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Original Article

Egg qualities and haematological indices of laying birds fed chromium picolinate and vitamin C supplemented diets

F.B. Adebayo[1], O.A. Adu[1], *O.D. Oloruntola[2] and C.A. Chineke[1]

[1]Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria
[2]Department of Animal Science, Adekunle Ajasin University, Akungba-Akoko, Nigeria

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***Correpsonding author**
O.D. Oloruntola
olugbenga.oloruntola@aaua.edu.ng

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Abstract

Two hundred and twenty-four Noiler pullets (20 weeks of age) were randomly distributed into 8 dietary groups to determine the effects of chromium picolinate (CrPic) and vitamin C supplementations in layers. A basal diet was sundered into eight like portions, supplemented and tagged diet 1: 0 mg/kg CrPic; diet 2: 0.4 mg/kg CrPic; diet 3: 0.8 mg/kg CrPic; diet 4: 1.2 mg/kg CrPic, diet 5: 200 mg/kg vitamin C; diet 6: 0.4 mg CrPic+200 mg vitamin C; diet 7: 0.8 mg CrPic+200 mg vitamin C and diet 8: 1.2 mg CrPic+200 mg vitamin C. General linear model procedure for complete randomized design with 4 CrPic levels (0 mg/kg, 0.4 mg/kg, 0.8 mg/kg and 1.2 mg/kg) x 2 vitamin C levels (0 mg/kg and 200 mg/kg) factorial treatments' arrangement was used. The eggshell thickness was affected ($P<0.05$) by the CrPic supplementation. Albumen height, albumen length and haught units recorded in the birds fed diets 6, 7 and 8 were similar ($P>0.05$) but higher ($P<0.05$) than those fed diet 1. The packed cell volume, red blood cell count, haemoglobin concentration, mean cell haemoglobin concentration, mean cell volume, mean cell haemoglobin and white blood cell count were affected ($P<0.05$) by the CrPic and vitamin C supplementation. In conclusion, the external egg qualities were not affected by CrPic and vitamin C supplementation. The albumen height, albumen length and haught improved with CrPic and vitamin C in the laying birds.

INTRODUCTION

Layers production plays an all-important part in food security of the world's population (Saki *et al*., 2010). Eggs provide a very high-quality protein that contains all nine essential amino acids in the correct proportions needed by the body for best growth and maintenance. However, the quality of eggs and the laying hens' health status are influenced by environmental factors, such as ambient temperature and nutrition (Adu *et al*., 2017). Remarkably, the layers' nutrition plays notable roles that affect internal and external egg qualities (Wang *et al*., 2017a).

Some minerals and vitamins such as chromium (Cr), vitamin C (Torki *et al*., 2014) and Zn (Chand *et al*., 2014) are suitable and proven supplements that can reduce the adverse effects of environmental factors on the egg quality. Chromium is involved in metabolic pathways of nutrients through heightening of the insulin activity (Amata, 2013). It also subscribes to the anabolic profile (Lien *et al*., 2004) and lessens oxidative stress and lipid peroxidation (Şahin *et al*., 2010) in laying hens exposed to heat stress. Chromium chelates with organic compounds (e.g. Chromium picolinate, Chromium nicotinate, and Chromium propionate) have higher bioavailability and lower toxicity than Cr in inorganic form (Piva *et al*., 2003).

Vitamin C, on the other hand, is among the nutritional factors paramount for laying hen's performance and egg quality. It is indispensable to sustain regular body metabolic activities and reaching physiological requirements (Saki *et al*., 2010). Vitamin C involvement in various biochemical activities and tasks is also connected to reversible oxidation and reduction characteristics in the endogenous cells. Moreover, vitamin C has a vital position in reacting with all aggressive oxygen species and transferring radical equivalents from lipid phases to hydrophilic compartments (Saki *et al*., 2010).

In previous studies, supplemental dietary Cr (Şahin *et al*., 2004; Khan *et al*., 2014) and vitamin C (Ahmed *et al*., 2008; Torki *et al*., 2014) improved egg production, feed efficiency, and egg quality effectively, even in birds reared under heat or cold stress (Şahin *et al*. 2004; Khan *et al*. 2014). More interestingly, the combination of 250 mg of vitamin C and 400 µg of Cr yielded the high positive upshot on the performance and egg

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quality traits of laying hens (Şahin and Onderci, 2002).

However, there is the dearth of information about the interactive effect of dietary supplementation of CrPic and vitamin C in laying hens raised under tropical environmental conditions. The objective of this study was to evaluate the effects of dietary supplementation of CrPic, and vitamin C, singly or in combined forms, on the egg quality traits, and haematological indices of laying hens raised under a typical tropical high ambient temperature (28 - 31°C).

MATERIALS AND METHODS

Ethical approval

The study was executed under the authorization to use animal and animal protocol approved by the Research and Ethics Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria.

Study site

The study was carried out in the dry season, between January and February, 2020, at the Avian Unit of the Teaching and Research Farm, The Federal University of Technology, Akure, Nigeria. The study site has geographical coordinates of 7° 17' North and 5° 9' East (Mapzoom, 2015). The mean yearly rainfall is 1524 mm, the atmospheric temperature ranges between 28 and 31°C, while the mean yearly relative humidity is about 80 % (Adu *et al*., 2017). The wet and dry bulb temperatures of the experimental pen were determined in the morning and evening daily. The average daily temperature-humidity index (THI) of 34.08°C ± 1.36 was recorded for the experimental pen.

Experimental design and diet

The dietary treatments were arranged in a 2 x 4 factorial arrangement of completely randomized design; comprising 2 vitamin C inclusion levels (0.0 mg/kg and 200 mg/kg) and 4 CrPic inclusions (0.0 mg CrPic, 0.4 mg/kg CrPic, 0.8 mg/kg CrPic and 1.2 mg/kg CrPic). The CrPic supplement (AK Scientific, Union City, CA, USA), vitamin C powder (Avondale Laboratories Limited, Banbury, England) and other feed ingredients were procured from local markets in Nigeria.

A basal diet was formulated for the laying birds (Table 1) and analyzed for proximate compositions (AOAC, 1995) after which the basal diet was divided equally into eight portions, coded diets 1 to 8 and supplemented as follows: Diet 1: Control, Diet 2: 0.4 mg/kg CrPic, Diet 3: 0.8 mg/kg CrPic, Diet 4: 1.2 mg/kg CrPic, Diet 5: 200 mg/kg vitamin C, Diet 6: 0.4 mg/kg CrPic and 200 mg/kg vitamin C, Diet 7: 0.8 mg/kg CrPic and 200 mg/kg vitamin C, and Diet 8: 1.2 mg CrPic and 200 mg/kg vitamin C.

Table 1 Composition of the basal experimental diet

Ingredients	Quantity (g/kg)
Maize	400.00
Soybean meal	240.00
Maize bran	140.00
Wheat offal	100.00
Limestone	85.20
Bone meal	23.00
Lysine	3.00
Methionine	3.50
*Layer premix	2.50
Table salt (NaCl)	2.80
Calculated values (g/kg)	
Crude protein	176.30
Crude fibre	41.90
Crude fat	44.70
Calcium	39.00
Phosphorus	5.10
Lysine	12.10
Methionine	6.20
Metabolizable energy (kcal/kg)	2592.80
Analyzed values (g/kg	
Crude protein	177.24
Crude fat	45.01
Metabolizable energy (kcal/kg)	2614.32

* 2.5 kg of layer premix contains: Vit. A (10000000 iu), Vit. B1 (2000 mg), Vit. D3 (2500000 iu), Vit. E (12000 iu), Niacin (15000 mg), Vit. B6 (1500 mg), Vit. B12 (10 mg), Vit. K3 (2000 mg), Biotin (20 mg), Chlorine Chloride (150000 mg), Folic Acid (600 mg), Panthothenic Acid (7000 mg), Manganese (80000 mg), Selenium (150 mg), Iodine (1000 mg), Magnesium (100 mg), Iron (40000 mg), Copper (0 mg), Zinc (60000 mg), BHT (700 g), Ethoxyquine (500 g).

Experimental birds

Two hundred and twenty-four Noiler pullets of 20 weeks of age were randomly distributed into 8 dietary groups (28 pullets/group; 4 birds/replicate). The birds were housed in two tiers battery cages and managed under strict biosecurity system. The birds were fed (101 - 115 g/bird/day) twice daily following Isa brown management guide; while water was provided *ad libitum*. Prophylactic treatments and vaccinations were administered following the vaccination schedule.

Data collection

Collection of eggs was carried out from each treatment on day 21 (thrice/day), after the onset of lay, and subsequently on the 7-day interval for 6 weeks for external and internal egg qualities determination. A total of 28 eggs per treatment, 4 eggs per replicate were randomly selected on the collection day.

The egg height, weight, yolk weight, shell weight, and shell membrane were determined with a sensitive scale calibrated in grams. The egg length, width, albumin height, albumen width, albumen length, yolk height, yolk width, and yolk length were measured with Vernier caliper calibrated in centimeters. The albumen weight was estimated as the difference between the total egg weight and sum shell weights and the yolk weight. The yolk index was determined as the percentage of the fraction of yolk height to yolk width (Oluyemi and Robert, 2000); while the haught unit (HU) was also determined using the formula given by (Oluyemi and Robert, 2000) as follows: $HU=100\log(H+7.57-1.7W^{0.37})$. Where H= albumen height in mm; W= weight of eggs in grams.

On the final day of the feeding experiment, 7 birds were randomly selected per group (1 bird/replicate). After that, their blood samples were collected with syringe and needle into EDTA for haematology within 120 mins post collection following the procedures described by Cheesbrough (2000).

Statistical analysis

All data on egg qualities and blood parameters were subjected to analysis of variance using General Linear Model procedures for complete randomized design with 4 CrPic levels x 2 vitamin C levels factorial arrangement of treatments. The data were tested for CrPic, vitamin C and interaction of CrPic with vitamin C. When the treatment outcome was significant (P<0.05), means were separated using Duncan's multiple range test of SPSS.

RESULTS

The external egg quality parameters measured in this study were not significantly (P>0.05) affected by the levels of CrPic, vitamin C and their interaction (Table 2). The higher (P<0.05) eggshell thickness recorded for birds fed 0.0mg/kg CrPic supplemented diet was similar (P>0.05) for those fed 0.8mg/kg CrPic, 0.4mg/kg CrPic, and 1.2mg/kg CrPic supplemented diets.

Table 3 shows the effects of chromium picolinate and vitamin C supplementations on layer birds' internal egg qualities. The internal egg qualities were not affected (P>0.05) by the CrPic, and vitamin C supplementations. However, the CrPic x vitamin C was significant (P<0.05) for albumen height, albumen height, yolk weight and haught unit. The albumen height, albumen length, yolk weight and haught units recorded in the birds fed the diets supplemented with the combinations of CrPic and vitamin C (diets 6, 7 and 8) were similar (P>0.05) but higher (P<0.05) than those fed the control diet. In the same vein, the yolk weights of the birds fed the diets 5, 6, 7 and 8 were

Table 2: Effect of dietary Chromium Picolinate and vitamin C supplementation on external egg quality of layer birds

Diets	Cr Pic (mg/kg)	Vitamin C (mg/kg)	Egg weight (g)	Egg length (cm)	Egg width (mm)	Shell weight (g)	Shell membrane (mm)	Shell thickness (mm)	Shell and membrane (mm)	ES index
1	0.0	0.0	51.19±1.41	3.97±0.10	2.68±0.04	4.55±0.16	1.08±0.05	0.30±0.01	5.63±0.19	69.81±1.11
2	0.4	0.0	48.83±1.04	3.85±0.06	2.59±0.04	4.49±0.10	0.96±0.05	0.29±0.01	5.45±0.11	67.43±1.53
3	0.8	0.0	48.73±1.40	3.95±0.09	2.58±0.04	4.46±0.18	0.96±0.05	0.28±0.02	5.42±0.19	65.74±1.68
4	1.2	0.0	49.35±1.20	3.86±0.08	2.65±0.03	4.61±0.13	1.03±0.04	0.30±0.01	5.64±0.14	68.75±1.26
5	0.0	200	48.84±1.01	3.88±0.05	2.63±0.03	4.69±0.16	1.06±0.06	0.31±0.02	5.75±0.17	67.82±0.44
6	0.4	200	51.85±0.95	3.97±0.05	2.67±0.05	4.73±0.11	1.06±0.07	0.27±0.01	5.79±0.13	67.47±1.53
7	0.8	200	50.01±1.76	3.90±0.07	2.68±0.06	4.57±0.18	1.00±0.06	0.28±0.01	5.57±0.20	68.69±1.27
8	1.2	200	48.58±2.39	3.80±0.14	2.61±0.08	4.50±0.18	1.05±0.05	0.29±0.01	5.55±0.20	69.00±1.07
P value			0.31	0.58	0.38	0.68	0.73	0.63	0.72	0.69
	0		50.02±0.88	3.92±0.06	2.65±0.02	4.62±0.11	1.07±0.04	0.31±0.01[a]	5.69±0.12	67.81±0.58
	0.4		50.34±0.76	3.91±0.04	2.63±0.03	4.61±0.08	1.01±0.04	0.28±0.01[ab]	5.62±0.09	67.45±1.06
	0.8		49.37±1.11	3.93±0.05	2.63±0.04	4.51±0.12	0.98±0.04	0.28±0.01[ab]	5.49±0.13	67.21±1.07
	1.2		48.97±1.31	3.83±0.08	2.63±0.04	4.55±0.11	1.04±0.03	0.30±0.01[ab]	5.60±0.12	68.87±0.81
	P value		0.78	0.61	0.93	0.87	0.35	0.04	0.69	0.58
		0	49.53±0.63	3.91±0.04	2.63±0.02	4.53±0.07	1.01±0.02	0.29±0.01	5.54±0.08	67.43±0.70
		200	49.82±0.81	3.89±0.04	2.65±0.03	4.62±0.08	1.04±0.03	0.29±0.01	5.66±0.09	68.24±0.56
		P value	0.77	0.71	0.51	0.37	0.38	0.57	0.28	0.37
	Cr Pic level*Vitamin C P value		0.28	0.61	0.28	0.70	0.69	0.65	0.64	0.60

Means with a different superscript in the same column are significantly (P<0.05) different; CrPic: Chromium picolinate.

similar (P>0.05), but significantly higher than the control diet (Table 3).

Table 4 shows the effects of dietary CrPic and vitamin C supplementations on the haematological indices of the birds. The packed cell volume (PCV) were affected (P<0.05) by the

Table 3: Effect of dietary Chromium Picolinate and vitamin C supplementation on internal egg quality of layer birds

Diets	Cr Pic (mg/kg)	Vitamin C (mg/kg)	Albumen Height (mm)	Albumen weight (g)	Albumen length (mm)	Yolk height (mm)	Yolk weight (g)	Yolk length (mm)	Yolk index	Egg mass	Haught unit
1	0	0	0.63±0.04^{c}	14.70±0.85	6.17±0.10^{c}	1.80±0.05	12.05±0.35^{c}	2.99±0.06	0.61±0.02	35.16±1.58	63.94±0.04^{bc}
2	0.4	0	0.72±0.03^{b}	13.27±0.70	6.22±0.11^{bc}	1.77±0.03	12.07±0.30^{c}	2.81±0.06	0.63±0.02	33.73±1.22	65.05±0.02^{b}
3	0.8	0	0.73±0.02^{b}	12.27±0.73	6.24±0.20^{bc}	1.76±0.03	12.23±0.42^{bc}	2.95±0.08	0.60±0.02	34.35±1.04	65.05±0.03^{b}
4	1.2	0	0.70±0.02^{b}	12.89±0.97	6.33±0.18^{bc}	1.78±0.02	12.37±0.15^{bc}	2.87±0.06	0.63±0.02	34.91±1.17	65.03±0.03^{b}
5	0	200	0.70±0.02^{b}	12.94±0.74	6.28±0.18^{bc}	1.75±0.04	12.77±0.33^{ab}	2.80±0.04	0.63±0.02	35.01±1.19	66.04±0.02^{b}
6	0.4	200	0.73±0.03^{a}	14.15±0.72	6.84±0.15^{a}	1.79±0.03	12.93±0.29^{a}	2.94±0.06	0.61±0.02	35.19±1.30	70.95±0.02^{a}
7	0.8	200	0.74±0.02^{a}	13.03±1.15	6.60±0.16^{a}	1.70±0.04	12.94±0.30^{a}	2.94±0.08	0.59±0.02	33.72±1.04	70.99±0.04^{a}
8	1.2	200	0.71±0.03^{ab}	12.26±1.62	6.45±0.37^{ab}	1.68±0.08	12.52±0.24^{ab}	2.79±0.17	0.62±0.03	32.41±2.18	69.04±0.04^{a}
P value			0.04	0.61	0.04	0.64	0.04	0.46	0.76	0.45	0.03
	0		0.67±0.02	13.82±0.58	6.68±0.11	1.78±0.03	12.28±0.26	2.89±0.04	0.62±0.01	35.09±0.97	63.99±0.02
	0.4		0.69±0.02	13.71±0.50	6.50±0.11	1.78±0.02	12.50±0.22	2.87±0.04	0.62±0.01	34.46±0.89	64.00±0.02
	0.8		0.70±0.02	12.65±0.67	6.45±0.13	1.73±0.03	12.68±0.25	2.95±0.06	0.59±0.01	34.04±0.72	66.02±0.02
	1.2		0.71±0.02	12.58±0.93	6.44±0.20	1.73±0.04	12.67±0.14	2.83±0.09	0.62±0.02	33.66±1.24	65.03±0.02
	P value		0.43	0.43	0.62	0.58	0.62	0.59	0.45	0.76	0.50
		0	0.69±0.02	13.28±0.42	6.44±0.08	1.78±0.02	12.47±0.16	2.90±0.03	0.62±0.01	34.54±0.62	63.02±0.02
		200	0.70±0.01	13.09±0.55	6.59±0.12	1.73±0.03	12.49±0.15	2.87±0.05	0.61±0.01	34.08±0.74	65.00±0.02
		P value	0.62	0.78	0.29	0.12	0.94	0.52	0.70	0.64	0.57
	Cr Pic level*Vitamin C P value		0.04	0.49	0.04	0.60	0.02	0.30	0.75	0.56	0.01

Means with a different superscript in the same column are significantly (P<0.05) different; CrPic: Chromium picolinate;

Table 4: Effect of dietary Chromium Picolinate and vitamin C supplementation on the haematological indices of layer birds

Diets	Cr Pic (mg/kg)	Vitamin C (mg/kg)	PCV %	RBC (x10^{6}/l)	MCHC (g/dl)	MCV (fl)	MCH (pg/cell)	HB (g/dl)	WBC (x10^{9}/l)
1	0.0	0.0	25.00 ± 0.58^{c}	3.13 ± 0.61^{c}	32.00 ± 0.58^{b}	79.02 ± 0.01^{ab}	25.34 ± 0.01^{bc}	8.34 ± 0.01^{bc}	1.80 ± 0.06^{c}
2	0.4	0.0	23.33 ± 0.88^{c}	3.30 ± 0.06^{bc}	32.43 ± 0.43^{b}	70.68 ± 0.08^{b}	26.01 ± 0.02^{b}	7.66 ± 0.01^{d}	3.17 ± 0.09^{a}
3	0.8	0.0	20.00 ± 0.58^{c}	3.43 ± 0.09^{bc}	32.13 ± 0.09^{b}	58.31± 0.27^{c}	27.49 ± 0.32^{b}	6.66 ± 0.33^{d}	2.37 ± 0.19^{b}
4	1.2	0.0	24.33 ± 0.67^{c}	3.14 ± 0.09^{c}	31.03 ± 0.04^{bc}	77.48 ± 0.15^{ab}	27.52 ± 0.11^{b}	8.13 ± 0.33^{bc}	2.23 ± 0.09^{b}
5	0.0	200	29.00 ± 0.58^{ab}	3.76 ± 0.03^{a}	31.30 ± 0.06^{bc}	77.12 ± 0.01^{ab}	30.63 ± 0.01^{a}	9.60 ± 0.03^{b}	1.37 ± 0.09^{d}
6	0.4	200	32.30 ± 0.53^{a}	3.68 ± 0.07^{a}	34.17 ± 0.88^{a}	87.77 ± 0.02^{a}	31.81 ± 0.02^{a}	10.65 ± 0.01^{a}	2.60 ± 0.06^{a}
7	0.8	200	32.00 ± 0.58^{a}	3.80 ± 0.12^{a}	34.19 ± 0.12^{a}	84.21 ± 0.01^{a}	31.34 ± 0.02^{a}	10.61 ± 0.03^{a}	1.09 ± 0.01^{b}
8	1.2	200	31.67 ± 0.88^{a}	3.77 ± 0.09^{a}	34.20 ± 0.06^{a}	84.01 ± 0.01^{a}	30.78 ± 0.01^{a}	10.53 ± 0.01^{a}	1.60 ± 0.06^{c}
P value			0.01	0.01	0.01	0.01	0.01	0.01	0.01
	0.0		26.00 ± 0.58	3.55 ± 0.63	32.65 ± 0.39	73.23 ±12.26^{ab}	25.49 ± 4.09^{ab}	8.67 ± 0.15	1.58 ± 0.11^{d}
	0.4		26.17 ± 1.35	3.42 ± 0.07	33.78 ± 0.44	76.52 ± 0.55^{a}	26.41 ± 0.18^{a}	8.98 ± 0.30	2.88 ± 0.14^{a}
	0.8		26.00 ± 2.71	3.56 ± 0.08	32.17 ± 0.44	73.03 ± 7.31^{ab}	26.62 ± 2.43^{a}	8.97 ± 0.75	2.13 ± 0.13^{b}
	1.2		27.00 ± 0.58	3.47 ± 0.11	33.51 ± 0.05	77.81 ± 2.69^{a}	26.55 ± 0.91^{a}	8.83 ± 0.27	1.92 ± 0.15^{c}
	P value		0.41	0.13	0.17	0.00	0.00	0.24	0.00
		0.0	23.67 ± 0.77^{b}	3.14 ± 0.26^{b}	32.77 ± 0.21	75.33 ± 3.37	21.77 ± 1.12^{b}	7.08 ± 0.16^{b}	2.39 ± 0.16^{a}
		200	28.92 ± 0.65^{a}	3.88 ± 0.11^{a}	32.59 ± 0.32	74.53 ± 2.71	30.27 ± 0.19^{a}	9.65 ± 0.18^{a}	1.87 ± 0.14^{b}
		P value	0.00	0.00	0.53	0.20	0.00	0.00	0.00
	Cr Pic level*Vitamin C P value		0.00	0.00	0.00	0.00	0.00	0.00	0.68

Means with a different superscript in the same column are significantly (P<0.05) different; CrPic: Chromium picolinate; PCV: Packed cell volume; RBC: Red blood cell; HBc: Haemoglobin concentration; MCV: Mean cell volume; MCH: Mean cell haemoglobin; WBC: White blood cells.

vitamin C supplementation and CrPic x vitamin C interaction. The PCV of the birds on diets 5, 6, 7 and 8 were similar (P>0.05) but higher (P<0.05) than the control and the other diets. Vitamin C supplementation and CrPic x vitamin C interaction improved (P<0.05) the red blood cell (RBC) counts of the birds. The birds on diets 5, 6, 7 and 8 had similar (P>0.05) RBC counts but higher (P<0.05) than those on the control and the other diets. The CrPic x vitamin C interaction was significant (P<0.05) for the mean cell haemoglobin concentration (MCHC). The MCHC for the birds fed diets 6, 7 and 8 were similar (P>0.05) and higher than those fed the other diets. The mean cell volume (MCV) and mean cell haemoglobin (MCH) significantly (P<0.05) increased with the CrPic, vitamin C and CrPic x vitamin C interaction. The birds fed CrPic x vitamin C supplemented diets had higher MCV and MCH values than those on the control diet. The vitamin C supplementation and CrPic x vitamin C interaction were significant (P<0.05) for haemoglobin (Hb) concentration. The Hb concentration was significantly (P<0.05) higher in the birds fed diets 6, 7 and 8 than those fed the control and other diets. The white blood cell (WBC) counts were significantly (P<0.05) affected by the CrPic and vitamin C supplementation. The CrPic dietary supplementation increased the WBC count, compared to the control and the other inclusion levels. In contrast, the vitamin C supplementation reduced the WBC counts in the birds.

DISCUSSION

Previous studies which showed that chromium and vitamin C supplementations can improve some egg quality parameters (Ahmed *et al*., 2008; Torki *et al*., 2014; Karami *et al*., 2018) contradict the results reported in this study, where the CrPic and vitamin C supplementation did not promote any improvement in all the external egg qualities measured. This suggests that the efficiency of these dietary supplements in enhancing the egg qualities could have been affected by some other factors such as breeds and age. Therefore, the indifference in the external egg qualities recorded between the supplemented diets and the control implies the safety of these supplements and their respective supplementation levels used in this study.

The significant interaction of CrPic x vitamin C in producing notable differences in the albumen height, albumen weight, yolk weight and haught unit as observed in this study showed that these supplements complement each other to improve some internal egg qualities of the birds. Previous studies reported that chromium and vitamin C improved the internal egg quality of laying hens (Şahin and Küçük, 2001; Şahin *et al*., 2001). The supplemental chromium plays a major role in ameliorating heat stress by shifting metabolic balance more towards anabolism through its involvement in the auto-amplification mechanism of insulin action (Vincent, 2000); but Cr are loss irreversibly in birds under stress, leading to Cr marginal deficiency (Amatya *et al*., 2004). However, vitamin C, besides being a primary antioxidant in plasma and within cells, it can also interrelate with the plasma membrane by donating electrons to the tocopherol radical (Anderson *et al*., 2001; Gursu *et al*., 2004). Chromium also functions as an antioxidant and may influence metabolism of vitamin C by protecting ascorbate from oxidative destruction. It is known that insulin plays a role in vitamin C transportation in the red blood cells while glucose competitively inhibits vitamin C transportation (Şahin *et al*., 2001). Therefore, Cr indirectly promotes vitamin C transportation by improving insulin effectiveness (Şahin *et al*., 2001).

The egg quality of laying birds can be affected by the mineral composition of some of the feed ingredients. For instance, limestone, one of the vital feed ingredients of layer birds, contains 10 - 45 mg/kg vanadium (Make, 2005) while 10 mg/kg is the maximum tolerable level for vanadium in poultry diets (Henry and Miles, 2001). Mineral vanadium has been reportedly noted as the primary nutritional factors affecting internal egg quality (Wang *et al*., 2017a, b). Notably, vanadium was reported to reduce the internal egg qualities such as albumen and haugh units of eggs by inhibiting the magnum's motility during egg formation (Henry and Miles, 2001). However, the ascorbic acid and chromium's ability to counteract the deleterious effects of vanadium has been investigated, and ascorbic acid supplementation was found effective in ameliorating the adverse effects of mineral vanadium (Wang *et al*., 2017b). This explains the improved albumen height; albumen length and haught unit recorded in the experimental layer birds fed diets 2, 3, 4, 5, 6, 7 and 8 due to the positive interaction of CrPic and vitamin C supplements.

Egg yolk is considered a vital source of nutrients and the primary storage site of trace minerals for the embryo and hatchling. Therefore, the yolk height or weight is considered one of the factors responsible for gigantic chick due to the embryos' superior nutritional set-up in the course of incubation (Zita *et al*., 2012). By implication, the improved egg yolk weight recorded in this study in the experimental laying birds due to the interaction of CrPic and vitamin C supplementation suggested the possible role of these supplements in improving chick's weight.

As earlier reported by Oloruntola *et al*. (2018), nutrition has significant effects on animals' haematological indices. Oxidative stress is also a significant cause of red blood cells dysfunction (Asmah *et al*., 2015). Therefore, the improved PCV, RBC, and Hb concentrations recorded in birds on diets supplemented with 200 mg/kg vitamin C and interaction of vitamin C and CrPic could be linked with these supplements' antioxidant properties (Onderci *et al*., 2003; Adenkola and Angani 2017). This result is in tandem with the findings of Adenkola and Angani (2017), who recorded improved RBC, PCV and Hb concentrations in chickens fed vitamin C supplemented diet compared to the control. The MCHC measures the mean concentration of haemoglobin in a single red blood cell. The improved MCHC caused by the interaction of CrPic and vitamin C supplementation; improved MCV caused by the CrPic and the interaction of CrPic and vitamin C; improved MCH caused by the CrPic and vitamin C and their interaction also signalled positive effect of these supplements on the normal haemopoiesis.

White blood cells are cells of the immune system involved in the body's protection against infectious diseases and foreign invaders. Some vitamins such as vitamins A, C, E, among others, play vital roles in the normal function of immune systems by altering the transcription of multiple enzyme genes or contributing to antioxidant activities (Shojadoost *et al*., 2021). This explains the observed variations in the WBC counts of birds fed both CrPic and vitamin C supplemented diets than the control in this study. Adebayo *et al*. (2020) had earlier reported variation in the WBC differentials due to CrPic supplementation.

CONCLUSION

In conclusion, the external egg qualities were not affected by 0.4 - 1.2 mg/kg CrPic and 200 mg/kg vitamin C supplementation. The albumen height, albumen length and haught improved with CrPic (0.4 -1.2 mg/kg CrPic) and vitamin C (200 mg/kg) supplements interaction. The CrPic and vitamin C supplementation support the normal haematopoiesis in the layer birds.

Conflicts of interest: The authors declare no conflicts of interest.

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