and 36 days. Then, on the 35th and 42nd days, blood samples were taken from three birds in each repetition, after integration, they were collected before the antibody test and transferred to the labo-

ratory (Seidavi et al., 2014). Antibody titer against SRBC was measured using Vanderzipp hemagglutination method (Pourhossein et al., 2015). To measure total anti-SRBC titer, 50 microliters of serum sample was mixed with 50 microliters of phosphate buffered saline (PBS) inside the microtiter plate, serial dilutions of the chicken serum were preparedf rom 1:2 to 1:256, and then 50 microliters of chicken serum were prepared in each well as a 2% SRBC suspension. Plates were placed at room temperature for 4 to 5 hours before reading. The titers were expressed as log2 of the highest dilution, which indicates complete agglutination (Pourhossein et al., 2015).

Newcastle disease antibody titers (NDV; Live LaSota strain; Vetrina, Zagreb, Croatia) and influenza were measured on days 28 and 42. In this way, blood was taken from three broiler chickens in each repetition and collected after pooling before the antibody test. Hemagglutination inhibition test (HI) was performed on the samples based on the OIE standard. 96-well microplates were used for the experiment. At first, 25 µL of PBS was added to each well. Then 25 microliters of bird serum were poured into the first well of a 96-well plate and its dilution was done until the last well.

Twenty five microliters of Newcastle and influenza antigens were added to the wells. Then the microplate was placed on a mechanical shaker for 1 minute and incubated at 25°C for 30 minutes. In the next step, 25 microliters of 1% red blood cells were added to all wells and the microplate was again placed on a mechanical shaker for 15 seconds. Then the microplate was placed at 25°C for 30 minutes and the results were recorded. A 4-unit antigen (Pasouk, Iran) was used to perform the hemagglutination inhibition (HI) test (Seidavi et al., 2014). Titers were expressed as log2 of the highest dilution indicating agglutination. The 1% erythrocytes were obtained from pathogen-free chickens. On day 42, blood samples were taken from three birds per replicate, and the samples were pooled and tested for white blood cell count and differential. Then, in each repetition, two pieces of birds with a weight close to the average were slaughtered. The weight of the spleen, and bursa of Fabricius was measured.

Meat sensory taste evaluation

Sensory evaluation of meat samples according to the method of Kim et al., (2009) and based on a combination of indicators of fat content, juiciness,tenderness, color, odor, chewing ability, elasticity, oral sensation and general acceptance using a five-point hedonic method with a score of 5 points (5: points very good, 4: good, 3: average, 2: bad and 1: very bad) (Kim, 2009). For the sensory evaluation of the samples, 10person evaluator group of trained sensory evaluators and food industry experts was used (Baston and Barna, 2010). The first step was to thaw the samples overnight in the refrigerator, then from all repetitions of each experimental treatment, three pieces of meat were cut into approximate 10x10x10 mm pieces and cooked. The oven (Electric oven, SI4 854 C IX - F096799, Hamburg, Germany) was set to a temperature of 75 °C. No additives or seasonings such as salt were added to the samples; also, to neutralize the sensory senses between the samples, bread and water at room temperature were used (Komprda, 2003).

Fatty acid profile of breast meat

In order to measure fatty acids on the 42nd day, one breast meat sample from each treatment were taken to the laboratory. The breast muscle was separated, ground and stored at -20°C in a freezer (Emersun freezer, NRF3292D Emersun Co, Tehran, Iran). The fat content of the samples was extracted by the method of Folchet al., (1957). Briefly, a mixture of two solvents, chloroform and methanol, was prepared ata ratio of 2:1.Then one gram of the mixed samples was weighed and poured into test tubes. After that, 15 ml of the prepared solvent was added,mixed thoroughly and stored in the refrigerator for 24 hours. After storing in the refrigerator, 5 ml of distilled water was added to the samples to create 3 phases in the test tube. After separation,the lower phase, which contains the soluble fat and chloroform, was poured into special centrifuge tubes (Sorvall super T21, Du Point Co, USA) and centrifuged at 25°C for 15 minutes at a speed of 3000 rpm to completely separate the chloroform and soluble fat phases. This process was done twice and at the end after the final centrifugation the lower phase contained only chloroform and soluble fat was separated and poured into a clean laboratory container. After pouring the solution into the test tube, the tube was placed under a laboratory hood at a temperature of 70°C and nitrogen gas was blown on it to evaporate the chloroform. Then 50 mg of extracted pure fat was removed and subjected to base and acid methylation in two stages. First, the fat sample was exposed to a 0.5 M methoxide solution in methanol at a temperature of 50°C for 30 minutes. Then it was

placed under the influence of hydrochloric acid solution in methanol (ratio 1:1) for 30 minutes at 50°C. By adding hexane to the solution, the produced fatty acid methyl ester was dissolved in hexane. Then, after adding anhydrous sodium sulfate and final dehydration, the dissolved in hexane methyl esters of fatty acids were passed through a special filter and prepared for injection into the gas chromatograph 296 (Agilent 7890B GC System, Santa Clara, CA 95051, United States) column. C13 fatty acids were used as standards. Pure nitrogen was used as a carrier gas for injection into the gas chromatography device at a ratio of 1:50. The temperature program of the oven (Electric oven, SI4 854 C IX - F096799, Hamburg, Germany) was kept constant at 100°C for 4 minutes, reached a temperature of 240°C at a rate of 3°C/min and then kept at a constant temperature of 240°C for 20 minutes. The temperature at the sample injector position was 225°C and the detector temperature was 250°C. The analysis time of each sample was 71 minutes. Nitrogen gas pressure inside the column was 2.2, hydrogen gas pressure was 0.5 and air pressure was 0.4 kg/m3.

Cecal microflora

To investigate cecal microbial flora, two birds from each treatment were slaughtered and sampled on day 42, and finally 8 final samples were merged with each other. In order to take samples, the abdominal cavity was opened, the right and left cecum were separated with sterile scissors, and, immediately using a sampler, one gram of the contents of the digestive tract was removed, mixed with 9 ml of diluted physiological saline, and transferred to a sterile container. The sample was then mixed well with phosphate buffer and frozen at -20°C until counting *Escherichia coli*, total coliforms, *Lactobacillus* spp. and *Clostridium* spp.(Dibaji et al., 2014). A special culture medium was used to determine the number of different bacteria. For this purpose, in order to determine the total frequency of forms, the total number of anaerobic bacteria, *E. coli* and *Lactobacillus* from culture media, VRBL(Violet Red Bile Lactose Agar), WC (Wilkins-Chalgren anaerobe agar), MRS(de Man,Rogosa, Sharpe Agar) and EMB (Eosin Methylen Blue agar)agar was used. Serial dilution was used to create 10^{-5}, 10^{-6} and 10^{-7} dilutions to count *Escherichia coli*, coliforms, *Lactobacillus* and *Clostridium* bacteria in dilutions of 10^{-2}, 10^{-3} and 10^{-4} of the cecum contents. Then, 300 µlof each dilution were inoculated and incubated for 24 hours at 37°Cto determine the colonies based on colony forming units (CFU). Data were log2 transformed before analysis (Dibaji et al., 2014).

Statistic analysis

Statistical analysis was performed using SPSS statistics software. The comparison between groups was obtained in the form of a completely randomized design using one-way analysis of variance (ANOVA) and then Duncan's multi-range test with $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Growth performance and economic efficiency

Supplemental effects of different levels of vitamin C on the function of broilers are summarized in Tables 2 and 3. While there was no statistical difference between groups on feed intake, weight gain and feed conversion ratio in all three starter (1- 10 d of age), grower (11-24 d of age) and finisher (25-42 d of age) periods in broilers ($P> 0.05$), but numerically the VC250 treatment showed the highest performance level growth compared to the control and VC500. The VC250 recipient broilers showed the lowest feed intake, along with the highest weight gain and the best feed conversion ratio in the starter period, grower and finisher (Table 2). Also, feed intake, daily weight gain and feed conversion ratio were not affected by experimental treatments ($P> 0.05$), but data showed that the lowest feed intake, the highest weight gain and the lowest feed conversion ratio were observed in the VC250 group (Table 3). Ascorbic acid plays a role in many of the essential functions in broilers These results match the study of Lopes et al., (2020) which examined the effects of the low levels of ascorbic acid supplements on performance, relative weight of the organs in Cobb 500 commercial strain broilers,without observing significant differences between experimental treatments, which beneficial effects on body weight gain and FCR were observed in the primary phase. These results are contradicting those of Ghafarifarsani et al., (2022) and Osowe et al., (2022).

In this present study weight gain increased significantly after 10 days of age. In addition, during the period after hatching, broilers are affected by many stresses (transportation, vaccination, adaptation to water and feed), which disrupts the synthesis and metabolism of ascorbic acid in broilers (Rafiee, 2016). Therefore, vitamin C supplementation at this time can lead to better weight gain. On the other hand, the results observed for feed conversion ratio without sig-

Table 2. Growth performance (mean ±SEM) of of Arbor Acres® Plus broilers at starter, grower, finisher, and whole periods of age, which fed diets containing different levels of vitamin C

Trait / Treatment	Feed intake (g/chick/day)	Weight 1 chick (g/chick)	Weight gain (g/chick/day)	Feed conversion ratio (g/g)
	Starter period of age (1st-10th days of age)			
Vitamin C (0 mg/kg)	30.648 [a]	214.525 [a]	17.235 [a]	1.780 [a]
Vitamin C (250 mg/ kg)	31.110 [a]	214.500 [a]	17.198 [a]	1.810 [a]
Vitamin C (500 mg/ kg)	31.585 [a]	219.000 [a]	17.685 [a]	1.788 [a]
SEM	0.624	3.402	0.345	0.052
P	0.588	0.580	0.561	0.915
Contrast		**Pr> F**		
Vitamin C (0 with 250 and 500 mg/kg)	0.383	0.606	0.637	0.775
Vitamin C (250 with 500 mg/kg)	0.603	0.374	0.344	0.767
Vitamin C (0 with 250mg/kg)	0.612	0.996	0.940	0.693
Vitamin C (0 with 500 mg/kg)	0.315	0.376	0.380	0.921
	Grower period of age (11st-24th days of age)			
Vitamin C (0 mg/kg)	85.745 [a]	701.325 [a]	34.770 [a]	2.468 [a]
Vitamin C (250 mg/ kg)	87.833 [a]	737.725 [a]	37.373 [a]	2.363 [a]
Vitamin C (500 mg/ kg)	85.598 [a]	701.525 [a]	34.468 [a]	2.498 [a]
SEM	1.412	16.579	1.237	0.093
P	0.486	0.255	0.242	0.579
Contrast		**Pr> F**		
Vitamin C (0 with 250 and 500 mg/kg)	0.588	0.390	0.467	0.749
Vitamin C (250 with 500 mg/kg)	0.292	0.157	0.131	0.331
Vitamin C (0 with 250mg/kg)	0.323	0.155	0.171	0.445
Vitamin C (0 with 500 mg/kg)	0.942	0.993	0.866	0.824
	Finisher period of age (25th-42nd days of age)			
Vitamin C (0 mg/kg)	152.905 [a]	2017.523 [a]	73.243 [a]	2.098 [a]
Vitamin C (250 mg/ kg)	142.055 [a]	2098.808 [a]	75.975 [a]	1.893 [a]
Vitamin C (500 mg/ kg)	150.975 [a]	2015.647 [a]	72.835 [a]	2.070 [a]
SEM	5.837	60.036	2.783	0.126
P	0.411	0.557	0.697	0.486
Contrast		**Pr> F**		
Vitamin C (0 with 250 and 500 mg/kg)	0.394	0.602	0.740	0.469
Vitamin C (250 with 500 mg/kg)	0.308	0.352	0.445	0.344
Vitamin C (0 with 250mg/kg)	0.221	0.363	0.505	0.278
Vitamin C (0 with 500 mg/kg)	0.820	0.982	0.919	0.880
	Total period of age (1st-42nd days of age)			
Vitamin C (0 mg/kg)	100.390 [a]	2017.523 [a]	46.918 [a]	2.145 [a]
Vitamin C (250 mg/ kg)	95.273 [a]	2098.808 [a]	48.883 [a]	1.963 [a]
Vitamin C (500 mg/ kg)	99.300 [a]	2015.647 [a]	46.838 [a]	2.122 [a]
SEM	2.907	60.036	1.385	0.104
P	0.455	0.557	0.522	0.437
Contrast		**Pr> F**		
Vitamin C (0 with 250 and 500 mg/kg)	0.406	0.602	0.591	0.443
Vitamin C (250 with 500 mg/kg)	0.352	0.352	0.323	0.306
Vitamin C (0 with 250mg/kg)	0.244	0.363	0.341	0.247
Vitamin C (0 with 500 mg/kg)	0.796	0.982	0.968	0.882

[*] Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05.
SEM= Standard Error of Means.

Table 3. Economical performance (mean ±SEM) of Arbor Acres® Plus broilers at 42nd day of age fed diets containing the different levels of vitamin C

Trait / Treatment	Weight of 1 chick (g/chick)	Feed cost per kg live weight (Rial/kg)	Feed cost per kg live weight ($/kg)	European production index
Vitamin C (0 mg/kg)	2017.523 [a]	59580.350 [a]	0.232 [a]	219.675 [a]
Vitamin C (250 mg/ kg)	2098.808 [a]	56074.682 [a]	0.220 [a]	245.293 [a]
Vitamin C (500 mg/ kg)	2015.647 [a]	59928.833 [a]	0.233 [a]	221.370 [a]
SEM	60.036	2669.960	0.010	15.422
P	0.557	0.551	0.637	0.454
Contrast		**Pr> F**		
Vitamin C (0 with 250 and 500 mg/kg)	0.602	0.640	0.637	0.488
Vitamin C (250 with 500 mg/kg)	0.352	0.334	0.420	0.301
Vitamin C (0 with 250mg/kg)	0.363	0.377	0.420	0.270
Vitamin C (0 with 500 mg/kg)	0.982	0.928	1.000	0.939

[*] Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05.
SEM= Standard Error of Means.

nificant differences between the experimental treatments was observed in whole period (1.9-2.0).

The positive effect of the VC250 treatment on the feed conversion ratio obtained in this study may occur due to feeding conditions. The ascorbic acid used in this study was complexed with methionine, and that may have provided a greater antioxidant effect or it may have provided more stability to the vitamin molecule for a longer period. Either of which may have provided more potency for the vitamin. However, if this was the case, one might expect that the 500 mg dose of vitamin C would produce better results, as there have been no reports of negative effects of vitamin C in the diet.

The results for feed costs per kilogram of live weight in dollars and Iran riyals, the weight of the chick per day 42 (grams per chick) and the European production index presented in Table 3 did not show a significant effect of vitamin C supplementation and no advantage of increasing supplementation from 250 to 500 mg/kg (Table 3). These findings do not agree with the results of Tavakoli et al.(2021). However, Kucuk et al.(2003) reported that under the natural conditions, vitamin C had a slight effect on the traits that match the results of the present study.

Carcasses characteristics, digestive organs, and intestinal components

The effect of experimental treatments on carcass characteristics is summarized in tables 4 and 5. Live body weight, defeathered body weight, empty abdominal carcass weight, and eviscerated carcass were not affected by experimental treatments (P>0.05) (Table 4). However,the relative weight of breast was affected in broilers fed vitamin C (P< 0.05). On the other hand, comparison of different vitamin C (0, 250 and 500 mg/kg)levels showed that the relative weight of the breast was affected with consumption vitamin C (P< 0.05) and the greatest weight was demonstrated in VC250 treatment (P<0.05). Dietary treatments had no effect on other relative weights of carcass, including the relative weight of thighs, wings, abdominal fat, pancreas, gizzard and heart (P> 0.05) (Table 5).

According to the findings of Jeburet et al.(2017), the use of vitamins C, E, aspirin and sodium chloride in broiler's diet led to positive effects on digestibility, relative weight and some carcass characteristics. These results can be related to the antioxidant properties of the compounds by removing free radicals and thus improving the relative weight and characteristics of the carcass. In fact, the use of vitamin C can eliminate all oxygen species and have a protective effect against inhibiting the antioxidant enzymes, which can improve production performance(Hajati et al.,2015). In accordance with findings of this presents study, Rafiee et al.(2016) reported that the effect of different levels of lemon powder and vitamin C on the relative weight of gizzard, pancreas, small intestine and cecum was not significant.

As shown in Table 6, the results showed that the use of two different levels of vitamin C had no significant effect on some parts of the intestine such as relative weight of duodenum, jejunum and ileum (P>0.05), but numerically, the relative weight of duodenum and jejunum in the control group was higher than the VC250 and VC500 groups. Also, the rela-

Table 4. Economically relevant carcass characteristics (mean ±SEM) of Arbor Acres® Plus broilers at 42nd day of age fed diets containing the different levels of vitamin C

Treatment \ Trait	Live body weight (g)	Defeather body weight (g)	Empty abdominal carcass weight (g)	Eviscerated carcass (%)	Relative weight of breast (%)	Relative weight of drumsticks (thighs) (%)	Relative weight of wings (%)	Relative weight of abdominal fat (%)
Vitamin C (0 mg/kg)	2193.750 [a]	1738.750 [a]	1440.000 [a]	82.310 [a]	26.845 [b]	25.558 [a]	7.543 [a]	1.220 [a]
Vitamin C (250 mg/ kg)	2116.250 [a]	1691.250 [a]	1440.000 [a]	84.885 [a]	30.013 [a]	24.640 [a]	7.180 [a]	1.428 [a]
Vitamin C (500 mg/ kg)	2127.500 [a]	1692.500 [a]	1393.750 [a]	82.460 [a]	28.262 [ab]	21.880 [a]	7.392 [a]	1.845 [a]
SEM	118.440	93.171	107.635	2.292	0.607	1.943	0.091	0.281
P	0.884	0.920	0.941	0.683	0.016	0.415	0.058	0.324
Contrast				**Pr> F**				
Vitamin C (0 with 250 and 500 mg/kg)	0.632	0.690	0.864	0.639	0.013	0.359	0.047	0.257
Vitamin C (250 with 500 mg/kg)	0.947	0.992	0.768	0.473	0.071	0.341	0.134	0.321
Vitamin C (0 with 250mg/kg)	0.654	0.726	1.000	0.447	0.005	0.746	0.020	0.614
Vitamin C (0 with 500 mg/kg)	0.701	0.733	0.768	0.964	0.132	0.213	0.275	0.150

[*] Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05.
SEM= Standard Error of Means.

Table 5. Mean (±SEM) of organ characteristics of Arbor Acres® Plus broilers at 42nd day of age fed diets containing the different levels of vitamin C

Treatment \ Trait	Relative weight of pancreas %	Relative weight of gizzard (%)	Relative weight of heart (%)
Vitamin C (0 mg/kg)	0.395 [a]	2.310 [a]	0.620 [a]
Vitamin C (250 mg/ kg)	0.385 [a]	2.315 [a]	0.570 [a]
Vitamin C (500 mg/ kg)	0.345 [a]	2.450 [a]	0.628 [a]
SEM	0.040	0.166	0.037
P	0.665	0.800	0.512
Contrast		**Pr> F**	
Vitamin C (0 with 250 and 500 mg/kg)	0.559	0.729	0.648
Vitamin C (250 with 500 mg/kg)	0.502	0.579	0.297
Vitamin C (0 with 250mg/kg)	0.865	0.983	0.361
Vitamin C (0 with 500 mg/kg)	0.404	0.566	0.888

[*] Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05.
SEM= Standard Error of Means.

tive weight of ileum in the VC500 treatment group showed the highest amount compared to the control and VC250 groups (Table 6). On the other hand, consumption of this vitamin at 250 and 500 levels had no significant effect on the length of duodenum, jejunum and ileum (P>0.05). Whereas, in comparison of different vitamin C (0, 250 and 500 mg/kg)levels the relative weight of duodenum was increased in broilers fed vitamin C (P< 0.05) and VC250 treatment was the highest relative weight of duodenum.

In another study, by adding 250 mg of vitamin C in diet of broilers under heat stress, no significant effect was observed on the weight and relative length of the intestine (Alba et al., 2015). However, these results are in contrast to Pečjak et al. (2022) findings.

Blood constitutes and liver enzymes

The comparison of the average results of blood parameters is summarized in Table 7. The results indicate that there is no significant effect of different levels of vitamin C on the amount of VLDL, LDL, total protein, and triglycerides (P>0.05), but HDL and total cholesterol were affected in broilers fed vitamin C (P< 0.05). However, numerically the lowest level of these parameters belongs to the control group. Similarly, in comparison among different levels of vitamin C (0, 250 and 500 mg/kg), the amount of HDL and total cholesterol was affected in the blood of chickens fed the VC250 treatment compared to those in the control treatment and the VC500 treatment (P< 0.05). Blood albumin was not affected by different levels of vitamin C in the diet of broilers (P>0.05).

Recently, experiments have shown that antioxidants prevent the reduction of cholesterol in the body, which is consistent with the results of the present study. Normally, liver cells carry important proteins

Table 6. Intestinal segments (mean ±SEM) of Arbor Acres® Plus broilers at 42nd day of age fed diets containing the different levels of vitamin C

Small intestine / Treatment	Duodenum	Jejunum	Ileum
		Relative weight (%)	
Vitamin C (0 mg/kg)	1.085 [a]	1.835 [a]	1.410 [a]
Vitamin C (250 mg/ kg)	0.898 [a]	1.803 [a]	1.487 [a]
Vitamin C (500 mg/ kg)	0.933 [a]	1.745 [a]	1.530 [a]
SEM	0.058	0.092	0.103
P	0.101	0.789	0.714
Contrast		**Pr> F**	
Vitamin C (0 with 250 and 500 mg/kg)	0.039	0.601	0.453
Vitamin C (250 with 500 mg/kg)	0.677	0.670	0.776
Vitamin C (0 with 250mg/kg)	0.046	0.809	0.606
Vitamin C (0 with 500mg/kg)	0.094	0.508	0.430
		Length (mm)	
Vitamin C (0 mg/kg)	375.000 [a]	967. 500 [a]	977. 500 [a]
Vitamin C (250 mg/ kg)	320.000 [a]	907. 500 [a]	942. 500 [a]
Vitamin C (500 mg/ kg)	365.000 [a]	872. 500 [a]	975.000 [a]
SEM	17. 640	33. 010	41.850
P	0.116	0.176	0.808
Contrast		**Pr> F**	
Vitamin C (0 with 250 and 500 mg/kg)	0.166	0.087	0.723
Vitamin C (250 with 500 mg/kg)	0.104	0.472	0.596
Vitamin C (0 with 250mg/kg)	0.054	0.230	0.568
Vitamin C (0 with 500mg/kg)	0.697	0.072	0.967

[*] Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05. SEM= Standard Error of Means.

Table 7. Blood constitutes (mean ±SEM) of Arbor Acres® Plus broilers at 42nd day of age fed diets containing the different levels of vitamin C

Blood constitutes / Treatment	VLDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	Total Protein (g/dl)	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	Albumin (g/dl)
Vitamin C (0 mg/kg)	18.750 [a]	25.750 [a]	68.250 [b]	3.548 [a]	95.000 [a]	117.250 [b]	1.813 [a]
Vitamin C (250 mg/ kg)	20.250 [a]	33.250 [a]	76.250 [a]	3.308 [a]	103.250 [a]	137.000 [a]	1.783 [a]
Vitamin C (500 mg/ kg)	20.750 [a]	29.500 [a]	67.250 [b]	3.623 [a]	104.250 [a]	123.500 [b]	1.842 [a]
SEM	0.750	2.700	1.618	0.102	3.478	2.332	0.045
P	0.201	0.201	0.006	0.127	0.175	0.001	0.654
Contrast				**Pr> F**			
Vitamin C (0 with 250 and 500 mg/kg)	0.089	0.123	0.111	0.524	0.070	0.001	1.000
Vitamin C (250 with 500 mg/kg)	0.648	0.351	0.003	0.056	0.843	0.002	0.370
Vitamin C (0 with 250mg/kg)	0.190	0.081	0.006	0.129	0.127	<0.001	0.648
Vitamin C (0 with 500mg/kg)	0.092	0.351	0.672	0.614	0.092	0.090	0.648

[*] Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05. SEM= Standard Error of Means; VLDL Cholesterol = very low density lipoprotein cholesterol; LDL Cholesterol = Low Density Lipoproteins Cholesterol ; HDL Cholesterol= high- density lipoproteins cholesterol.

in the structure of lipoproteins such as VLDL. This means that VLDL is not converted to LDL, which is the most important carrier of cholesterol in the blood. Therefore, antioxidants prevent of this conversion in liver cells. so, the lack of oxidation of apoprotein B in VLDL means the continuation of lipoprotein metabolism and LDL formation (Krauss, 2004).

In accordance with the results of the present study, it was shown that antioxidants such as vitamin C, E, and beta-carotene increase triglycerides, apolipoprotein B, and LDL therefore, the degradation of apoprotein B is increased by peroxidation and decreased by antioxidants (Hallfrisch et al.,1994).Tavakoli et al. (2021) and Abdulameer et al. (2019) results are con-

sistent with these findings.

The results of the effect of vitamin C consumption on liver enzymes are presented in Table 8. The results showed that creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were decreased by adding vitamin C in broilers diet (P<0.05).Also, comparison results of the various levels of vitamin C (0, 250 and 500 mg/kg)showed that the levels of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) in broilers receiving VC500 treatment were affected and decreased in the blood(P<0.05). The amount of creatine phosphokinase in the VC500 treatment is lower than either control and VC250 treatment, whereas the VC250 treatment is. Also, the level of LDH in the VC250 treatment was the higher level compared to the other two treatments. However, the VC500 treatment had lower LDH than control treatment.

Due to its antioxidant properties, vitamin C protects lymphoid tissues and increases their efficiency. Compounds with antioxidant properties, such as vitamin C, act by strengthening the immune system and lead to the improvement of blood metabolites. Liver is the main place of metabolism by many enzymes. Damage to the cell membrane or the death of liver cells results in increases the amount of liver enzymes in the blood, and the amount of this increase is a sign of the extent of liver lesions. The liver tissue damage and increased concentration of liver enzymes have already been reported (Huang et al.,2018).Compounds with antioxidant properties prevent damage to the liver and other internal organs by preventing oxidative stress and protect cell membranes from oxidative stress (Horvath and Babinszky, 2018).

Immunity responses

The effect of the experimental treatments on the immune system is summarized in Tables 9, 10 and 11. The investigation of the amount of blood cells related to the immune response is presented in Table 9.Comparison of the average results of white and red blood cells, hemoglobin, HTC, MCV, MCH and MCHC was not affected by different levels of vitamin C in the diet of broilers (P>0.05).In contrast,comparison of various levels of vitamin C (0, 250 and 500 mg/kg) demonstrated that red blood cells decreased with increasing vitamin C in broilers diet (P<0.05), with this reduction being not significant compared to the group that did not receive vitamin C. Amount of all variables in the control treatment was higher than VC250 and VC500 treatments. Comparison of the average results of white blood cells related to the immune system, including the percentage of monocytes and eosinophiles, was not affected by the experimental treatments (P>0.05). However, numerically, the percentage of monocytes and eosinophilesin the VC250 and VC500 treatments was lower than the control treatment (Table 10). The percentage of lymphocytes and heterophils was affected by different levels of vitamin C (P<0.05) with the percentage of heterophil cells higher in control and VC250 treatments than in the VC500 treatment. Comparison of lymphocytes showed that the VC500 treatment was the highest percentage compared to the VC250 and the control treatments respectively (P<0.05) (Table 10). Also, comparison of various levels of vitamin C (0, 250 and 500 mg/kg) showed that vitamin C has been effective inheterophil, lymphocyte and monocytes cells (P<0.05), and the highest decrease has been observed at the VC500 level.

Table 8. Liver enzymes (mean ±SEM) of Arbor Acres® Plus broilers at 42nd day of age fed diets containing the different levels of vitamin C*

Enzymes / Treatment	Creatine phosphokinase (U/L)	Lactate dehydrogenase (U/L)
Vitamin C (0 mg/kg)	3988.750 [a]	472.500 [ab]
Vitamin C (250 mg/ kg)	4420.250 [a]	534.500 [a]
Vitamin C (500 mg/ kg)	3259.250 [b]	402.500 [b]
SEM	213.385	27.190
P	0.012	0.023
Contrast	**Pr> F**	
Vitamin C (0 with 250 and 500 mg/kg)	0.582	0.907
Vitamin C (250 with 500 mg/kg)	0.003	0.007
Vitamin C (0 with 250mg/kg)	0.186	0.141
Vitamin C (0 with 500 mg/kg)	0.038	0.102

*Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05.
SEM= Standard Error of Means.

Table 9. Immune response (mean ±SEM) of Arbor Acres® Plus broilers fed diets containing the different levels of vitamin C

Parameters / Treatment	White blood cells (n ×10³/mL)	Red blood cells (n ×10⁶/mL)	Hemoglobin (g/dl)	HCT (%)	MCV (Fl)	MCH (pg)	MCHC (g/dl)
Vitamin C (0 mg/kg)	10475.000 [a]	2366250.000 [a]	12.098 [a]	32.750 [a]	138.250 [a]	51.000 [a]	36.925 [a]
Vitamin C (250 mg/ kg)	9750.000 [a]	2261250.000 [a]	11.378 [a]	30.750 [a]	136.100 [a]	50.275 [a]	36.975 [a]
Vitamin C (500 mg/ kg)	7950.000 [b]	2230500.000 [a]	11.328 [a]	30.500 [a]	136.700 [a]	50.775 [a]	37.100 [a]
SEM	289.036	45445.480	0.320	0.890	1.811	0.653	0.061
P	<0.001	0.141	0.219	0.202	0.697	0.732	0.171
Contrast			**Pr> F**				
Vitamin C (0 with 250 and 500 mg/kg)	0.058	0.001	0.090	0.083	0.425	0.567	0.167
Vitamin C (250 with 500 mg/kg)	0.643	0.001	0.914	0.846	0.820	0.601	0.182
Vitamin C (0 with 250mg/kg)	0.136	0.109	0.146	0.146	0.422	0.452	0.577
Vitamin C (0 with 500mg/kg)	0.063	<0.001	0.123	0.107	0.560	0.813	0.074

[*] Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05.
SEM= Standard Error of Means; HCT = hematocrit; MCV = mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration.

Table 10. Immune response (mean ±SEM) of Arbor Acres® Plus broilers fed diets containing the different levels of vitamin C*

Parameters / Treatment	Heterophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Vitamin C (0 mg/kg)	41.750 [a]	54.250 [c]	3.000 [a]	1.000 [a]
Vitamin C (250 mg/ kg)	37.250 [a]	59.500 [b]	2.750 [a]	0.500 [a]
Vitamin C (500 mg/ kg)	31.500 [b]	66.500 [a]	1.750 [a]	0.250 [a]
SEM	1.514	1.341	0.391	0.323
P	0.003	<0.001	0.109	0.296
Contrast		**Pr> F**		
Vitamin C (0 with 250 and 500 mg/kg)	0.003	<0.001	0.151	0.148
Vitamin C (250 with 500 mg/kg)	0.025	0.005	0.103	0.597
Vitamin C (0 with 250mg/kg)	0.064	0.021	0.661	0.301
Vitamin C (0 with 500mg/kg)	0.001	<0.001	0.050	0.134

*Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05.
SEM= Standard Error of Means.

Researchers reported an increase in the number of lymphocytes and a decrease in the ratio of heterophils to lymphocytes by adding lemon leaf powder and vitamin C to the diets of broilers under heat stress (Rafiee et al., 2016). Other researchers reported a 5.8% increase in the number of lymphocytes and a 10.8% decrease in the ratio of heterophil to lymphocyte by adding 200 mg of vitamin C to the diet of broilers, and stated that it indicates the role of vitamin C in increasing the bird's tolerance to heat stress. They suggested that this was probably due to the protective effect of vitamin C against free radicals and the strengthening of the immune system of broilers (Attia et al., 2017). In addition, vitamin C plays a key role in the synthesis of leukocytes, especially phagocytes and neutrophils, which form a major part of the poultry immune system. Ascorbic acid's ability to transfer electrons allows it to have exceptional antioxidant properties and helps maintain the integrity of various cells, including lymphocytes, by protecting them from damage caused by free radicals generated in response to toxins or infection (Nimse et al., 2015). Phagocytosis of damaged cells and antigens as well as the formation, storage and maturation of lymphocytes are two main mechanisms which are protected against infection by the immune system of adult birds (Smith et al., 2014). Since immunosuppression and cytotoxicity are both effects of adrenocorticotropic hormone, by adding ascorbic acid to the diet, due to its ability to reduce the level of adrenocorticotropic hormone, an improvement of the immune system can be expected in chickens. In addition, by increasing the activity of the hexose monophosphate pathway, ascorbic acid leads to an increase in the growth of lymphoid cells, and as a result, the production of antibodies increases (Pardue et al., 1985).

Our results indicate that antibody titers against Newcastle virus, influenza and SRBC were not affected by experimental treatments (P>0.05) (Table 11). Various factors affect the functioning of the immune

system, among which ascorbic acid is one of the most important. Previous studies have shown that ascorbic acid can increase plasma IgG and IgM immunoglobulin levels as well as lysozyme enzyme activity)Yao, 2014(. In addition, research showed that the gene regulatory activities of ascorbic acid may play a role in the immunomodulatory function of lymphocytes by regulating the expression of immunoglobulins)Zhu et al.,2019), they found that the addition of 3 mg of ascorbic acid (in eggs) increased IgG on day 21 and IgM on day 1 and 21.Although the role of ascorbic acid in lymphocytes is less clear, research has shown that the gene regulatory effect of ascorbic acid may play a role in the immunomodulatory function of lymphocytes by regulating the expression of immunoglobulins. According to Gan et al.)2018(, increased ascorbic acid and DHA concentrations in the spleen due to ascorbic acid dietary supplements may be a contributing factor in increasing CD^{+4} T lymphocyte counts and IgG concentrations. B cells can differentiate to produce IgM and ascorbic acid has been shown to facilitate this process. Due to the increased number of CD^{+4} T lymphocytes, B cells may become more active. Additionally, ascorbic acid can increase antibody formation as part of the immune response to infection. Supplementing 200 mg/kg of ascorbic acid in the feed has led to an increase in Newcastle virus preventive antibodies and a decrease in lymphocytes in poultry (Gouda et al., 2020). Also, an increase in humoral and cellular immunity has been reported in chickens fed with a diet containing 300 mg of ascorbic acid per kg of feed. The availability of ascorbic acid is required for the systemic presence of IFNs and immunoglobulins (Chand et al., 2014). Moreover, ascorbic acid supplements improve blood CD^{+8} and IgM levels. Different doses of ascorbic acid with 50-200 mg/ kg in the feed have been found to have different effects on post-vaccination antibodies that protect against NDV and infectious bursal disease virus (IBDV) Lohakare et al., 2005).

In this study, birds that received 250 mg/kg of ascorbic acid had more antibodies against IBDV than birds in the control group. It showed that ascorbic acid has good effects in improving antibody-induced immunity in broilers ($P<0.05$). These results are consistent with Abdulameer (2019) who showed a significant increase in primary and secondary response against SRBC in broilers. From the comparison of the average antibody titer against SRBC among different treatments, it can be concluded that vitamin C increases the sensitivity of the immune system, and when sheep red blood cells entered the body, a significant response was observed in treatments containing vitamin C.

The bursa and spleen are among the organs that affect the immune system of birds, and any defect in the immune system can affect the development and evolution of these organs. The effect of the experimental treatments on the organs related to the immune system is summarized in Table 12. The relative weight of the liver, spleen and bursa of Fabricius was not affected by the experimental treatments ($P>0.05$), although the VC250 and VC500 groups were not distinguishable, but relative weight of bursa in both of them were higher than the control group. However,

Table 11. Immune response (mean ±SEM) of Arbor Acres® Plus broilers fed diets containing the different levels of vitamin C*

Treatment / Antibody titer	Antibody titer against first injection of Newcastle (28th day of age) (lg2)	Antibody titer against first injection of Newcastle (42nd day of age) (lg2)	Antibody titer against Influenza (28th day of age) (lg2)	Antibody titer against Influenza (42nd day of age) (lg2)	Total antibody against Sheep Red Blood Cell (35th day of age) (IgT)	Total antibody against Sheep Red Blood Cell (42nd day of age) (IgT)
Vitamin C (0 mg/kg)	1.000 [a]	1.500 [a]	2.000 [a]	2.500 [a]	2.000 [a]	2.500 [a]
Vitamin C (250 mg/ kg)	1.500 [a]	1.500 [a]	2.500 [a]	2.000 [a]	2.500 [a]	2.000 [a]
Vitamin C (500 mg/ kg)	1.500 [a]	1.000 [a]	2.000 [a]	1.500 [a]	2.000 [a]	2.500 [a]
SEM	0.236	0.236	0.289	0.333	0.289	0.408
P	0.274	0.274	0.405	0.161	0.405	0.622
Contrast			**Pr> F**			
Vitamin C (0 with 250 and 500 mg/kg)	0.117	0.409	0.497	0.099	0.497	0.629
Vitamin C (250 with 500 mg/kg)	1.000	0.167	0.251	0.316	0.251	0.409
Vitamin C (0 with 250mg/kg)	0.167	1.000	0.251	0.316	0.251	0.409
Vitamin C (0 with 500mg/kg)	0.167	0.167	1.000	0.062	1.000	1.000

* Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at $P<0.05$.
SEM= Standard Error of Means.

Table 12 .Immunity related organ (mean ±SEM) of Arbor Acres® Plus broilers at 42nd day of age fed diets containing the different levels of vitamin C

Organs / Treatment	Relative weight of liver (%)	Relative weight of spleen (%)	Relative weight of bursa of Fabricius (%)
Vitamin C (0 mg/kg)	2.820 [a]	0.135 [a]	0.168 [a]
Vitamin C (250 mg/ kg)	2.803 [a]	0.112 [a]	0.217 [a]
Vitamin C (500 mg/ kg)	2.708 [a]	0.118 [a]	0.193 [a]
SEM	0.181	0.011	0.016
P	0.895	0.334	0.133
Contrast		**Pr> F**	
Vitamin C (0 with 250 and 500 mg/kg)	0.775	0.158	0.082
Vitamin C (250 with 500 mg/kg)	0.718	0.746	0.288
Vitamin C (0 with 250mg/kg)	0.946	0.167	0.050
Vitamin C (0 with 500mg/kg)	0.670	0.273	0.288

[*] Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at P<0.05.
SEM= Standard Error of Means.

the relative weight of the liver and spleen, were less than the control treatment. In other words, increasing the amount of ascorbic acid consumption from 250 to 500 mg/kg did not affect the relative weights of organs related to the immune system (P>0.05).Butin comparison to various levels of vitamin C (0, 250 and 500 mg/kg), it was observed that adding 250 mg/kg vitamin C to the broilers diet led to an increase in relative weight of bursa (P<0.05).The reports of Rafiee et al.(2016) also observed no difference in spleen weight after supplementing broiler diets with 230 mg/kg vitamin C, which is consistent with the results of the present study.

The spleen is the largest immune organ in poultry, and plays a more important role in cellular and humoral immunity in birds than in other animals, as birds lack lymphatic arteries and lymph nodes (Rafiee et al., 2016). Different levels of lemon powder and vitamin C on the relative weight of spleen and liver was not significant, but it increased the relative weight of the bursa (Rafiee et al., 2016). Despite of this reports another study, adding 200 and 400 mg /kg of vitamin C to the diet of broiler chickens were significant effect on the relative weight of liver, spleen, or bursa and was improved immune system (Tavakoli et al., 2021). However, Chand et al.(2014) found an increase in the weight of the bursa and spleen, although the supplement used by these researchers was 300 mg/kg ascorbic acid. According to Gan et al.(2018), the effects of ascorbic acid supplementation in the diet of broiler breeder have greatly reduced the ability to synthesize L-gluconolactone oxidase, therefore, it can be concluded that the addition of ascorbic acid in poultry diet improves immunity, health and performance.

Meat sensory taste evaluation

The effect of experimental treatments on meat taste evaluation is summarized in Tables 13. General acceptance was different in all 3 treatments and VC250 chickens had the highest acceptance rate compared to both control and VC500 treatments, while the lowest rate was observed in the VC500 treatment (P<0.05). Similarly, the highest general acceptance in comparison to different levels of vitamin C (0, 250 and 500 mg/kg), was at VC250 level (P<0.05). On the other hand, the experimental treatments resulted in a significant difference on the chewing ability trait, and the control treatment showed the highest level compared to the VC250 and VC500 treatment meats (P<0.05). Fat content, juiciness, tenderness, color, odor, elasticity and oral sensation traits were not affected by experimental treatments (P>0.05) (Table 13). Despite of this, in comparison to different levels of vitamin C (0, 250 and 500 mg/kg), color of meat was affected by vitamin C (P<0.05). Furthermore, using of 250 and 500 mg/kg of vitamin C were affected chewing ability C (P<0.05) and elasticity was decreased at VC500 level (P<0.05). In addition, this comparison also showed that consumption of vitamin C in 500 mg/kg diets was reduced elasticity of meat (P<0.05).

Numerically, the fat content of breast meat in the control and VC250 treatments was lower than the amount compared to the VC500 treatment. The addition of 250 mg of vitamin C per kg of diet led to an increase in tenderness and a decrease in odor compared to the control group. Juiciness was not affected by different levels of vitamin C (P>0.05) and the highest tenderness was observed in chickens fed with the control treatment, follow by those fed VC500 with

VC250 being the least tender (Table 13).

Studies have shown that the antioxidant compounds in the diet lead to an increase in the water retention capacity (Dalólio et al., 2020),Since the tenderness is related to the myofibrils of the tissue, and the increase in water secretion during the storage period reduces the water storage capacity in the tissue, reducing the ability of the tissue to maintain and store water causes the nutritional value of the meat to be lost, which depends on the degradation of the tissue proteins. In addition, the pH of meat can affect the structure of myofibrils and, as a result, the water holding capacity and the color of the meat (Christensen, 2003). Shrinkage of contractile fibers at lower pH reduces the water binding ability and ultimately leads to a decrease in water holding capacity and a lighter meat color. During the oxidation of meat fat, many decomposition reactions occur and a wide range of different molecules such as aldehydes, ketones, alchohols, peroxides, and hydrocarbons are produced, which are the main chemicals responsible for the change in taste and the smell in meat (Zouari et al.,2010).These reports are consistent with the results of Tavakoli et al. (2021), and contradict the results of Omidi et al. (2022). Therefore, it seems that vitamin C, with its antioxidant properties, has reduced the oxidation of meat and the production of the mentioned compounds, and as a result, it has improved the qualitative characteristics of meat and its taste.

Fatty acid profile of breast meat

The effect of the experimental treatments on the fatty acid profile of breast meat is summarized in Tables 14, the amount of C12 fatty acid without double bond was zero in three treatment groups. Fatty acid C18:3, C20:0, C20:1, C22:0 and C22:1 in the control group, is zero percent, but C14:0, C16:0, C18:0, and C18:3 fatty acids have shown a significant decrease by increasing consumption of vitamin C in the diet ($P<0.05$). In general, the investigation of the main effects of the experimental treatments on the fatty acid profile showed that with the increase in the level of vitamin C, the amount of unsaturated fatty acids increased and at the same time a decrease in the amount of saturated fatty acids was observed.

The fatty acids cis-11, 14-eicosadienoic acid, cis-8,11,14-eicosatrienoic acid and cis-11,14,17 eicosatrienoic acid are ideal standard fatty acids for biological studies. They are mainly found at low levels in animal tissues (Huang et al., 2018; Wang et al., 2012). The fatty acid cis-11,14-eicosadienoic acid is

Table 13. Evaluation sensory traits mean (±SEM) of breast meat at 42nd days of age in Arbor Acres® Plus broilers fed diets containing the different levels of vitamin C*

Treatment \ Sensory	Fat content	Juiciness	Tenderness	Color	Odor	Chewing ability	Elasticity	Oral Sensation	General acceptance
Vitamin C (0 mg/kg)	3.250 [a]	4.000 [a]	3.250 [a]	3.250 [a]	3.000 [a]	4.750 [a]	4.500 [a]	4.500 [a]	4.250 [b]
Vitamin C (250 mg/ kg)	3.250 [a]	3.750 [a]	4.000 [a]	3.750 [a]	3.250 [a]	3.750 [b]	4.500 [a]	4.000 [a]	5.000 [a]
Vitamin C (500 mg/ kg)	4.000 [a]	3.750 [a]	4.000 [a]	4.250 [a]	4.000 [a]	3.500 [b]	3.500 [a]	4.000 [a]	3.500 [c]
SEM	0.312	0.204	0.276	0.250	0.363	0.264	0.289	0.289	0.220
P	0.201	0.622	0.141	0.057	0.184	0.019	0.057	0.405	0.003
Contrast					**Pr> F**				
Vitamin C (0 with 250 and 500 mg/kg)	0.351	0.343	0.053	0.036	0.193	0.006	0.190	0.190	1.000
Vitamin C (250 with 500 mg/kg)	0.123	1.000	1.000	0.190	0.178	0.519	0.036	1.000	0.001
Vitamin C (0 with 250mg/kg)	1.000	0.409	0.087	0.190	0.638	0.025	1.000	0.251	0.039
Vitamin C (0 with 500mg/kg)	0.123	0.409	0.087	0.019	0.083	0.008	0.036	0.251	0.039

* Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at $P<0.05$.
SEM= Standard Error of Means.

Table 14. Profile of breast fatty acids Arbor Acres® Plus broilers at 42nd day of age fed diets containing different amounts of vitamin C

	Profile of breast fatty acids (%)																	
Trait / Treatment	Lauric acid C12:0 (%)	Myristic Acid Methyl Ester C14:0 (%)	Silicic Acid C15:0 (%)	Monosilicic Acid C15:1 (%)	Palmitic Acid C16:0 (%)	Palmitoleic Acid Methyl Ester C16:1 (%)	Margaric acid C17:0 (%)	Heptadecanoic acid C17:1(%)	Stearic Acid Methyl Ester C18:0 (%)	Oleic Acid Methyl Ester C18:1 (%)	Linoleic Acid Methyl Ester C18:2 (%)	Linolenic Acid Methyl Ester C18:3 (%)	trans Linolenic Acid Methyl Ester C18:3t (%)	Arachidic acid C20:0 (%)	cis-11, 14-Eicosadienoic Acid Methyl Ester C20:1 (%)	Behenic acid C22:0 (%)	Erucic acid. C22:1 (%)	Other ISIRI 4091 (%)
Vitamin C (0 mg/kg)	0	0.58	0.11	0.03	28.41	6.68	0.02	0.01	6.15	45.46	12.43	0	0.07	0	0	0	0	0.05
Vitamin C (250 mg/ kg)	0	0.52	0.12	0.08	25.44	6.04	0.06	0.02	5.7	44.81	16.05	0.42	0	0.06	0.41	0.05	0.1	0.12
Vitamin C (500 mg/ kg)	0	0.49	0.16	0.04	24.8	6.26	0.05	0.03	5.2	45.7	16.24	0.45	0	0.05	0.4	0.04	0.02	0.07

Table 15. Cecum microflora Arbor Acres® Plus broilers at 42nd day of age fed diets containing different amounts of vitamin

Trait / Treatment	E. coli (log10 CFU/gr)	Coliform (log10 CFU/gr)	Lactobacil (log10 CFU/gr)	Clostridium (log10 CFU/gr)
Vitamin C (0 mg/kg)	1.204	2.064	2.524	0
Vitamin C (250 mg/ kg)	1.863	1.924	2.772	0
Vitamin C (500 mg/ kg)	1.415	2.422	1.672	0

produced from linoleic acid by the enzyme delta-9 elanase and can be converted into dihomo-γ-linolenic acid, arachidonic acid, siadonic acid and other unsaturated fatty acids. cis-11,14-eicosadienoic acid can modulate the metabolism of unsaturated fatty acids and is responsible for the response of macrophages to inflammatory stimuli. Cis-11,14-eicosadienoic acid together with other monounsaturated and polyunsaturated fatty acids can inhibit the binding of leukotriene B4 to the neutrophil membrane, which is part of these anti-inflammatory activities (Huang et al., 2018). In fact, vitamin C act as antioxidants in the body so that through rapid oxidation, it forms a compound (dehydroascorbic acid), this compound protects the cell membrane and unsaturated fatty acids of the membrane against the oxidation of free radicals (Huang et al.,2018). Tavakoli et al. (2020) results confirmed the present findings which contradict with Amer et al. (2021) results.

Cecal microflora

The effects of vitamin C on intestinal cecal bacteria population are summarized in Table 15. The results indicate that the population of *E. coli* and total coliform bacteria were lowest in the cecum of birds fed at the 250 mg/kg diet amount compared to the other two treatments. However, the lowest lactobacillus population was observed in the cecum of the VC500 treated birds. On the other hand, there was no difference among treatments for total clostridiums (P> 0.05). These results are consistent with the results of Tavakoli et al. (2021) and Saracila et al.(2020). By increasing free radicals, biological cells oxidize and destroy, so it can cause several intestinal disorders (Tavakoli et al., 2021). Antioxidants, such as vitamin C, may improve problems associated to disorders in intestinal tissues and improve performance characteristics (Wang et al., 2008).

CONCLUSION

Based on the conditions of this study, the addition of vitamin C at the level of 250 mg/kg in the diet of broilers of the Arbor Acres commercial strain may lead to the improvement of feed intake, weight gain and feed conversion ratio, as well as the lowest cost of each kilogram of live chicken and the best European production index. The results of the present research showed that the use of vitamin C led to the improvement of carcass characteristics, intestinal components and blood parameters in the birds fed 250 mg ascorbic acid / kg of diet and also improved the quality of the breast meat profile by improving the immune system. Therefore, according to these results, the use of 250 mg/kg of vitamin C is recommended for use as an antioxidant compound and as an economical growth stimulant in the diet.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ETHICAL APPROVAL

The use and care of birds in this study were approved by the Rasht Branch, Islamic Azad University from the point of Ethical issue. All the experimental procedures described herein were also approved by the Rasht Branch, Islamic Azad University. Care was taken to minimize the number of birds used.

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