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Effects of dietary vitamin C supplementation on growth performance, carcass characteristics, gastrointestinal organs, liver enzymes, abdominal fats, immune response, and cecum microflora of broiler chickens

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Abstract

Introduction: Increasing the concentrations of blood glucose leads to decrease appetite and decreased the concentration of stimulant in the hunger centre of the bird's hypothalamus and consequently, decrease feed intake. High intakes of vitamin C may alter the blood glucose concentration, although the evidence is contradictory. The aim of this study was to evaluate the effects of increasing vitamin C concentrations (0, 200 and 400 mg/kgDM) on performance, carcass and digestive organ characteristics, blood plasma components, liver enzymes, immune system, and cecal microbial flora of broilers. **Material and method:** The study was based on a completely randomized design with three treatments and four replicates of 10 chickens per pen. The study lasted42 days and started with 120 one-day-old male chickens of commercial Ross 308 strain. Treatments were compared usingDuncan's multiple-range test. **Results and discussion: C**hicks fed a diet containing 200 mg/kg vitamin C had the highest feed intake and weight gain as well as the best feed conversion ratio, the lowest cost per kilogram of live body and the best European production factor. The effect of vitamin C was significant on the weights of live body, featherless, full abdomen carcass, empty abdomen carcass, relative crop, and relative breast ($P<0.05$). Moreover, the highest increase was related to 200 mg vitamin C /kg diet. The effect of different levels of vitamin C on blood parameters and liver enzymes of broilers was not significant ($P>0.05$). In addition, the percentage of neutrophils and lymphocytes was significantly higher ($P<0.05$), and the highest percentage of neutrophils was also observed in chicks' fed the level of 200 mg/kg vitamin C. The mean results of blood antibody titers against SRBC did not show a significant difference ($P>0.05$), except for 35 days of age, which was significant ($P <0.05$). Based on the results of the present study, the use of 200 mg/kg vitamin C to supplement the diet of Ross 308 strain broiler chickens is recommended. **Conclusions.** Blood parameters were insignificantly affected in vitamin C diet fed birds; nonetheless, the experimental diet improved the immune system of animals and reduced the fat content of ventricular area; thereby improving the quality of carcass meat. According to our observations,

utilization of 200 mg vitamin C/kg diet is recommended as an antioxidant compound and inexpensive growth promoter.

Keywords: abdominal fats, ascorbic acid, blood metabolites, chick, immunity, liver enzymes, performance.

Introduction

Feed efficiency is one of important factors in reducing the production costs of poultry industry. In addition to economic efficiency of production, quality of the meat is similarly vital (Grashorn2007; Hulan et al. 1988; Miller et al. 1969). Broilers store large amounts of fat in the body due to genetic characteristics as well as synthesis of large amounts of triglycerides and lipoproteins in the liver, thus lowering the quality of product (Griffin et al. 1991). Additionally, fatty acids may be altered in the liver cells, in the form of triglycerides, enter very low-density lipoproteins (VLDL) and transfer to other tissues via the bloodstream (Post-Beittenmiller 1996).

The concentration of glucose in the blood determines feed consumption in poultry. Increasing the concentrations of blood glucose leads to appetite suppression and lower concentration of stimulant in the hunger center of the hypothalamus hence decreasing feed intake (Shurlockand Forbes 1981; FerketandGernat 2006). Handful studies have shown the beneficial effect of high intake of vitamin C (more than 400 mg / kg) on blood glucose concentration in broiler chickens. In disagreement, Branch (1999) reported that high vitamin c intakes can lead to higher blood glucose levels. Vitamin C may also have further benefits in poultry suffering environmental stress. Under these conditions, vitamin C inhibits the activity of 21-hydroxylase and 11–β hydroxylase (key enzymes in the biochemical pathways of corticosteroids) (Brake 1989). Thus, vitamin C can prevent the negative effects of stress on immune system and performance of poultry (PardueandThaxton 1986). However, further research under farm conditions is required to better establish the benefits of vitamin C. The aim this study was to investigate of the effect of vitamin C on growth performance, carcass characteristics and digestive organs, blood plasma components, liver enzymes, carcass fat, immune system, and microbial flora of the cecum of broilers.

Materials and Methods

The study was conducted at a broiler farm in Masal, Iran. The experiment was performed with 120 one-day-old male chicks of the commercial Ross 308 strain with an average weight of 45±2g. The study was based on a completely randomized design with three treatments and four replicates of 10 chickens per pen for 42 days. The three treatments were addition of (1) no vitamin C (VC0 mg/kg diet), (2) 200 mg (VC200 mg/kg DM) and (3) 400 mg/kg DM additional vitamin C (VC400 mg/kg). All three groups were fed the same basal diet containing the minimum recommended nutrients from the Ross 308 feed guide (Manual 2012) (Table 1). The chicks were raised in 1 × 1 m cages on a bed of cellulose roll and fed the experimental diets for 42 days. Each cage contained a cylindrical feeding container and a manual chicken drinker. The temperature in the pens was 33 ºC for the first week, gradually dropped to 23 ºC by day 18 and was constant thereafter. Room humidity was set to 65 to 70% throughout the study period and included chicks were given 23 hours of light exposure and one hour of darkness. Feed and water were ad-libitum throughout the study. All birds were vaccinated against infectious bronchitis (10 days old), Newcastle disease (4, 21 and 35 days old) and infectious Bursal disease (12 days old). All the vaccines were obtained from the Razi Vaccine and Serum Institute (Karaj, Iran).

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

Ingredients (g/kg as-fed)	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
Corn gluten	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 compounds			
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
Calcium (%)	1. 04	0. 95	0. 92
Available phosphorus (%)	0. 52	0. 47	0. 41

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.

Growth performance and economic efficiency

The weight gain of chickens was calculated on a pen basis for days from 1 to 10, 11 to 24 and 25 to 42 days using a digital scale (Tozin Kala, Iran) with an accuracy of ±0. 1 g. At the end of each period (starter 1 to 10 days, grower 11 to 24 days and finisher 25 to 42 days), the amount of feed left in each feed container was weighed. The amount of consumed feed was calculated by deducting waste from the amount of feed in each period. Feed conversion ratio was calculated by dividing the feed consumption rate by the weight gain for the three periodsand for the whole period (Sigolo et al. 2019).

European production factor (EPF) was calculated using the following formula:

EPF= 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), feed was removed for two hours and two birds from each replicate with a weight close to the pen mean weight were slaughtered and weights were measured using a digital scale (A&D GF-300, A&D Weighing Design and Manufacture, San Jose, California) with an accuracy of 0.01 g (Shabani et al. 2015). Measured weight parameters included: featherless carcass, full abdomen carcass, empty abdomen carcass, abdominal fat, breast, thigh, wing, and gastrointestinal organs (pancreas, heart, gizzard, liver, abdominal fat, duodenum, jejunum, and ileum).

Parameters of blood plasma component and liver enzymes

On day 42, two birds with a weight near to the mean were selected and 5 ml of blood was taken from the wing vein. The samples were held for 12 hours at room temperature, centrifuged at 5, 000 rpm for 3 minutes (before serum separation). Serum was stored at -20˚C until further analysis. Analyses were done using Pars Azmoon commercial kits with an autoanalyzer (Hitachi 917, Japan) (Golrokh et al. 2016). The measured metabolites included glucose, triglycerides, cholesterol, total protein, albumin, globulin, creatine kinase, lactate dehydrogenase, VLDL (very-low-density lipoprotein), HDL (high-density lipoprotein), LDL (Low-density lipoprotein), alanine transferase, and alkaline phosphatase.

Immune responses

To investigate humoral immunity, the broiler chickens were immunized against SRBC (sheep red blood cell) by Lemer method (Lerner et al. 1971). To prepare the suspension, blood samples were taken from three sheep and poured into a glass container with EDTA. A 2% SRBC suspension was then prepared in PBS. SRBC injection was performed on 28 and 36 days of age to two birds per pen. Hundred microliters of the 2% SRBC solution was injected intravenously via the wing vein. Blood samples were taken on days 35 and 42 (Seidavi et al. 2014). Measurement of antibody titers against SRBC was done using the Van derzipp hemagglutination method (Pourhossein et al. 2015). To measure total anti-SRBC titre, 50 µL of serum sample was mixed with 50 µL phosphate-buffered saline (PBS) inside the microtiter plate and serial dilutions from 1:2 to 1:256 were prepared from the chicken serum followed by 50 µL per well of 2% SRBC suspension. The plates were placed at room temperature for 4 to 5 hours before reading. Titers were expressed as log2 of the highest dilution showing complete agglutination (Pourhossein et al. 2015).

Newcastle disease (NDV) and influenza antibody titers were measured on days 28 and 42. Blood was taken from two birds per pen and pooled before antibody testing. The hemagglutination inhibition (HI) test was performed on the samples according to OIE standard. 96-well microplates were used for the experiment. Initially 25 µL of PBS were 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 performed until the last well. Then, 25 µL of Newcastle and influenza antigens were added to the wells. Afterward the microplate was put on the mechanical shaker for 1 minute and then, incubated at 25˚C for 30 min. In the next step, 25 microliters of 1% red blood cells were added to all the 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). The titers were expressed as log2 of the highest dilution showing agglutination. The 1% red blood cell was obtained from specific pathogen free chicks.

On day 42, blood samples were taken from two birds of each pen, pooled, and tested for white blood cell count and differential. Then, two birds per replicate with a weight near to the mean were slaughtered. The

weights of spleen, bursa of Fabricius and thymus were measured by a digital scale with an accuracy of 0.01 g (A&D GF-300 digital scale balance (310 gr × 0. 001 gr, A&D Weighing Design and Manufacture, San Jose, CA), (Shabani et al. 2015).

Microbial flora

To investigate cecal microbial flora, two birds of each treatment were slaughtered on day 42. The abdominal cavity was opened and the right and left cecum were separated with sterile scissors, the contents of each chick was combined and frozen at -20˚C until *E. coli* enumeration (Dibaji et al. 2014). Serial dilution in distilled water was used to create 10^{-3}, 10^{-4}, and 10^{-5} dilutions of the caecal contents. Then, 300 µl of each dilution were taken and inoculated on to Eosin methylene blue Agar and incubated at 37˚C for 24 hours before colonies were determined based on CFU (Colony Forming Units). Data were log transformed before analysis (Dibaji et al. 2014).

Statistical Analysis

All data collected during the experiment and laboratory traits were analysed by proc SAS statistical software. The comparison of the means was performed with Duncan's multiple-range test with $P<0.05$ being used as the threshold for statistical significance.

Results and Discussion

Growth performance

The effects of supplementing with different levels of vitamin C on broiler performance are summarised in Tables 2 and 3. While chicks in both VC200 and VC400 groups had higher feed intakes and weight gains and lower FCR than unsupplemented chicks in the VC0 group, no diffeernces were observed between the two supplemented groups. Although the study showed that vitamin C supplementation increased chick performance, there was no evidence that increasing supplementation from 200 mg/kgto 400 mg/kg provided any additional benefit.

Results for weight of chick at day 42 (gr/chick), and European production factor showed similar significant effect of vitamin C supplementation, but no benefit was observed from increasing supplementation from 200 to 400 mg/kg. These findings are consistent with the results of Lohakareet et al. (2005) and Ciftciet et al. (2005). However, Kucuket et al. (2003) reported that under natural conditions vitamin C had insignificant effects on these traits. As there were no apparent differences between the two groups supplenented with vitamin C, feed cost per kg of live weight was the lowest in the experimental group of 200 mg/kg diet.

Table 2- Growth performance (mean ±SEM) of Ross 308 broilers at starter, grower, finisher, and whole periods of age, which fed diets containing different levels of vitamin C

	1st-10th days of age			11st-24th days of age			25th-42nd days of age			1st-42nd days of age		
Vitamin C (mg/kg)	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed Conversion ratio	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio
0	18. 10	9. 65	1. 88^{a}	48. 40^{b}	32. 71^{c}	1. 48^{a}	144. 84^{b}	68. 57^{b}	2. 12^{a}	82. 52^{b}	42. 59^{b}	1. 94^{a}
200	19. 20	12. 75	1. 51^{b}	61. 72^{a}	56. 40^{a}	1. 10^{b}	167. 56^{a}	79. 08^{a}	2. 12^{a}	96. 96^{a}	55. 73^{a}	1. 74^{b}
400	17. 40	11. 30	1. 58^{b}	60. 11^{a}	47. 93^{b}	1. 25^{b}	166. 36^{a}	84. 59^{a}	1. 97^{b}	95. 48^{a}	54. 92^{a}	1. 74^{b}
P-value	0. 55	0. 16	0. 03	0. 02	0. 0001	0. 001	0. 004	0. 002	0. 03	0. 0003	0. 0001	0. 0001
SEM	1. 136	1. 04	0. 08	2. 91	1. 68	0.05	3. 80	2. 26	0. 04	1. 66	1. 15	0. 02

[*] Means within each column of dietary treatments with no superscript letter or at least one common superscript letter does not differ significantly ($P\geq0.05$); SEM: Standard Error of Means

Table 3- Economical performance (mean ±SEM) of Ross 308 broilers at 42nd day of age fed diets containing the different levels of vitamin C

Vitamin C (mg/kg)	Weight of 1 chick at 42th days of age (g/chick)	Feed cost per kg live weight (Rial/kg)	European production factor
0	1828. 75[b]	52943. 00[a]	224. 90[b]
200	2380. 50[a]	48447. 20[b]	325. 72[a]
400	2346. 50[a]	49016. 50[b]	321. 19[a]
P-value	0. 0001	0. 0008	0. 0001
SEM	48. 20	582. 34	8. 90

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter does not differ significantly (P≥0.05); SEM: Standard Error of Means

Carcass characteristics, carcass fat, and digestive organs

The effect of the experimental treatments on carcass characteristics is summarised in Tables 4 and 5. Carcass weights were different across all three treatment groups, with chicks in the VC400 had higher weights than both control and VC200 groups, except for eviscerated carcass weight, the two supplemented groups were not separable though both were higher than the control in body weight, featherless carcass weight, full and empty abdomen weight with a reverse linear relation (P<0.05) which was consistent with the results of Lohakare et al. (2005). Relative weights of organs and muscles were also affected by vitamin C supplementation; but, increasing supplementation rate from 200 to 400 mg/kg did not affect these relative weights. In fact, the use of vitamin C removed all oxygen variants and had a protective effect against inhibition of body's antioxidant enzymes, which in turn can improve production performance (Hajati et al. 2015).

Table 4- Economically relevant carcass characteristics (mean ±SEM) of Ross 308 broilers at 42nd day of age fed diets containing the different levels of vitamin C

Vitamin C (mg/kg)	Live body weight (g)	Defeather body weight (g)	Full abdomen carcass weight (g)	Empty abdomen carcass weight (g)	Eviscerated carcass (%)	Relative weight of crop (%)	Relative weight of breast (%)	Relative weight of drumsticks (thighs) (%)	Relative weight of wings (%)	Relative weight of abdominal (%)
0	2267. 50[c]	2031. 75[c]	1869. 25[c]	1596. 50[c]	78. 54[b]	0. 44[a]	26. 93[b]	21. 10[b]	8. 27	1. 66[a]
200	2667. 50[b]	2465. 00[b]	2295. 00[b]	2032. 00[b]	82. 41[a]	0. 35[b]	32. 32[a]	27. 06[a]	7. 41	0. 43[b]
400	2921. 25[a]	2721. 00[a]	2561. 00[a]	2256. 00[a]	82. 91[a]	0. 36[b]	31. 89[a]	27. 27[a]	7. 77	0. 32[b]
P-value	0. 0001	0. 0001	0. 0001	0. 0001	0. 0001	0. 02	0. 005	0.01	0. 29	0. 0001
SEM	60. 45	59. 08	61. 97	54. 88	0. 46	0. 02	0. 93	1. 10	0. 36	0. 06

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter does not differ significantly (P≥0.05); SEM: Standard Error of Means

Table 5- Mean (±SEM) of organ characteristics of Ross 308 broilers at 42nd day of age fed diets containing the different levels of vitamin C

Vitamin C (mg/kg)	Relative weight of pancreas (%)	Relative weight of gizzard (ventriculus) (%)	Relative weight of heart (%)	Relative weight of liver (%)	Relative weight of proventriculus (%)
0	0. 32	2. 18[a]	0. 75	2. 56	0. 49[a]
200	0. 28	1. 75[b]	0. 50	2. 71	0. 39[b]
400	0. 29	1. 72[b]	0. 45	2. 34	0. 37[b]
P-value	0. 37	0. 005	0. 06	0. 25	0. 003
SEM	0. 02	0. 08	0. 08	0. 15	0. 02

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter does not differ significantly (P≥0.05); SEM: Standard Error of Means

Parts of intestine

As shown in Table 6, the results showed that the use of two different levels of vitamin C had a significant effect on some intestinal parts such as relative weight of rectum, duodenum, and ileum (P<0.05). Relative weight of cloaca and duodenum was higher in VC400 chicks than in the control group (P<0.05); but relative weight of ileum at the level 200 of vitamin C was not significant (P>0.05). In addition, the relative weight of jejunum between the two different levels of this vitamin showed a statistically significant difference as compared with the control group, (P<0.05). According to the findings of Jeburet et al. (2017), the use of vitamins C, E, aspirin, and sodium chloride in the diet of broilers led to positive effects on digestibility, relative weight, and some characteristics of the carcass, which was consistent with the findings of Stilborn et al. (1988). These results could be related to the antioxidant properties of the compounds by removing free radicals, thus improving relative weight and carcass characteristics. However, the use of this vitamin had no significant effect on relative weight of colon and cecum (P≥0.05).

Table 6- Intestinal segments (mean ±SEM) of Ross 308 broilers at 42nd day of age fed diets containing the different levels of vitamin C

Vitamin C (mg/kg)	Relative weight of cloaca (%)	Relative weight of duodenum (%)	Relative weight of jejunum (%)	Relative weight of ileum (%)	Relative weight of colon (%)	Relative weight of right cecum (%)	Relative weight of left cecum (%)
0	0. 24[a]	0. 73[a]	1. 30[a]	0. 54[b]	0. 38	0. 19	0. 19
200	0. 22[a]	0. 72[a]	0. 92[c]	0. 67[a]	0. 32	0. 16	0. 16
400	0. 18[b]	0. 57[b]	1. 16[b]	0. 47[b]	0. 33	0. 16	0. 16
P-value	0. 005	0.01	0. 0002	0. 003	0. 16	0. 11	0. 14
SEM	0. 009	0. 03	0. 04	0. 03	0. 02	0.01	0.01

[*] Means within each column of dietary treatments with no superscript letter or at least one common superscript letter does not differ significantly (P≥0.05); SEM: Standard Error of Means

Blood parameters and digestive enzymes

The results of using two different levels of vitamin C on blood parameters and liver enzymes are shown in Tables 7 and 8. The results showed that the use of vitamin C had no significant effect on blood parameters and liver enzymes (P≥0.05), which was consistent with the results of Abdulameer (2019). Although the use of vitamin C had no significant effect on triglycerides and glucose levels (P≥0.05), but the lowest amount was related to the use of VC 200, which was consistent with the results of Sahinet al (2002). No increase in serum glucose has been reported with increasing vitamin C levels in the diet. However, it can be assumed that due to the production of ascorbic acid from glucose in poultry, the need for blood glucose to synthesize ascorbic acid decreases with increasing the levels of this vitamin in the diet, and this may be due to higher levels of glucose by using higher levels of vitamin C (Krauss. 2004). Recently, medical tests have shown that antioxidants prevent the body from lowering cholesterol, which is consistent with the results of the present study. Naturally, liver cells dissolve key proteins in the structure of the lipoproteins such as VLDL. This means that VLDL is not converting to VDL, which is the most important carrier of cholesterol in blood. Antioxidants prevent this from happening in liver cells (Krauss 2004). So, no oxidation of apoprotein B in VLDL means continuing the path of lipoprotein metabolism and LDL formation (Krauss 2004). In 2002, heart health studies stated that the antioxidants such as vitamins C, E, and beta-carotene increase triglycerides, Apolipoprotein B, and LDL, which is consistent with the results of the present study. Therefore, the breakdown of apoprotein B is increased by peroxidation and reduced by antioxidants (Hallfrisch et al. 1994).

Table 7- Blood constitutes (mean ±SEM) of Ross 308 broilers at 42nd day of age fed diets containing the different levels of vitamin C

Vitamin C (mg/kg)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	VLDL (Very low density lipoprotein) (mg/dl)	HDL Cholesterol (High Density Lipoproteins) (mg/dl)	LDL Cholesterol (Low Density Lipoproteins) (mg/dl)	HDL /LDL	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
0	131. 25	76. 25	15. 28	72. 25	36. 00	0. 50	197. 75	3. 80	2. 48	1. 33
200	162. 50	60. 75	12. 15	90. 50	50. 75	0. 56	175. 00	3. 73	2. 33	1. 40
400	142. 25	78. 25	15. 65	79. 75	43. 50	0. 52	185. 50	3. 65	1. 95	1. 70
P-value	0. 42	0. 60	0. 60	0. 26	0. 48	0. 74	0. 59	0. 95	0. 72	0. 53
SEM	16. 24	13. 03	2. 60	7. 38	8. 29	0.05	15. 34	0. 33	0. 47	0. 24

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter does not differ significantly (P≥0.05); SEM: Standard Error of Means

Table 8- Liver enzymes (mean ±SEM) of Ross 308 broilers at 42nd day of age fed diets containing the different levels of vitamin C

Vitamin C (mg/kg)	Alkaline phosphatase (U/L)	Alanine transaminase (IU/L)	Lactate dehydrogenase (IU/L)	Creatine kinase (IU/L)
0	5048. 30	239. 25^{b}	4280. 00	10051. 00
200	4289. 30	372. 75^{ab}	5125. 00	26996. 00
400	3701. 30	420. 25^{a}	6085. 00	35800. 00
P-value	0. 40	0.05	0. 25	0. 22
SEM	666. 18	46. 49	713. 68	9826. 39

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter does not differ significantly (P≥0.05); SEM: Standard Error of Means

Immune system

The effect of adding different levels of vitamin C in diet on the function of the humoral immune system in response to SRBC antigen injection and antibody titer against Newcastle disease and influenza virus is shown in Tables 9 and 10. The results showed that the use of two different levels of vitamin C did not have a significant effect on the percentage of neutrophils and lymphocytes, but there was a statistically significant difference compared with the control group (P<0.05). The highest percentage of neutrophils was observed in VC 200 group. It is probably due to the protection effect of vitamin C on lymphocytes against free radicals and the strengthening of the immune system of broiler chickens. In addition, according to the findings of Ahmed et al. (2009), vitamin C plays a key role in the synthesis of leukocytes especially phagocytes and neutrophils, which make up the bulk of the poultry's immune system. Comparing the mean results of antibody titers against SRBC did not show a significant difference (P≥0.05), except for 35 days old broiler, VC 400mg/kg which was significantly higher than the control group (P<0.05). This agrees with the results of Abdulameer et al. (2019) and Mirzaporet et al. (2016) showing a significant increase in primary and secondary response against SRBC in broilers. From the comparison of the means of antibody titer against SRBC among different treatments, it can be inferred that vitamin C increases the sensitivity of the immune system, and when sheep's red blood cells enter the body, there is a significant response in the treatments containing vitamin C. In addition, the use of this vitamin had no significant effect on antibody titers against Newcastle disease and influenza virus (P≥0.05). According to the results of the present study, the highest antibody titer was related to high levels of this vitamin, which was consistent with the results of Abdulameeet et al. (2019). According to the results of Table 10, the use of two levels of vitamin C did have a significant effect on the relative weight of thymus and bursa of Fabricius (P<0.05).

Table 9- Immune response (mean ±SEM) of Ross 308 broilers fed diets containing the different levels of vitamin C

Vitamin C (mg/kg)	White blood cells (42nd day of age) ($n \times 10^3$/mL)	Neutrophils (42nd day of age) (%)	Lymphocytes (42nd day of age) (%)	Eosinophils (42nd day of age) (%)	Antibody against Newcastle disease (28th day of age) (lg 2)	Antibody against Newcastle disease (42nd day of age) (lg 2)	Antibody against avian influenza (28th day of age) (lg 2)	Antibody against avian influenza (42nd day of age) (lg 2)	Antibody against sheep red blood cell (35th day of age)	Antibody against sheep red blood cell (42nd day of age)
0	5250. 00	14. 25^{b}	80. 00^{a}	5. 50	4. 00	6. 00	2. 50	4. 50^{ab}	5. 50^{a}	6. 75
200	1100. 00	47. 25^{a}	46. 50^{b}	6. 25	2. 75	4. 75	2. 25	3. 50^{b}	6. 50^{a}	8. 00
400	2075. 00	40. 25^{a}	55. 00^{b}	5. 25	4. 25	5. 75	2. 50	5. 75^{a}	4. 00^{b}	7. 25
P-value	0. 09	0. 001	0. 001	0. 93	0. 19	0. 30	0. 77	0. 02	0. 002	0. 13
SEM	1231. 05	4. 19	4. 35	1. 90	0. 57	0. 57	0. 28	0. 43	0. 33	0. 39

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter does not differ significantly (P≥0.05); SEM: Standard Error of Means

Table 10- Immunity related organ (mean ±SEM) of Ross 308 broilers at 42nd day of age fed diets containing the different levels of vitamin C

Vitamin C (mg/kg)	Relative weight of thymus (%)	Relative weight of spleen (%)	Relative weight of bursa of Fabricius (%)
0	0. 49^{a}	0. 13	0. 20^{a}
200	0. 35^{b}	0. 10	0. 08^{b}
400	0. 35^{b}	0. 10	0. 06^{b}
P-value	<0. 0001	0. 27	<0. 0001
SEM	0.01	0.01	0. 006

* Means within each column of dietary treatments with no superscript letter or at least one common superscript letter does not differ significantly (P≥0.05); SEM: Standard Error of Means

Microbial flora

Table 11- Sacrosemicroflora Ross 308 broilers at 42nd day of age fed diets containing different amounts of vitamin C

Vitamin C (mg/kg)	*Escherichia coli* bacteria (log10 CFU/gr)
0	8. 66
200	8. 4
400	8. 0

4. Conclusion

Dietary feeding of vitamin C in broiler chickens of Ross 308 strain improved feed intake, weight gain, conversion ratio, cost per kilogram of live chicken meat and European production factor. Although vitamin C was not very effective on blood parameters, it improved the immune system and reduced the fat content of the ventricular area as well as the quality of carcass meat. Therefore, according to the results of this experiment, it is recommended to use 200 mg/kg vitamin C in diet as an antioxidant compound, which is an inexpensive promoter of growth.

The effect of vitamin C on the population of *E. coli* is shown in Table 11. The results showed that the population of *E. coli* decreased with the use of high levels of vitamin C, which is consistent with the findings of Hajatiet et al. (2015). Increasing free radicals oxidizes and destroys biological cells, hence causing several intestinal tissue disorders (Ocak et al. 2008; Sahin et al. 2003). The presence of some antioxidants, including vitamin C may solve the problems related to intestinal disorders and improve the performance traits (Wang et al. 2008), which is consistent with the results of Nosratiet et al. (2017).

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اثرات مکمل جیره‌ای ویتامینc بر عملکرد رشد، صفات لاشه، اندام‌های گوارشی، آنزیم‌های کبدی، چربی‌بطنی، سیستم ایمنی و فلور میکروبی جوجه‌های گوشتی

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چکیده

زمینه مطالعاتی: ویتامینc برای کاهش تجمع چربی در بدن و حفظ سلامت طیور مفید است. **هدف:** این آزمایش جهت بررسی بهبود عملکرد رشد، خصوصیات لاشه و اندام‌های گوارشی، اجزای پلاسمای خون، آنزیم‌های کبدی، سیستم ایمنی، فلورمیکروبی سکوم و پروفایل اسیدهای‌چرب گوشت سینه جوجه‌های گوشتی با افزودن ویتامینc به جیره مورد بررسی قرار گرفت. **روش کار:** سه سطح مختلف ویتامینC (۰، ۲۰۰ و ٤۰۰ میلی‌گرم در کیلوگرم خوراک) در ۳ تیمار و ٤ تکرار و ۱۰ قطعه جوجه در هر پن به مدت ٤۲ روزبا استفاده از ۱۲۰ قطعه جوجه نر یک‌روزه گوشتی سویه تجاری راس ۳۰۸درقالب طرح کاملاًتصادفی مورد آزمایش قرار گرفت. **نتیجه:** نتایج نشان داد که در دوره ۱ تا ٤۲ روزگی، جوجه‌های تغذیه شده با جیره حاوی ۲۰۰ میلی‌گرم در کیلوگرم ویتامین C دارای بیشترین خوراک مصرفی و افزایش وزن و بهترین ضریب تبدیل غذایی نسبت به سایر تیمارها بودند. همچنین طبق جدول کمترین هزینه تمام شده هر کیلوگرم مرغ زنده و بهترین شاخص اروپایی مربوط به همین سطح از ویتامین C بود. اثر ویتامینC بر وزن زنده، وزن بدون پر، وزن لاشه شکم پر، وزن نسبی چینه دان و وزن نسبی سینه معنی‌دار بود (P< ۰/۰۵) و بیشترین افزایش مربوط به سطح ۲۰۰ میلی‌گرم در کیلوگرم ویتامین C بود. از طرفی استفاده از سطوح متفاوت ویتامین Cبر پارامترهای خونی و آنزیم‌های کبدی جوجه‌های گوشتی معنی‌دار نبود (P> ۰/۰۵). علاوه بر آن اثر تیمارها بر درصد نوتروفیل و لنفوسیت معنی‌دار شد(P< ۰/۰۵) و بیشترین درصد نوتروفیل در سطح ۲۰۰ میلی‌گرم در کیلوگرم ویتامین C مشاهده شد. مقایسه میانگین‌های نتایج حاصل از تیتر آنتی بادی علیه SRBC تفاوت معنی‌داری را نشان نداد (P> ۰/۰۵)، بجز ۳۵ روزگی که معنی‌دار شد (P< ۰/۰۵)، ولی از نظر عددی بیشترین تیتر آنتی بادی مربوط به سطح ۲۰۰ میلی‌گرم در کیلوگرم ویتامین C بود. **نتیجه گیری نهایی:** براساس نتایج تحقیق حاضر، استفاده از سطح ۲۰۰ میلی‌گرم در کیلوگرم ویتامین Cجهت مکمل سازی جیره غذایی جوجه‌های گوشتی سویه راس ۳۰۸ توصیه می-شود.

واژگان کلیدی: جوجه گوشتی، اسیداسکوربیک، سیستم ایمنی، عملکرد، فراسنجه‌های خونی و آنزیم‌های کبدی