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Effects of Horsetail (*Equisetum arvense*), Vitamin C and Organic Zinc Supplements on Growth Performance, Carcass Yield, Serum Biochemical Values and Antioxidant Status of Broiler Chickens

Research Article

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ABSTRACT

This study was conducted to evaluate the effect of horsetail (HT), vitamin C (VC) and organic zinc (OZ) supplements on growth performance, carcass yield, serum biochemical values and antioxidant status of broiler chickens. A total of 480, one-day-old male broiler chicks (Arbor Acres Plus) were allocated to 8 treatments diets in a 2 × 2 × 2 factorial experiment including 2 levels of HT (0 and 0.5%), 2 levels of VC (0 and 250 mg/kg) and 2 levels of OZ (0 and 60 mg/kg). Results showed that feed intake (FI) was affected by HT and HT × VC × OZ interaction during starter period. During the finisher period, consumption of 250 mg/kg VC resulted in highest FI as compared with other treatments. During the whole experimental period, FI was affected by dietary incorporation of HT, VC, OZ, and their interaction. Moreover, body weight gain (BWG) was influenced by dietary incorporation of HT, VC, OZ, and their interaction during the starter period. Furthermore, greater proportional liver weight was observed in OZ birds. Proportional abdominal fat pad weight was decreased in VC birds as compared to control birds. Likewise, feeding HT diets decreased serum low-density lipoprotein cholesterol (LDL-c). Dietary supplementation of VC increased activity of serum glutathione peroxidase (GSH-Px) and the highest amount of total antioxidant capacity (TAC) was also observed in chicks fed HT. Basically, it can be concluded that dietary supplementation of 60 mg/kg OZ can improve the liver function and these data suggest that 0.5% HT may have a beneficial effect on serum antioxidant in broilers.

KEY WORDS broiler, glutathione peroxidase, horsetail, low-density lipoprotein cholesterol, total antioxidant capacity.

INTRODUCTION

Increasing the productive ability and health of broiler chickens is the primary goal of investigators and producers in poultry production farms. Hence, the primary objective of poultry nutrition is to provide a nutritionally balanced mixture of ingredients to support the maintenance, growth, reproductive performance, meat quality and health of the bird at an acceptable cost. One of the most effective ways for a profitable poultry industry is using feed additives and supplements (Hatab, 2011). Horsetail (HT) is a survivor of a very ancient group of vascular plants, the Sphenophyta, which has a history reaching back to the Upper Devonian, approximately 377 million years ago. This plant occupies a wide range of habitats in many countries, including the north of Iran. HT contains a high amount of polyphenols, fixed and volatile oils and a variety of pharmacologically active substances, such as benzyl acetate and Linalool (Tufarelli *et al*. 2021). Broilers require VC for the synthesis of steroid hormones, amino acid and mineral metabolism

and to maintain immunity and to respond to physiological stress. Endogenous vitamin C (VC) synthesis is usually considered not sufficient for the biological demands of poultry, especially during extrem environmental conditions. The kidneys are the principal organ for chickens to synthesize VC, but cannot synthesize adequate amount of VC until 15 days of age. Vaccination is also a stressor that can hinder with adequate biosynthesis of VC in chickens, thus dietary supplementation of VC is often beneficial (Amakye-Anim *et al*. 2000).

Zinc is the other important micro element that plays, directly or indirectly, a vital role in the growth and development of broilers and so it is important to meet the minimum requirements of zinc in broiler diets. The standard source for zinc supplementation in poultry diets has been zinc sulfate, but in recent years, organic sources of zinc has become common usage due to their potential higher bioavailability and less environmental impact through manure loading (Martin, 2016). Because of the higher bioavailability of organic zinc (OZ) sources as compared to inorganic forms (oxide and sulphate), more attention has been paid to the OZ sources (Abd El-Hack *et al*. 2017). Dietary Zn supplementation more than recommended levels, either from organic or inorganic sources, may positively impact the birds' appetite, productive and reproductive efficiency, skeletal and skin health. So, the objective of current study was to evaluate the effect of dietary supplementation of HT, VC and OZ alone or in combination on performance, carcass yield and serum biochemical values and antioxidant status of broiler chickens.

MATERIALS AND METHODS

Procedures related to animal care, handling and sampling were conducted under the approval of Institutional Animal Care and Use Committee of Urmia University (Urmia, Iran). Four hundred and eighty, one-day-old male chicks (Arbor Acres Plus, 42±1 g) were provided from a local hatchery and used in 8 treatments with 6 replicates and 10 birds in each replicate in a factorial arrangement 2 × 2 × 2 based a completely randomized design. The corn and soybean meal used for formulating the experimental diets were analyzed for nutrients by near-infrared spectroscopy (NIR) in Evonik Industries laboratory (North Rhine-Westphalia, Germany). The broiler diets were formulated (Table 1) based on nutrient recommendation for Arbor Acres Plus during the starter (1–10 d), grower (11–24 d) and finisher (25–42 d) periods. The HT (0 and 0.5%), VC (0 and 250 mg/kg) and OZ (0 and 60 mg/kg) were the main factors. HT powder was provided from a Darvash Giah Khazar medicinal herbs complex company (Gilan, Rasht, Iran) and analysed for the active components (Albadri, 2016) before the experiment (Table 2).

The VC (50% pure) used in this trial was provided from a commercial company (Rooyan Darou Pharmaceutical, Iran). The OZ (35 mg/kg zinc) was provided by Danesh Pazhohan Novin Khorak Company (Urmia, Iran). All birds had free access to feed and water throughout the experiment. A 23L: 1D h light program was applied throughout the experiment.

Table 1 Composition of experimental diets

Ingredients, %	Starter (0-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Maize	55.88	59.09	64.96
Soybean meal (44% CP)	39.28	35.67	30.42
Soybean oil	0.68	1.49	1.18
Dicalcium phosphate	1.64	1.45	1.27
Calcium CO_3	1.14	1.06	1.00
Lysine-HCL	0.23	0.19	0.17
DL-methionine	0.23	0.19	0.16
Threonine	0.11	0.07	0.05
Vitamin-mineral mix[1]	0.50	0.50	0.50
Sodium chloride	0.31	0.29	0.29
Total	100	100	100
Calculated analysis			
Metabolisable energy, kcal/kg	2850	2945	3000
Crude protein, %	21.85	20.42	18.5
Fiber, %	3.9	3.70	3.50
Calcium, %	0.91	0.83	0.74
Available phosphorus, %	0.45	0.41	0.37
Chloride, %	0.22	0.22	0.22
Sodium, %	0.14	0.14	0.14
Methionine, %	0.53	0.48	0.44
Lysine, %	1.36	1.22	1.10
Methionine + cysteine, %	1.02	0.94	0.86

[1] Supplied per kilogram of diet: vitamin A: 9000 U; vitamin D_3: 2000 U; vitamin E: 18 U; vitamin B_{12}: 0.15 mg; Riboflavin: 6.6 mg; Calcium pantothenate: 10 mg; Niacin: 30 mg; Choline: 500 mg; Biotin: 0.1 mg; Thiamine: 1.8 mg; Pyridoxine: 3 mg; Folic acid: 1 mg; vitamin K_3: 2 mg; Antioxidant (ethoxyquin): 100 mg; Zinc: 50 mg; Manganese oxide: 100 mg; Copper: 10 mg; Fe: 50 mg; I: 1 mg and Se: 0.2 mg.

The body weight and feed intake (FI) of the birds in each replicate pen were determined at the end of starter, grower and finisher periods and body weight gain (BWG), FI and FCR were calculated based on hen day.

At the end of the experiment (day 42), two birds from each replicate were randomly selected and then slaughtered. Carcass, liver, abdominal fat, heart, gizzard, pancreas, proventriculus, spleen and bursa of Fabricius were weighted and their relative weights to live body weight were determined. At slaughter, blood samples were collected from the birds for determination serum glucose, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid, triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations.

Table 2 Nutrient content[1] and phytochemical[2] of horsetail

Specification	Value
Dry matter (%)	92.14
Crude protein (%)	13.53
Crude fat (%)	1.56
Crude fiber (%)	11.90
Ash (%)	18.34
Calcium (%)	1.32
Phosphorus (%)	0.20
Magnesium	0.97
Sodium (%)	0.72
Starch (%)	11.7
Sugar (%)	3.54
Carbohydrate (%)	46.81
Selenium (mg/100 g)	1.00
Iron (mg/100 g)	29.40
Copper (mg/100 g)	4.30
Manganese (mg/100 g)	1.50
Zinc (mg/100 g)	7.50
Total Polyphenols (%)	1.46
Tannins (%)	0.16
Vitamin E (µg/100 g)	70
Gross energy (kcal kg^{-1})	2673
Linalool (%)	0.51
Benzyl acetate (%)	4.32
Cyclamen aldehyde (%)	2.51
Dimethylbenzylcarbinyl butyrate (%)	11.51
2-Nonenal, 2-pentyl (%)	0.19
Undecalactone (%)	81.80

[1] All Nutrient values analysis by ViroMed Central Analytical Lab which is also called the Samaneh Payesh Salamat laboratory complex located in Pardis technology park, Tehran, Iran.
[2] Phytochemical analysis by KSP Lab which is also called the Kimia Shengerf Pars laboratory complex located in Tehran, Iran.

The amount of serum indices were measured with a spectrophotometer (Alcyon 300, USA) using commercial kits (Pars Azmon, Iran).

The total antioxidant capacity (TAC) and activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were determined in serum samples using RANDOX kits (Germany) according to the manufacturer's instruction. Serum SOD activity was assayed by the xanthine oxidase method (Tufarelli *et al.* 2022), which monitors the degree of inhibition of nitroblue tetrazolium reduction by O_2-generated by xanthine and xanthine oxidase; the absorbance was read at 550 nm using a spectrophotometer (UV-1201, Shimadzu, Japan). Serum lipid peroxidation (LP) was determined using the method proposed by Kei (1978) and Yagi (1984), but with 1,1,3,3-tetraethoxypropane as the standard. The malondialdehyde (MDA) forms a pink-colored complex with thiobarbituric acid (TBA). The absorbance of solution containing the complex was measured at 532 nm using a spectrophotometer (UV-1201, Shimadzu, Japan). The Serum LP values were expressed in terms of MDA as µmol/L serum.

Statistical analysis

Data were analyzed as a 2 × 2 × 2 factorial arrangement using the GLM procedure of SAS 9. 4 packages (SAS, 2013). The statistical model included all fixed main effects and interactions of dietary HT (0 and 0.5%), VC (0 and 250 mg/kg) and OZ (0 and 60 mg/kg). Pen was considered the experimental unit for performance criteria. Effects were considered significant at a probability of 5%. Difference among the treatment means was tested for significance using Tukey's multiple-range test.

RESULTS AND DISCUSSION

The effects of experimental diets on FI of broiler chickens are shown in Table 3. FI were affected (P<0.05) by dietary addition of HT during starter period. Consumption of 0.5% HT increased the FI during starter- and total periods. Dietary VC inclusion increased the FI during the finisher and total periods. Dietary OZ inclusion only increased FI during total period. Increasing the VC to 250 mg/kg, caused the higher FI at the 0.0 HT but it did not change the FI at 0.5% HT (HT×VC interaction) during starter and total periods. Moreover, increasing the HT to 0.5%, led the higher FI at the 0.0 OZ but did not affected the FI at 60 mg/kg OZ (HT×OZ interaction) during starter and total periods. Increasing the VC to 250 mg/kg, increased the FI at the 0.0 OZ but did not change the FI at 60 mg/kg OZ (VC×OZ interaction) during finisher and total periods. In addition, rather than combination of all three supplements together, inclusion of HT and OZ during starter period, VC and VC along with OZ during finisher period and all treatments except control during the total period increased the FI (HT×VC×OZ interaction).

During the starter period, BWG was improved by dietary incorporation of HT, VC and OZ (Table 4, P<0.05). The improvements due to the all there supplements was not changed at presence of the others (HT×VC, HT×OZ, VC×OZ and HT×VC×OZ). However, during the other phases (grower, finisher and total period), BWG was not affected by HT, VC and OZ or their interactions (P>0.05). The effects of dietary supplements on FCR in broiler chicks have been presented in Table 5. No effects of HT, VC and OZ or their interactions was detected on FCR during the different growth phases (P>0.05). The influence of dietary supplements on carcass traits in male broiler chicks was shown in Tables 6 and 7. The various carcass yield factors (carcass, breast and thigh) and organ weight (heart, spleen, gizzard, pancreas, proventriculus and bursa) did not differ significantly except for liver and abdominal fat (P<0.05). We have observed significantly (P<0.05) increased on liver weight in birds fed OZ in comparison with controls.

Table 3 Effect of experimental diets on feed intake (g/d/bird) of broiler chickens

Item	Starter (0-10 d)	Grower (11-24 d)	Finisher (25-42 d)	Total (1-42 d)
HT %				
0	23.88[b]	72.59	169.98	88.82[b]
0.5	25.59[a]	73.75	171.42	90.25[a]
SEM	0.30	0.50	1.09	0.44
VC (mg/kg)				
0	24.38	72.77	169.05[b]	88.74[b]
250	25.09	73.57	172.35[a]	90.34[a]
SEM	0.30	0.50	1.09	0.44
OZ (mg/kg)				
0	24.34	72.59	169.24	88.73[b]
60	25.13	73.76	172.15	90.35[a]
SEM	0.30	0.50	1.09	0.44
HT × VC × OZ				
0 × 0 × 0	23.12[b]	70.34	162.56[b]	85.34[b]
0 × 250 × 0	23.90[ab]	73.15	172.62[a]	89.89[a]
0.5 × 0 × 0	24.65[ab]	73.34	170.51[ab]	89.50[a]
0.5 × 250 × 0	25.70[a]	73.54	171.28[ab]	90.18[a]
0 × 0 × 60	23.81[ab]	73.52	172.07[ab]	89.80[a]
0.5 × 0 × 60	25.95[a]	73.91	171.07[ab]	90.31[a]
0 × 250 × 60	24.71[ab]	73.38	172.68[a]	90.26[a]
0.5 × 250 × 60	26.07[a]	74.22	172.80[a]	91.03[a]
SEM	0.61	1.00	2.18	0.88
P-value				
HT	0.01	0.11	0.35	0.02
VC	0.11	0.27	0.04	0.01
OZ	0.08	0.10	0.07	0.01
HT × VC	0.01	0.24	0.11	0.01
HT × OZ	0.01	0.13	0.18	0.02
VC × OZ	0.19	0.20	0.03	0.01
HT × VC × OZ	0.01	0.23	0.04	0.002

HT: Horsetail; VC: vitamin C and OZ: organic zinc.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of experimental diets on body weight gain (g/d/bird) of broiler chickens

Item	Starter (0-10 d)	Grower (11-24d)	Finisher (25-42d)	Total (1-42 d)
HT %				
0	20.94[b]	55.56	72.40	49.73
0.5	22.05[a]	55.50	75.13	51.04
SEM	0.37	1.44	2.42	0.86
VC (mg/kg)				
0	20.81[b]	55.42	72.51	49.58
250	22.18[a]	55.64	75.72	51.18
SEM	0.37	1.44	2.42	0.86
OZ (mg/kg)				
0	20.83[b]	55.25	73.10	49.73
60	22.16[a]	55.81	75.13	51.04
SEM	0.37	1.44	2.42	0.86
HT × VC × OZ				
0 × 0 × 0	17.95[b]	54.48	67.74	46.73
0 × 250 × 0	21.91[a]	55.81	74.64	50.79
0.5 × 0 × 0	21.46[a]	55.84	73.61	50.18
0.5 × 250 × 0	22.00[a]	55.23	76.39	51.21
0 × 0 × 60	22.01[a]	56.00	73.32	50.44
0.5 × 0 × 60	21.83[a]	55.73	75.34	50.97
0 × 250 × 60	21.89[a]	55.97	73.90	50.59
0.5 × 250 × 60	22.91[a]	55.55	77.96	52.14
SEM	0.75	2.88	4.85	1.72
P-value				
HT	0.05	0.97	0.32	0.22
VC	0.01	0.91	0.35	0.19
OZ	0.02	0.78	0.55	0.28
HT × VC	0.04	0.94	0.56	0.33
HT × OZ	0.03	0.95	0.69	0.42
VC × OZ	0.001	0.95	0.67	0.32
HT × VC × OZ	0.002	0.98	0.90	0.55

HT: Horsetail; VC: vitamin C and OZ: organic zinc.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of experimental diets on feed conversion ratio (FCR) of broiler chickens

Item	Starter (0-10 d)	Grower (11-24d)	Finisher (25-42d)	Total (1-42 d)
HT %				
0	1.15	1.32	2.41	1.79
0.5	1.17	1.34	2.30	1.78
SEM	0.02	0.03	0.08	0.03
VC (mg/kg)				
0	1.18	1.32	2.39	1.79
250	1.14	1.34	2.31	1.77
SEM	0.02	0.03	0.08	0.03
OZ (mg/kg)				
0	1.18	1.32	2.36	1.79
60	1.13	1.34	2.34	1.78
SEM	0.02	0.03	0.08	0.03
HT × VC × OZ				
0 × 0 × 0	1.31	1.30	2.51	1.83
0 × 250 × 0	1.09	1.32	2.32	1.77
0.5 × 0 × 0	1.14	1.33	2.37	1.78
0.5 × 250 × 0	1.20	1.34	2.25	1.77
0 × 0 × 60	1.08	1.31	2.39	1.79
0.5 × 0 × 60	1.18	1.33	2.30	1.78
0 × 250 × 60	1.13	1.35	2.42	1.79
0.5 × 250 × 60	1.14	1.37	2.25	1.76
SEM	0.05	0.06	0.16	0.06
P-value				
HT	0.67	0.26	0.32	0.57
VC	0.32	0.56	0.50	0.57
OZ	0.19	0.73	0.85	0.81
HT × VC	0.54	0.88	0.66	0.86
HT × OZ	0.46	0.93	0.76	0.93
VC × OZ	0.33	0.90	0.81	0.90
HT × VC × OZ	0.13	0.99	0.95	0.99

HT: Horsetail; VC: vitamin C and OZ: organic zinc.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effect of dietary treatments on proportional carcass and internal organ weights[1] of broiler chickens at day 42 of age

Item	Carcass	Breast	Thigh	Heart	Spleen	Liver
HT %						
0	61.58	26.21	19.88	0.49	0.09	1.89
0.5	65.14	24.59	21.09	0.43	0.10	1.91
SEM	0.88	0.69	0.68	0.01	0.004	0.04
VC (mg/kg)						
0	62.32	25.15	19.43	0.43	0.10	1.87
250	64.40	25.33	19.78	0.48	0.09	1.91
SEM	0.88	0.69	0.68	0.01	0.004	0.04
OZ (mg/kg)						
0	62.86	25.20	19.21	0.46	0.10	1.82[b]
60	63.86	25.80	20.36	0.47	0.09	1.96[a]
SEM	0.88	0.69	0.68	0.01	0.004	0.04
HT × VC × OZ						
0 × 0 × 0	60.70	23.73	16.19	0.45	0.11	1.68
0 × 250 × 0	62.48	23.51	18.56	0.50	0.09	1.80
0.5 × 0 × 0	64.76	27.26	21.07	0.41	0.10	1.90
0.5 × 250 × 0	63.49	26.30	21.03	0.46	0.09	1.92
0 × 0 × 60	60.91	26.32	19.94	0.42	0.09	2.11
0.5 × 0 × 60	62.92	25.79	21.94	0.42	0.11	1.81
0 × 250 × 60	62.23	24.82	19.22	0.54	0.08	1.96
0.5 × 250 × 60	69.39	25.79	20.32	0.48	0.09	1.98
SEM	2.85	1.59	1.19	0.03	0.009	0.09
P-value						
HT	0.08	0.15	0.06	0.07	0.35	0.88
VC	0.31	0.51	0.99	0.07	0.07	0.58
OZ	0.62	0.72	0.37	0.87	0.59	0.05
HT × VC	0.23	0.46	0.19	0.06	0.14	0.23
HT × OZ	0.30	0.23	0.12	0.50	0.52	0.10
VC × OZ	0.55	0.90	0.65	0.10	0.19	0.10
HT × VC × OZ	0.48	0.69	0.43	0.12	0.44	0.08

[1] Carcass traits presented as percentage of live body weight.
HT: Horsetail; VC: vitamin C and OZ: organic zinc.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effect of dietary treatments on proportional internal organ weights[1] of broiler chickens at day 42 of age

Item	Abdominal fat	Gizzard	Pancreas	Proventriculus	Bursa
HT %					
0	0.99	2.55	0.21	0.43	0.14
0.5	0.86	2.46	0.24	0.43	0.16
SEM	0.04	0.06	0.008	0.01	0.009
VC (mg/kg)					
0	1.15[a]	2.46	0.22	0.42	0.14
250	0.78[b]	2.58	0.24	0.44	0.16
SEM	0.04	0.06	0.008	0.01	0.009
OZ (mg/kg)					
0	0.98	2.69	0.22	0.44	0.14
60	0.88	2.33	0.23	0.41	0.16
SEM	0.04	0.06	0.008	0.01	0.009
HT × VC × OZ					
0 × 0 × 0	1.36[a]	2.23	0.20	0.45	0.10
0 × 250 × 0	0.75[b]	2.65	0.20	0.44	0.17
0.5 × 0 × 0	1.00[ab]	2.56	0.22	0.43	0.14
0.5 × 250 × 0	0.75[b]	2.39	0.24	0.45	0.14
0 × 0 × 60	0.95[ab]	2.25	0.23	0.39	0.17
0.5 × 0 × 60	0.94[ab]	2.09	0.21	0.43	0.16
0 × 250 × 60	0.75[b]	2.65	0.25	0.41	0.13
0.5 × 250 × 60	0.74[b]	2.43	0.26	0.42	0.15
SEM	0.09	0.16	0.01	0.02	0.01
P-value					
HT	0.06	0.50	0.07	0.96	0.96
VC	0.02	0.14	0.46	0.24	0.44
OZ	0.21	0.38	0.59	0.20	0.15
HT × VC	0.09	0.08	0.10	0.71	0.74
HT × OZ	0.19	0.59	0.08	0.42	0.58
VC × OZ	0.06	0.10	0.73	0.36	0.07
HT × VC × OZ	0.04	0.15	0.14	0.39	0.20

[1] Carcass traits presented as percentage of live body weight.
HT: Horsetail; VC: vitamin C and OZ: organic zinc.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Treatments did not have any significant effect on the serum biochemical values (Table 8; P>0.05). Serum lipids concentration is presented in Table 9. The triglycerides, cholesterol and HDL-C level in serum was not affected by dietary treatments (P>0.05). LDL-c level was significantly lower in the HT than the control. LDL-c level was the same in group VC and OZ. Effect of VC × OZ × HT and their interaction were found nonsignificant (P>0.05). There were no significant effects on serum TSOD and MDA, whereas GSH-Px contents of hens fed VC were significantly higher (P<0.05) than control group (Table 10). Additionally, broiler chickens fed diets supplemented with HT showed higher TAC activity in the serum than the other treatments (P<0.05).

Improvements of FI by dietary HT may also be due to the active components of HT that enhance palatability of the ration. The benzyl acetate content of HT is mainly used in the flavour and fragrance industry. Another active compound of HT is linalool. *In vitro* studies have investigated the antimicrobial, antifungal and anti-inflammatory properties and also other results strengthen the suggestion that feeding linalool rich essential oils can be useful as a mean to attain relaxation and counteract anxiety (Barbarestani *et al*. 2020). These findings are in agreement with the study of Adaszynska-Skwirzynska and Szczerbinska (2019), who reported that FI was significantly improved by the addition of linalool in broiler chicken drinking water. Baghban-Kanani *et al*. (2016) reported that adding the medicinal plant in the broiler diets improved FI. These results were not in agreement with those reported by Tufarelli *et al*. (2021) who found that hens fed on diets with 0.25 and 0.50% of HT had no effect on FI. No comparable data were found in the available studies on the influence of HT on FI in broilers. The relative increase in FI in favor of birds on VC diets compared to control birds can be attributed to enhance of immune system through number of activities that could conceivably contribute to its immune-modulating effects. It is a highly effective antioxidant, due to its ability to readily donate electrons, thus protecting important biomolecules (proteins, lipids, carbohydrates, and nucleic acids) from damage by oxidants generated during normal cell metabolism and through exposure to toxins and pollutants (e.g., cigarette smoke). Vitamin C is also a cofactor for a family of biosynthetic and gene regulatory monooxygenase and dioxygenase enzymes (Carr and Maggini, 2017) which VC contributes and this agrees with the work of Shadman *et al*. (2022), who stated that VC in ration affect FI positively.

Table 8 Effect of treatments on some serum biochemical values concentration (mg/dL)

Item	Glucose	Total protein	AST	ALT	Uric acid
HT %					
0	231.70	3.81	303.42	4.25	8.30
0.5	232.00	3.69	300.83	3.95	7.42
SEM	2.98	0.12	8.93	0.29	0.50
VC (mg/kg)					
0	230.50	3.8	302.75	4.20	8.41
250	233.45	3.7	301.50	4.00	7.31
SEM	2.98	0.12	8.93	0.29	0.50
OZ (mg/kg)					
0	232.70	3.61	299.63	4.16	7.82
60	231.00	3.89	304.63	4.04	7.90
SEM	2.98	0.12	8.93	0.29	0.50
HT × VC × OZ					
0 × 0 × 0	232.50	3.53	293.67	4.83	8.88
0 × 250 × 0	235.16	3.65	300.33	4.00	7.55
0.5 × 0 × 0	230.00	3.68	300.83	3.83	7.87
0.5 × 250 × 0	233.16	3.60	303.67	4.00	6.98
0 × 0 × 60	228.16	4.28	310.33	4.00	8.95
0.5 × 0 × 60	230.33	3.73	306.17	4.16	7.93
0 × 250 × 60	231.00	3.80	309.33	4.02	7.81
0.5 × 250 × 60	234.50	3.75	292.67	3.83	6.91
SEM	5.97	0.24	17.86	0.58	1.01
P-value					
HT	0.94	0.47	0.83	0.48	0.23
VC	0.45	0.53	0.92	0.61	0.13
OZ	0.68	0.11	0.69	0.76	0.91
HT × VC	0.85	0.77	0.98	0.80	0.25
HT × OZ	0.88	0.24	0.85	0.82	0.67
VC × OZ	0.84	0.32	0.92	0.89	0.48
HT × VC × OZ	0.90	0.51	0.90	0.94	0.78

HT: Horsetail; VC: vitamin C and OZ: organic zinc.
SEM: standard error of the means.

Table 9 Effect of treatments on serum lipids concentration (mg/dL)

Item	Triglyceride	Cholesterol	HDL-c	LDL-c
HT %				
0	99.41	165.54	67.98	45.45[a]
0.5	100.41	164.79	70.29	38.70[b]
SEM	4.18	4.65	2.79	2.26
VC (mg/kg)				
0	101.29	165.50	68.18	44.45
250	98.54	164.83	70.10	39.70
SEM	4.18	4.65	2.79	2.26
OZ (mg/kg)				
0	100.91	165.37	68.14	43.45
60	98.91	164.95	70.06	40.70
SEM	4.18	4.65	2.79	2.26
HT × VC × OZ				
0 × 0 × 0	108.50	169.00	63.41	55.00
0 × 250 × 0	95.67	164.17	69.00	43.83
0.5 × 0 × 0	95.00	160.83	70.08	35.82
0.5 × 250 × 0	104.50	167.50	71.32	39.16
0 × 0 × 60	97.33	166.50	68.93	45.66
0.5 × 0 × 60	104.33	165.67	70.00	41.33
0 × 250 × 60	96.17	162.50	70.58	37.33
0.5 × 250 × 60	97.83	165.17	70.75	38.50
SEM	8.37	9.30	5.59	4.53
P-value				
HT	0.86	0.90	0.57	0.04
VC	0.64	0.91	0.61	0.14
OZ	0.73	0.94	0.62	0.39
HT × VC	0.84	0.93	0.82	0.06
HT × OZ	0.90	0.97	0.85	0.07
VC × OZ	0.93	0.98	0.90	0.45
HT × VC × OZ	0.90	0.90	0.95	0.09

HT: Horsetail; VC: vitamin C and OZ: organic zinc.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 10 Effect of dietary treatments on serum glutathione peroxidase (GSH-Px) activity, total superoxide dismutase (TSOD), total antioxidant capacity (TAC) and malondialdehyde (MDA)

Item	GSH-Px (u/gr Hb)	TSOD (u/gr Hb)	TAC (µmol/L)	MDA (µmol/L)
HT %				
0	44.00	4.14	4.10[b]	4.55
0.5	44.79	4.53	4.57[a]	4.17
SEM	0.41	0.15	0.14	0.14
VC (mg/kg)				
0	43.68[b]	4.14	4.16	4.55
250	45.10[a]	4.53	4.51	4.17
SEM	0.41	0.15	0.14	0.14
OZ (mg/kg)				
0	43.86	4.25	4.21	4.52
60	44.93	4.42	4.45	4.20
SEM	0.41	0.15	0.14	0.14
HT × VC × OZ				
0 × 0 × 0	41.90	3.78	3.56	5.24
0 × 250 × 0	44.48	4.40	4.33	4.36
0.5 × 0 × 0	44.39	4.28	4.41	4.32
0.5 × 250 × 0	44.68	4.53	4.55	4.18
0 × 0 × 60	44.40	4.12	4.13	4.50
0.5 × 0 × 60	44.05	4.36	4.54	4.16
0 × 250 × 60	45.23	4.24	4.39	4.11
0.5 × 250 × 60	46.03	4.97	4.76	4.03
SEM	0.82	0.31	0.28	0.28
P-value				
HT	0.11	0.08	0.02	0.06
VC	0.02	0.08	0.08	0.06
OZ	0.07	0.43	0.23	0.11
HT × VC	0.06	0.09	0.06	0.06
HT × OZ	0.15	0.28	0.08	0.10
VC × OZ	0.06	0.29	0.22	0.11
HT × VC × OZ	0.06	0.35	0.14	0.11

HT: Horsetail; VC: vitamin C and OZ: organic zinc.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Previously, Safavinia *et al.* (2021) confirmed that increased VC in ration increased immune system in birds and thus, increasing FI.

Ao *et al.* (2006) observed that a chelated Zn proteinate (Bioplex® zinc) increased FI with increasing levels (from 5 to 40 mg/kg diet). Liu *et al.* (2013) observed that broilers fed the diets enriched with Zn proteinate (10, 20, 40, or 80 mg/kg) had higher FI than the control. Wang *et al.* (2016) reported that FI increased without affecting feed efficiency due to supplementation of 600 mg/kg of OZ compared with the basal control diet (80 mg/kg) and other treatments.

In this study, during the starter period, BWG was influenced by dietary incorporation of HT, VC, OZ and their interaction, indicating the efficient utilization of feed. The findings recorded in present study are in agreement with those recorded by Edrise *et al.* (1986). Similarly, Lohakare *et al.* (2005) have evaluated the efficacy of supplemental VC on the performance of broiler chickens and reported significantly higher BW in the supplemental groups at higher levels as compared with controls. Rajput *et al.* (2009) have also recorded increased BW in broiler birds supplemented with VC at 500 mg/kg of feed in comparison with control birds. The control registered significantly less mean BW of broilers than the treatments, indicating thereby a significant beneficial effect of using VC in the diet of broilers. In contrast, Vathana *et al.* (2002) reported that during the first three weeks, no difference in BW among different treatment groups was detected. These contrasting results are presumably related to differences in strains of chickens and climatic conditions of rearing places. Burrell *et al.* (2004) reported that use of OZ in broiler diets and found an increase in BWG and BW. Higher biological efficacy of OZ in terms of increase in the growth was observed if Zn is bound by fibre and phytates in basal diets (Abd EL-Hack *et al.* 2017). Ao *et al.* (2011) observed that a chelated Zn proteinate (Bioplex® zinc) increased BWG with higher levels from 5 to 40 mg/kg diet. The study concluded that the supplemental Zn required for optimal broiler growth rate during the starting phase (1-21 days of age) was 9.8 mg/kg diet. Liu *et al.* (2011) stated that Zn proteinate had a significant beneficial effect on weight gain in broilers. Recently, Jahanian and Rasouli (2015) observed that partial substitution of inorganic Zn by ZnMet improved the BWG of broilers. However, Hudson *et al.* (2004) stated that OZ supplemented above NRC (1994) levels of 40 mg/kg had no impact on BWG of broiler breeders. Wang *et al.* (2016) evaluated a Zn-pectin oligosaccharide chelate (Zn-POS; containing 7% Zn) in a corn-soybean based diet (80 mg/kg of Zn) for broiler chickens, supplemented at the rate of 300, 600 and 900 mg/kg.

They reported that BWG increased with 600 mg/ kg of Zn-POS compared with the control and other treatments, although final BWG was similar among the treatments. Yalcinkaya *et al.* (2012) found no effect on broiler BWG with dietary OZ. Supplementation with Zn-acetate at 30 mg Zn/kg diet increased BWG in broiler chickens fed a corn-soybean-wheat diet compared with a basal diet (without Zn supplementation) (Nourozi *et al*. 2013). Yogesh *et al.* (2013) did not find significant effects on the performance of broiler chickens fed diets containing OZ. In the current study, dietary supplementation of HA at a dosage of 0.5% increased BWG of birds, indicating the efficient utilization of feed. These findings are in agreement with the study of Adaszynska-Skwirzynska and Szczerbinska (2019), who reported that BWG was significantly improved by the addition of linalool in broiler chicken drinking water. Interestingly, in the present experiment growth performance of broiler chickens fed the diets containing 0.5% HA was similar to the Barbarestani *et al.* (2020) study showing that growth performance of broiler chickens fed diets containing 600 mg/kg linalool was similar to broilers fed the diets supplemented with virginiamycin. Hussein (2013) reported that medicinal plants increased utilization of feed, resulting in enhanced growth. Similarly, several studies have been reported that the medicinal plants at levels of 0.5–1% in the broiler diets improved BWG, FI, and FCR (Gowda *et al.* 2009). Durrani *et al*. (2006) found that broilers fed medicinal plants had improved BWG compared to control. In contrast, El-Hakim *et al*. (2009) did not find any beneficial effects of adding medicinal plants to the feed of poultry.

In the present study, dietary supplementation of OZ increased the relative liver weight. This finding was consistent with previous reports that none of visceral organs was significantly affected by the level of zinc in the diet (Ahmadi *et al*. 2013). An explanation for increasing liver weight may be a consequence of a positive effect of OZ on digestion and absorption of nutrients in the gastrointestinal tract (GIT) or perhaps the higher bioavailability of zinc in the form of chelate. A further possible reason is that zinc retention was higher in the liver of broilers after absorption and entry to the portal of blood. Yalcinkaya *et al*. (2012) stated that supplementing broiler diets with OZ increased relative organ weights (liver, pancreas, and spleen) when compared with birds fed diets containing inorganic zinc. Jahanian and Rasouli (2015) stated that dietary replacement with ZnMet instead of inorganic Zn significantly improved the relative weight of the liver, decreased abdominal fat and increased carcass yield. From these studies, it appears that supplementation of VC, decreased the abdominal fat pad of broiler chickens. Tavakoli *et al*. (2021) reported that chicks fed a diet containing 200 or 400 mg/kg VC had the lower abdominal fat in comparison with controls. VC is associated with the conversion of body proteins and fat into energy and it is also known that VC is a catalyst in generating energy from fat molecules. In other words, VC plays a significant role in the poultry ability to oxidize fat and generate energy (Vathana *et al*. 2002). Another possible way that VC may help to lose abdominal fat is through iron absorption, in that VC is known to help the body absorb iron, the blood needs iron to carry oxygen to the muscles and oxygen helps the muscles to work more efficiently and burn more fat (Daneshyar *et al*. 2020). Our data showed that dietary supplementation of HT improved the serum lipid profile by reducing LDL-C concentration in serum compared to other dietary treatments.

The active substances in HT such as linalool have been reported to possess cholesterol-lowering effects (Barbarestani *et al*. 2020). Investigating the hypercholesterolemia properties of linalool, Cho *et al*. (2011) have shown that oral administrating of linalool to mice lowers 3-hydroxy-3-methyl glutaryl-CoA reductase protein expression (as a marker for hepatic cholesterol synthesis; HMG-CoA) leading to decreased total cholesterol and LDL-C concentrations. Similarly, other research studies with broilers and laying hens concerning the hypercholesterolemia properties of medicinal plants have suggested similar mechanisms such as decreased activity of HMGCoA reductase and cholesterol-7 hydroxylase fatty acid synthase, and inhibiting the activity of the pentose phosphate pathway by 6-amino nicotinamide (Chowdhury *et al*. 2018; Torki *et al*. 2018). According to antioxidant theory, when the concentrations of antioxidant vitamins (VC and vitamin E) decrease, lipid peroxidation increases in the plasma and tissues, leading to damage of cell membranes. In the present study, supplemental VC resulted in an increase in serum concentrations of GSH-Px, thus preventing the cell damage. VC is involved in several biochemical processes, and its function is related to its reversible oxidation and reduction characteristics in endogenous cells (Ghazi *et al*. 2015) such as mixed function oxidation involving incorporation of oxygen in the substrate. Not only is VC a primary antioxidant in plasma and within cells, but also can interact with the plasma membrane by donating electrons to the α-tocopheroxyl radical, a transplasma membrane oxidoreductase activity (McDowell, 1989). In the present study, increases in serum concentrations of VC was in accordance with the decrease in MDA concentration, indicating prevention of free radical production and consequently cell damage. VC helps reprocess glutathione by converting oxidized glutathione back to its active form. The increase in glutathione peroxidase activity in RBC of high VC supplemented groups may be due to the development of cellular defence mechanisms induced by peroxidative stress of VC in mice (Chen and Thacker, 1984). In the present study,

dietary supplementation of HT increased the activities of TAC in the serum. Several *in vitro* studies have shown that medicinal plant such as HT and its main bioactive compound, linalool, has potent antioxidant activities. For example, Gulcin *et al*. (2004) reported linalool had a powerful antioxidant activity comparable to standard antioxidants such as a-tocopherol, and butylated hydroxytoluene. These authors attributed the antioxidant activity of HT to its strong hydrogen donating and metal chelating ability, and concluded that the phenolic compounds are responsible for these antioxidant activities. Jabir *et al*. (2018) indicated that linalool exhibits antioxidant activity comparable to ascorbic acid, by donating hydrogen atoms and removing electrons from2,2-diphenyl-1-picrylhydrazyl (DPPH) and thus can be considered as a natural antioxidant source.

CONCLUSION

The dietary addition of 0.5% HT, 250 mg/kg VC and 60 mg/kg OZ, alone or in combination, improved FI during the starter period. On the basis of present findings, it is recommended that 60 mg/kg OZ may be used to improve liver function of broiler chicks. From the nutritional point of view, ration supplemented with VC at 250 mg/kg feed was found to produce the lowest abdominal fat pad of all the treatments. In addition, dietary supplementation of HT lowered LDL-c of broiler chickens. On the other hand, dietary inclusion of VC into the broiler diets could improve GSH-Px activity. Finally, the results presented in this study show an increase in the TAC of the broiler serum as a result of dietary supplementation of HT. However, further experiments using several doses of HT with large numbers of broilers should be conducted to confirm the appropriate doses of supplementation.

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REFERENCES

Abd El-Hack M.E., Alagawany M., Arif M., Chaudhry M.T., Emam M. and Patra A. (2017). Organic or inorganic zinc in poultry nutrition: a review. *World's Poult. Sci. J.* **73(4),** 904-915.

Adaszynska-Skwirzynska M. and Szczerbinska D. (2019). The effect of lavender (*Lavandula angustifolia*) essential oil as a drinking water supplement on the production performance, blood biochemical parameters, and ileal microflora in broiler chickens. *Poult. Sci.* **98(1),** 358-365.

Ahmadi F., Ebrahimnezhad Y., Sis N.M. and Ghiasi J. (2013). The effects of zinc oxide nanoparticles on performance, digestive organs and serum lipid concentrations in broiler chickens during starter period. *Int. J. Biosci*. **3(7),** 23-29.

Albadri H.M.B. (2016). Phytochemical Investigation of (*L. arvense equisetum*). Ph D. Thesis. Ministry of Higher Education, Al-Mustansiriyah Univ., Iraq.

Amakye-Anim J., Lin T.L., Hester P.Y., Thiagarajan D., Watkins B.A. and Wu C.C. (2000). Ascorbic acid supplementation improved antibody response to infectious bursal disease vaccination in chickens. *Poult. Sci.* **79(5),** 680-688.

Ao T., Pierce J.L., Pescatore A.J., Cantor A.H., Dawson K.A., Ford M.J. and Paul M. (2011). Effects of feeding different concentration and forms of zinc on the performance and tissue mineral status of broiler chicks. *British Poult. Sci*. **52(4),** 466-471.

Ao T., Pierce J.L., Power R., Dawson K.A., Pescatore A.J., Cantor A.H. and Ford M.J. (2006). Evaluation of Bioplex Zn® as an organic zinc source for chicks. *Int. J. Poult. Sci*. **5(9),** 808-811.

Baghban-Kanani P., Daneshyar M. and Najafi R. (2016). Effects of Cinnamon (*Cinnamomum zeylanicum*) and Turmeric (*Curcuma longa*) powder on performance, enzyme activity, and blood parameters of broiler chickens under heat stress. *Poult. Sci. J*. **4(1),** 47-53.

Barbarestani S.Y., Jazi V., Mohebodini H., Ashayerizadeh A., Shabani A. and Toghyani M. (2020). Effects of dietary lavender essential oil on growth performance, intestinal function, and antioxidant status of broiler chickens. *Livest. Sci*. **233,** 1-12.

Burrell A.L., Dozier W.A., Davis A.J., Compton M.M., Freeman M.E., Vendrell P.F. and Ward T.L. (2004). Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. *British Poult. Sci*. **45(2),** 225-263.

Carr A.C. and Maggini S. (2017). Vitamin C and immune function. *Nutrients*. **9(11),** 1211-1220.

Chen L.H. and Thacker R.R. (1984). An increase in glutathione peroxidase activity induced by high supplementation of vitamin C in rats. *Nutr. Res*. **4(4),** 657-664.

Cho S.Y., Jun H.J., Lee J.H., Jia Y., Kim K.H. and Lee S.J. (2011). Linalool reduces the expression of 3-hydroxy-3-methylglutaryl CoA reductase via sterol regulatory element binding protein-2-and ubiquitin-dependent mechanisms. *FEBS Lett.* **585(20),** 3289-3296.

Chowdhury S., Mandal G.P., Patra A.K., Kumar P., Samanta I., Pradhan S. and Samanta A.K. (2018). Different essential oils in diets of broiler chickens: 2. Gut microbes and morphology, immune response, and some blood profile and antioxidant enzymes. *Anim. Feed Sci. Technol*. **236,** 39-47.

Daneshyar M., Baghban-Kanani P. and Azimi-Youvalari S. (2020). The Importance of Minerals in Poultry Nutrition. Padina publication, Urmia, Iran.

Durrani F.R., Ismail M., Sultan A., Suhail S.M., Chand N. and Durrani Z. (2006). Effect of different levels of feed added turmeric (*Curcuma longa*) on the performance of broiler chicks. *J. Agric. Biol. Sci*. **1(2),** 9-11.

Edrise B.M., Khair-El-din A.W. and Soliman R. (1986). The immune potentiating effect of ascorbic acid against Newcastle disease in chicken. *Vet. Med. J*. **34(2),** 251-264.

El-Hakim A.S.A., Cherian G. and Ali M.N. (2009). Use of organic acid, herbs and their combination to improve the utilization of commercial low protein broiler diets. *Int. J. Poult. Sci*. **8(1),** 14-20.

Ghazi S., Amjadian T. and Norouzi S. (2015). Single and combined effects of vitamin C and oregano essential oil in diet, on growth performance, and blood parameters of broiler chicks reared under heat stress condition. *Int. J. Biometeorol*. **59,** 1019-1024.

Gowda N.K., Ledoux D.R., Rottinghaus G.E., Bermudez A.J. and Chen Y.C. (2009). Antioxidant efficacy of curcuminoids from turmeric (*Curcuma longa L.*) powder in broiler chickens fed diets containing aflatoxin B_1. *British J. Nutr*. **102(11),** 1629-1634.

Gulcin I., Şat İ.G., Beydemir Ş., Elmastaş M. and Küfrevioğlu Ö.İ. (2004). Comparison of antioxidant activity of clove (*Eugenia caryophylata Thunb*) buds and lavender (*Lavandula stoechas* L.). *Food Chem*. **87(3),** 393-400.

Hatab M.H.M. (2011). Studies on the efficacy of vitamin c and *Saccharomyces cerevisiae* in providing partial protection against ochratoxicosis in broiler chickens. Ph D. Thesis. Faculty of Agriculture, Cairo Univ., Cairo, Egypt.

Hudson B.P., Fairchild B.D., Wilson J.L., Dozier I.W.A. and Buhr R.J. (2004). Breeder age and zinc source in broiler breeder hen diets on progeny characteristics at hatching. *J. Appl. Poult. Res.* **13(1),** 55-64.

Hussein S.N. (2013). Effect of turmeric (*Curcuma longa*) powder on growth performance, carcass traits, meat quality, and serum biochemical parameters in broilers. *J. Adv. Biomed. Pathobiol. Res*. **3(2),** 25-32.

Jabir M.S., Taha A.A. and Sahib U.I. (2018). Antioxidant activity of Linalool. *Engin. Technol. J*. **36(1),** 64-67.

Jahanian R. and Rasouli E. (2015). Effects of dietary substitution of zinc methionine for inorganic zinc sources on growth performance, tissue zinc accumulation and some blood parameters in broiler chicks. *J. Anim. Physiol. Anim. Nutr*. **99(1),** 50-58.

Kei S. (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chim. Acta*. **90(1),** 37-43.

Liu Z.H., Lu L., Li S.F., Zhang L.Y., Xi L., Zhang K.Y. and Luo X.G. (2011). Effects of supplemental zinc source and level on growth performance, carcass traits, and meat quality of broilers. *Poult. Sci*. **90(8),** 1782-1790.

Liu S.B., Li S.F., Lu L., Xie J.J., Zhang L.Y., Wang R.L. and Luo X.G. (2013). The effectiveness of zinc proteinate for chicks fed a conventional corn-soybean meal diet. *J. Appl. Poult. Res*. **22(3),** 396-403.

Lohakare J.D., Ryu M.H., Hahn T.W., Lee J.K. and Chae B.J. (2005). Effects of supplemental ascorbic acid on the performance and immunity of commercial broilers. *J. Appl. Poult. Res.* **14(1),** 10-19.

Martin K.M. (2016). The effects of zinc supplementation from two sources on egg quality and bone health in laying hens. MS Thesis. University of Nebraska, USA.

McDowell L.R. (1989). Vitamins in Animal Nutrition Comparative Aspects to Human Nutrition Vitamin C. Academic Press, London, United Kingdom.

Nourozi E., Danesyar M. and Farhoomand P. (2013). Dietary supplementation effects of zinc acetate and magnesium sulfate on performance and antioxidant status of broilers under continuous heat stress. *Spanish J. Agric. Res*. **11(1),** 127-131.

Rajput A.B., Kolte B.R., Shisodiya J.M., Chandankhede J.M. and Chahande J.M. (2009). Effect of vitamin A, vitamin C, vitamin E and levamisole on performance of broilers. *Vet. World*. **2(6),** 225-234.

Safavinia L., Mazhari M., Esmaeilipour O. and Ziaei N. (2021). Study of the effect of vitamin c and *Carum copticum* seed powder diets on growth performance, blood metabolites, carcass characteristics, and meat quality in heat stressed broilers. *J. Vet. Res*. **76(3),** 291-303.

SAS Institute. (2013). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Shadman R., Ziaei N., Esmaeilipour O. and Ranjbar M. (2022). Comparison the effects of vitamin C with nano vitamin C supplement on growth performance, immune responses, intestine morphology and blood parameters of heat stressed broiler chickens. *J. Anim. Sci. Res*. **32(1),** 71-83.

Tavakoli M., Bouyeh M. and Seidavi A. (2021). 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. *J. Anim. Sci.* **31(1),** 67-78.

Torki M., Sedgh-Gooya S. and Mohammadi H. (2018). Effects of adding essential oils of rosemary, dill and chicory extract to diets on performance, egg quality and some blood parameters of laying hens subjected to heat stress. *J. Appl. Anim. Res*. **46(1),** 1118-1126.

Tufarelli V., Baghban-Kanani P., Azimi-Youvalari S., Hosseintabar-Ghasemabad B., Slozhenkina M., Gorlov I. and Laudadio V. (2021). Effects of horsetail (*Equisetum arvense*) and spirulina (*Spirulina platensis*) dietary supplementation on laying hens productivity and oxidative status. *Animals*. **11(2),** 335-342.

Tufarelli V., Baghban-Kanani P., Azimi-Youvalari S., Hosseintabar-Ghasemabad B., Slozhenkina M., Gorlov I., Viktoronova, F.M., Seidavi A. and Laudadio V. (2022). Effect of dietary flaxseed meal supplemented with dried tomato and grape pomace on performance traits and antioxidant status of laying hens. *Anim. Biotechnol*. **33(7),** 1525-1532.

Vathana S., Kang K., Loan C.P., Thingard G., Kabasa J.D. and Meulen U. (2002). Effect of vitamin C supplementation on performance of broiler chickens. Pp. 25-32 in Proc. Conf. Int. Agric. Res. Dev., Deutscher Tropentag Witzenhausen, University of Bonn, Germany.

Wang Z., Yu H., Wu X., Zhang T., Cui H., Wan C. and Gao X. (2016). Effects of dietary zinc pectin oligosaccharides chelate

supplementation on growth performance, nutrient digestibility and tissue zinc concentrations of broilers. *Biol. Trace Elem. Res*. **173,** 475-482.

Yagi K. (1984). Assay for blood plasma or serum. *Methods Enzymol*. **105,** 328-331.

Yalcinkaya I., Çinar M., Yildirim E., Erat S., Başalan M. and Güngör T. (2012). The effect of prebiotic and organic zinc alone and in combination in broiler diets on the performance and some blood parameters. *Italian J. Anim. Sci*. **11(3),** 55-61.

Yogesh K., Deo C., Shrivastava H.P., Mandal A.B., Wadhwa A. and Singh I. (2013). Growth performance, carcass yield, and immune competence of broiler chickens as influenced by dietary supplemental zinc sources and levels. *Agric. Res*. **2,** 270-274.