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The Performance, Antioxidant Status, Blood Chemistry Analysis and Tissue Histology of Broiler Birds Fed a Diet Containing Chromium Picolinate and Vitamin C

Research Article

F.B. Adebayo[1], O.A. Adu[1], C.A. Chineke[1], O.D. Oloruntola[2*], O.S. Omoleye[1], S.A. Adeyeye[3] and S.O. Ayodele[4]

[1] Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria
[2] Department of Animal Science, Adekunle Ajasin University, Akungb-Akoko, Nigeria
[3] Department of Animal Health and Production, Federal College of Agriculture, Akure, Nigeria
[4] Department of Agricultural Technology, Federal Polytechnic, Ado Ekiti, Nigeria

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*Correspondence E-mail: olugbenga.oloruntola@aaua.edu.ng
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ABSTRACT

This study looks into the effects of supplementing broiler chickens with chromium picolinate (CrPic) and vitamin C on their efficiency and antioxidant status without interfering with their essential metabolic functions and histology. Six hundred and forty Cobb 500 broiler chickens were randomly assigned to eight different dietary treatments, each with ten birds. Diets 1 to 8 were produced from a base diet that was divided into eight equal parts. Diets 1 to 4 were supplemented with 0 mg/kg CrPic, 0.4 mg/kg CrPic, 0.8 mg/kg CrPic, and 1.2 mg/kg CrPic, respectively. Supplements of 200 mg/kg vitamin C, 0.4 mg CrPic and 200 mg vitamin C, 0.8 mg CrPic and 200 mg vitamin C, and 1.2 mg CrPic and 200 mg vitamin C were given to the diets 5 to 8. Performance, carcass analysis, antioxidant parameters, serum-biochemistry, and histology were all investigated. Dietary supplements of 0.8 mg/kg CrPic and 200 mg vitamin C improved body weight gain, feed conversion ratio, slaughtered weight, and antioxidant enzyme status. Dietary vitamin C supplementation (200 mg/kg) reduced aspartate aminotransferase and alanine aminotransferase, while dietary CrPic supplementation (1.2 mg/kg) reduced blood glucose. However, dietary supplementation with 0.4 mg/kg CrPic decreased abdominal fat, while 1.2 mg/kg CrPic supplementation resulted in substantial hepatic inflammation and interstitial nephritis. In broiler development under tropical conditions, a mixture of 0.8 mg/kg CrPic and 200 mg vitamin C dietary supplementation is advanced.

KEY WORDS antioxidant, ascorbic acid, performance, poultry, stress, supplements.

INTRODUCTION

Minerals and vitamins serve essential roles in animals' diets and must be given sufficient amounts to ensure optimum growth, efficiency, and animal product quality (Sirri *et al.* 2016; Araujo *et al.* 2019; Tavakoli *et al.* 2020). Chromium's dietary use (Cr) for animal production is currently creating a lot of research interest. Since Cr dietary intake is generally low, researchers are now concentrating on finding ways to investigate the positive and beneficial effects of Cr dietary supplementation on animal performance, products, biological activities, and health status (Khan *et al.* 2014; Brooks *et al.* 2016). Cr's nutritional functions have been stated to include maintaining proper lipid and carbohydrate metabolism, stimulating the immune and antioxidant systems, reducing stress reactions, improving growth performance, and reducing carcass fat (Ognik *et al.* 2020; Tavakoli *et al.* 2020). Chromium dietary supplementation was pro-

posed to increase insulin sensitivity based on Cr altering the biological ability of insulin (Brooks *et al*. 2016). The glucose tolerance factor contains chromium as a significant component. As a result, Cr's ability to initiate insulin secretion allows for proper protein, carbohydrate, and lipid metabolic changes. Cr is also needed for the activation of enzymes and the stabilisation of protein and nucleic acid. As a result, Cr deficiency may be a factor in carbohydrate, lipid, and protein metabolism problems (Sahin *et al*. 2010). Chromium picolinate (CrPic) is one of the chromium varieties, and it was cited for its biological functions (Khan *et al.* 2014; Liu *et al.* 2015). Vitamin C (ascorbic acid) is mainly derived by poultry and some other mammals from glucose (Ahmadu *et al.* 2015; Gan *et al*. 2018). However, under stress conditions such as infestation, abnormal relative humidity, ambient temperature, and a highly effective rate, the birds' capacity to synthesise adequate ascorbic acid is hampered (Molnár *et al*. 2016). As a result, dietary vitamin C becomes essential for animal health. Vitamin C improves overall performance which are affected by stress by lowering adrenocorticotropic hormone and plasma corticosterone levels (Monacelli *et al.* 2017). Supplementing with ascorbic acid has been shown to increase resistance to diseases and infections (Takahashi *et al.* 1991; Monacelli *et al*. 2017). Vitamin C is essential for the biosynthesis of corticosterone, a hormone that boosts energy supply during stressful situations and wound healing, immunity, and histamine detoxification (Ahmadu *et al*. 2015).

Chromium has been found to play an important role in animal nutrition by raising body weight gain, improving feed quality, increasing carcass yield, and acting as a powerful antioxidant and hypocholesteric agent; excessive dietary levels, on the other hand, may cause toxicity or have negative health consequences (Ray, 2016). The results of animal studies on systemic toxicity have been published (Acharya *et al*. 2001; Ray, 2016; Sun *et al*. 2015; DesMarais and Costa, 2019). However, vitamin C has been shown to have anti-toxicity effects against toxic compounds by restoring toxin-induced changes in the damaged organs of poisoned laboratory animals (Awadalla, 2012); as a result, dietary CrPic and vitamin C supplementation can have a positive synergistic impact on broiler output (Nosrati *et al*. 2017). Sahiti *et al.* (2018) and Lin *et al.* (2018) previously proposed using ascorbic acid to reduce Cr toxicity. There have been few studies on dietary Cr supplementation in broiler chickens, and these studies were rarely performed in a traditional tropics setting. As a result, this study looked into the effects of dietary chromium picolinate supplementation, dietary vitamin C supplementation, and their interactions on broiler chicken results, antioxidant status, and serum biochemical indices.

MATERIALS AND METHODS

Study site

This research was carried out at FUTA's Teaching and Research Farm's Poultry Unit. The study was carried out in the study area at the dry season's height (i.e. between January and February 2020).

The wet and dry bulb temperatures in the experimental house were measured twice daily (in the morning and evening). The average daily temperature-humidity index (THI) for the experimental pen was 34.08 ˚C ± 1.36. The THI was measured as an indicator of enclosed broiler chickens' thermal comfort (Tao and Xin, 2003), making use of the formula:

$$THI = 0.85 \times T_{db} + 0.15 \times T_{wb}$$

Where:

T_{db}: dry bulb temperature (˚C).

T_{wb}: wet bulb temperature (˚C).

Sources of chromium picolinate and vitamin C

AK Scientific, Union City, CA, USA, manufactured the chromium picolinate powder (purity level=98%). Avondale Laboratories (Supplies and Services) Limited, Banbury, England, developed the L-ascorbic acid powder (purity level= 100 percent pure (USP/FCC grade).

Experimental diets and animals

For each of the starter (age 1-21 d) and finisher (age 22-42 d) phases, a base diet was formulated (Table 1). The proximate composition of the standard diets was investigated (AOAC, 1995). The basal diet was divided into eight equal parts, dubbed diets 1-8, and supplemented as follows in each phase:

Diet 1: No supplementation (control).

Diet 2: CrPic (0.4 mg) was added as a supplement.

Diet 3: CrPic (0.8 mg) was added as a supplement.

Diet 4: CrPic (1.2 mg) was added as a supplement.

Diet 5: Vitamin C (200 mg) was added as a supplement.

Diet 6: Vitamin C (200 mg) and CrPic (0.4 mg) was added as supplement.

Diet 7: Vitamin C (200 mg) and CrPic (0.8 mg) was added as supplement.

Diet 8: Vitamin C (200 mg) and CrPic (1.2 mg) was added as supplement.

A total of 640 Cobb 500 broiler chickens were randomly assigned to the eight experimental diets/treatment groups (80 birds per treatment; 10 birds per treatment). The birds were housed in individual cubicles/pens (200×100 cm) with a concrete floor and wood shavings for litter.

Table 1 Composition of the experimental diets

Ingredients (g/kg)	Starter (1-21 d)	Finisher (22-42 d)
Maize	524	594
Rice bran	0.000	60.0
Maize bran	70.0	0.000
Soybean meal	300	240
Soy oil	30.0	30.0
Fish meal	30.0	30.0
Limestone	5.00	5.00
Bone meal	30.0	30.0
Salt	3.00	3.00
Premix	3.00	3.00
Methionine	3.00	3.00
Lysine	2.50	2.50
Nutrient composition (g/kg)		
Crude protein*	222	200
Metabolizable energy (kcal/kg)	3019	3108
Methionine	6.80	6.60
Lysine	13.6	12.4
Available phosphorus	4.54	3.32
Calcium	10.1	9.90

* Determined / analysed composition.

The experimental house temperature was held at 32 ˚C ± 2 for the first seven days, then decreased by 2 ˚C every seven days after that until the house temperature reached 24 ˚C ± 2. Throughout the experiment, the birds had unlimited access to food and water.

Growth performance trial, collection of the sample, and laboratory analysis

The final body weight (FBW), body weight gain (BWG), and feed intake (FI) of the experimental birds were estimated, assessed, and reported on a pen-by-pen basis. On a pen-by-pen basis, the feed conversion ratio (FCR) was calculated as the birds' ratio of feed intake to body weight gain.

On day 42 of the experiment, three broiler chickens from each replication were chosen based on their relative weight to the average group weight, labelled, and fasted for 7 hours. Blood samples were taken from these birds and placed in plain sample bottles to be analysed for serum antioxidant enzymes and biochemical study. The concentrations of serum catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were calculated using the procedures of Aebi (1974), Rotruck *et al.* (1973), and Misra and Fridovich (1972), respectively. A Reflectron ®Plus 8C79 (Roche Diagnostic, GombH Mannheim, Germany), was used to determine serum biochemical concentrations (total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, cholesterol, and glucose). The farm's slaughterhouse was used to kill the chosen experimental birds.

The birds were stunned before having their right carotid artery and jugular vein cut with a sharp stainless knife and bleeding for four minutes. The birds were then scalded for 60 seconds at 61 degrees Celsius, de-feathered, manually eviscerated, and dressed. As a percentage of slaughtered weight, the dressed percentage (DP) was calculated (SW). The relative weights of internal organs (liver, lung, heart, kidney, spleen, and gizzard) and abdominal fat (AF) were calculated as percentages of the birds' total weight. Histological examinations were performed on the liver and kidney samples.

Histological studies

Liver and kidney samples were cut in half lengthwise and fixed in 10% neutral buffered formalin for 24 hours. Fixed tissue fragments were transferred through increasing alcohol solutions concentrations (70 percent, 90 percent, absolute ethanol), cleared with methyl benzoate, and embedded in paraffin wax in less than 24 hours. Hematoxylin and eosin were used to stain section 5 µm (HE staining). A light microscope was used to examine the tissues microscopically, while a digital camera was used to take the image (Oloruntola *et al.* 2017; Slaoui and Fiette, 2020).

Statistical analysis

All performance, digestibility, blood parameters, and carcass evaluation data were analysed using General Linear Model (GLM) procedures for a complete randomised design with 4 CrPic levels × 2 vitamin C levels factorial arrangement of treatments.

The data was analysed for CrPic, vitamin C, and CrPic-vitamin C interactions. When the treatment effect was significant (P<0.05), Duncan's multiple range test was used to distinguish the means in SPSS (2011).

RESULTS AND DISCUSSION

Compared with those birds fed the control diet, all other experimental diets recorded a better FBW (P<0.01) and BWG (P<0.01), except for the birds fed the 1.2 mg/kg CrPic supplemented diet who recorded the least FBW and BWG (Table 2). The 0.8 mg/kg CrPic + 200 mg/kg dietary supplementation (diet 7) produced s superior FBW (P<0.01) and BWG (P<0.01) compared to the rest diets. The 0.4 mg/kg and 0.8 mg/kg CrPic supplementations increased the FBW (P=0.00), and BWG (P=0.00) compared to 0.0mg/kg and 1.2 mg/kg CrPic supplementations. In the same vein, the 200 mg/kg vitamin C supplementation enhanced both the FBW (P=0.00) and BWG (P=0.00), compared to the 0.0 mg/kg vitamin C. The interaction of CrPic and vitamin C was also significant (P=0.00). The FI of the birds fed 1.2 mg/kg CrPic supplemented diet (diet 4) was lower (P<0.01) compared to those fed the control and the rest diets. The CrPic supplementation at 1.2 mg/kg level decreased the feed intake, while 200 mg/kg vitamin C supplementation supports increased FI. The best (P<0.01) FCR was recorded in birds fed 1.2 mg/kg CrPic supplemented diet (diet 4) compared to those fed the rest diets.

The CrPic supplementations (0.4 mg/kg. 0.8 mg/kg and 1.2 mg/kg) and vitamin C (200 mg/kg) significantly (P=0.00; P=0.00) improved the FCR. The interaction of CrPic and vitamin C was significant (P=0.00).

Table 3 shows the effect of CrPic and vitamin C supplementation on the slaughter analysis and relative weights of selected organ and carcass. The SW of the birds fed 1.2 mg/kg CrPic was lower (P=0.00) compared to the birds fed the control and the rest diets. However, the CrPic supplementation at 0.4 mg/kg and 0.8 mg/kg supports better SW compared to the 0.0 mg/kg and 1.2 mg/kg supplementations. Vitamin C supplementation increased (P<0.05) SW. The interaction of CrPic and vitamin C was significant (P=0.00). The AF relative weight was low (P<0.05) in the birds fed diet 6 (0.4 mg/kg CrPic and 200 mg/kg vitamin C) and 7 (0.8 mg/kg CrPic and 200 mg/kg vitamin C), compared to those fed the rest diets. CrPic supplementation at 0.4 mg/kg reduced (P<0.05) AF relative weight compared to those fed the control diet. The 1.2 mg/kg CrPic supplementation increased (P=0.00) the relative weights of the liver, lung, heart, kidney, spleen and gizzard.

However, the vitamin C supplementation reduced (P=0.00) the relative weights of these organs excepts for the spleen. The CrPic and vitamin C interaction was significant (P<0.05) for the relative internal organs examined in this study.

The serum CAT and GPx were affected (P<0.05) by the dietary treatment (Table 4). The CrPic supplementation at 1.2 mg/kg and vitamin C supplementation (200 mg/kg) increase (P<0.05) the serum CAT, compared to their various controls. Similarly, the serum GPx increased by 0.8 mg/kg and 1.2 mg/kg CrPic supplementation (P<0.04) and 200 mg/kg vitamin C supplementation (P=0.00).

Table 5 shows the serum biochemical indices of broiler chickens fed CrPic, and vitamin C supplemented diets. The total serum protein declined (P<0.05) with CrPic supplementations at 1.2 mg/kg, compared to the control, and the interaction of CrPic and vitamin C was significant (P<0.01). The albumin concentration was not affected (P>0.05) by both CrPic and vitamin C supplementation. However, the interaction of these supplements was significant (P<0.05). The serum albumin reduced significantly (P<0.05) in the birds fed diets supplemented with 0.4 mg/kg CrPic, 0.8 mg/kg CrPic and 1.2 mg/kg CrPic but picked up and similar to those fed the control diet in birds fed the diets supplemented with 0.8 mg/kg CrPic + 200 mg/kg vitamin C and 1.2 mg/kg CrPic + 200 mg/kg vitamin.

The CrPic dietary supplementation at 1.2 mg/kg produced significantly (P<0.05) lower globulin concentration than the control and other lower CrPic supplementation level. The 200 mg/kg vitamin C supplementation caused the reduction (P<0.05) of the AST. CrPic supplementation at 1.2 mg/kg produced an increased serum ALT concentration. However, vitamin C supplementation (200 mg/kg) caused a reduction (P<0.05) of the serum ALT. Also, CrPic X vitamin C was significant (P<0.05). The CrPic at 0.8 mg/kg and 1.2 mg/kg supplementations reduced the serum glucose concentration. Besides, the interaction of CrPic and vitamin C was significant (P<0.05).

Figure 1 shows the histological effects of CrPic and vitamin C supplementation on broiler liver. In the broiler chickens given 0 mg/kg CrPic (control group), 0.4 mg/kg CrPic, and 0.8 mg/kg CrPic dietary supplementations, the hepatocytes (H) appear polygonal and are disposed of in a sheet with a well-outlined nucleus.

The hepatocytes are separated by sinusoid (S) with thin endothelial lining, free from collections and mild leukocytosis (arrowhead). The Portal Region comprises branches of the hepatic portal vessels (HPV), and the bile duct (BD) appears normal.

Table 2 The performance of broiler chickens fed chromium picolinate (CrPic) and vitamin C supplemented diets (42 days of age)

Diets	CrPic (mg/kg)	Vitamin C (mg/kg)	Initial weight (g/bird)	Final body weight (g/bird)	Body weight gain (g/bird)	Feed intake (g/bird)	Feed conversion ratio
1	0.000	0.000	34.2	1844^{c}	1810^{c}	3474^{a}	1.92^{a}
2	0.400	0.000	33.0	2165^{ab}	2132^{ab}	3695^{a}	1.73^{ab}
3	0.800	0.000	33.5	2115^{ab}	2082^{ab}	3651^{a}	1.75^{b}
4	1.20	0.000	33.6	872^{d}	839^{d}	1377.87^{b}	1.64^{c}
5	0.000	200	32.4	2059^{b}	2027^{ab}	3620^{a}	1.78^{b}
6	0.400	200	33.3	2160^{ab}	2127^{ab}	3635^{a}	1.71^{ab}
7	0.800	200	32.6	2270^{a}	2238^{a}	3779^{a}	1.69^{ab}
8	1.20	200	32.9	2171^{ab}	2139^{ab}	3708^{a}	1.73^{ab}
SEM			0.250	90.4	90.5	160.9	0.020
P-value			0.750	0.010	0.010	0.010	0.010
	0.000		33.3	1952^{b}	1918^{b}	3547^{a}	1.85^{a}
	0.400		33.1	2163^{a}	2130^{a}	3665^{a}	1.72^{b}
	0.800		33.1	2193^{a}	2160^{a}	3715^{a}	1.72^{b}
	1.20		33.3	1522^{c}	1489^{c}	2543^{b}	1.68^{b}
	SEM		0.540	39.9	39.7	76.0	0.020
	P-value		0.980	0.000	0.000	0.000	0.000
		0.000	33.6	1749^{b}	1716^{b}	3050^{b}	1.76^{a}
		200	32.8	2165^{a}	2133^{a}	3686^{a}	1.72^{b}
		SEM	0.380	28.2	28.1	53.7	0.010
		P-value	0.160	0.000	0.000	0.000	0.000
	Cr Pic$_3$ × vitamin C						
		SEM	0.760	56.5	56.2	107.5	0.030
		P-value	0.610	0.000	0.000	0.000	0.000

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 3 The carcass traits and relative organ weights[1] of broiler chickens fed chromium picolinate (CrPic) and vitamin C supplemented diets (42 days of age)

Diets	CrPic (mg/kg)	Vitamin C (mg/kg)	SW (g/bird)	DP (% DW)	AF (% SW)	Liver (% SW)	Lung (% SW)	Heart (% SW)	Kidney (% SW)	Spleen (% SW)	Gizzard (% SW)
1	0.000	0.000	1871^{c}	88.4	0.730^{a}	1.69^{b}	0.510^{b}	0.380^{b}	0.610^{b}	0.070^{bc}	2.24^{b}
2	0.400	0.000	2192^{ab}	87.8	0.500^{ab}	1.41^{b}	0.410^{bc}	0.320^{bc}	0.440^{bc}	0.050^{c}	1.64^{cd}
3	0.800	0.000	2142^{ab}	93.5	0.810^{a}	1.59^{b}	0.470^{b}	0.380^{b}	0.350^{cd}	0.080^{bc}	1.80^{c}
4	1.20	0.000	899^{d}	81.4	0.630^{ab}	3.39^{a}	1.050^{a}	0.900^{a}	1.17^{a}	0.120^{a}	5.55^{a}
5	0.000	200	2086^{b}	84.9	0.940^{a}	0.91^{c}	0.210^{c}	0.260^{c}	0.230^{d}	0.040^{c}	1.28^{d}
6	0.400	200	2187^{ab}	82.4	0.250^{b}	1.58^{b}	0.400^{bc}	0.360^{b}	0.520^{bc}	0.100^{ab}	1.84^{c}
7	0.800	200	2297^{a}	91.5	0.260^{b}	1.49^{b}	0.400^{bc}	0.350^{b}	0.510^{bc}	0.070^{bc}	1.78^{c}
8	1.20	200	2198^{ab}	85.9	0.530^{ab}	1.71^{b}	0.420^{bc}	0.360^{b}	0.430^{bc}	0.080^{abc}	1.74^{c}
SEM			90.4	1.45	0.060	0.140	0.050	0.030	0.060	0.010	0.260
P-value			0.000	0.430	0.050	0.000	0.000	0.000	0.000	0.010	0.000
	0.000		1979^{b}	86.60	0.830^{a}	1.29^{b}	0.360^{b}	0.320^{b}	0.420^{b}	0.570^{b}	1.76^{b}
	0.400		2190^{a}	85.12	0.380^{b}	1.49^{b}	0.410^{b}	0.340^{b}	0.480^{b}	0.070^{ab}	1.74^{b}
	0.800		2220^{a}	92.47	0.530^{ab}	1.54^{b}	0.430^{b}	0.360^{b}	0.430^{b}	0.070^{ab}	1.79^{b}
	1.20		1549^{c}	83.64	0.580^{ab}	2.55^{a}	0.740^{a}	0.630^{a}	0.800^{a}	0.100^{a}	3.65^{a}
	SEM		39.9	2.89	0.110	0.090	0.050	0.020	0.040	0.010	0.090
	P-value		0.000	0.19	0.050	0.0000	0.000	0.000	0.000	0.000	0.000
		0.000	1776^{b}	87.8	0.670	2.02^{a}	0.610^{a}	0.500^{a}	0.640^{a}	0.080	2.81^{a}
		200	2192^{a}	86.2	0.490	1.42^{b}	0.360^{b}	0.330^{b}	0.420^{b}	0.070	1.66^{b}
		SEM	28.23	2.05	0.070	0.060	0.030	0.010	0.030	0.010	0.060
		P-value	0.000	0.590	0.130	0.000	0.000	0.000	0.000	0.530	0.000
	Cr Pic$_3$ × vitamin C										
		SEM	56.47	4.09	0.150	0.140	0.070	0.020	0.060	0.010	0.130
		P-value	0.000	0.650	0.140	0.000	0.010	0.000	0.000	0.020	0.000

SW: slaughter weight; DW: dressed weight; DP: dressed percentage and AF: abdominal fat.
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 The antioxidant enzyme status of broiler chickens fed chromium picolinate (CrPic) and vitamin C supplemented diets (42 days of age)

Diets	CrPic (mg/kg)	Vitamin C (mg/kg)	Catalase (kU)	Glutathione peroxidase (mg/mL)	Superoxide dismutase (%)
1	0.000	0.000	5.76^{c}	81.6^{c}	62.0
2	0.400	0.000	6.09^{c}	147^{ab}	64.0
3	0.800	0.000	8.17^{bc}	121^{bc}	48.7
4	1.20	0.000	9.70^{ab}	158^{ab}	46.7
5	0.000	200	10.4^{ab}	159^{ab}	68.6
6	0.400	200	10.9^{ab}	170^{a}	76.3
7	0.800	200	11.7^{a}	158^{ab}	64.6
8	1.20	200	12.4^{a}	158^{ab}	50.6
SEM			0.560	6.98	3.23
P-value			0.010	0.010	0.220
	0.000		8.09^{b}	120^{b}	65.3
	0.400		8.52^{b}	159^{ab}	70.2
	0.800		9.92^{ab}	140^{a}	56.7
	1.20		11.1^{a}	158^{a}	48.7
	SEM		0.690	9.72	5.99
	P-value		0.030	0.040	0.090
		0.000	7.45^{b}	127^{b}	55.3
		200	11.3^{a}	161^{a}	65.1
		SEM	0.490	6.92	4.24
		P-value	0.000	0.000	0.120
	Cr Pic$_3$ × vitamin C				
		SEM	0.980	13.84	8.47
		P-value	0.630	0.070	0.890

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 The serum biochemical indices[1] of broiler chickens fed chromium picolinate (CrPic) and vitamin C supplemented diets (42 days of age)

Diets	CrPic (mg/kg)	Vitamin C (mg/kg)	TP (g/dL)	ALB (g/dL)	GLO (g/dL)	AST (U/L)	ALT (U/L)	CREA (µmol/L)	CHOL (mmol/L)	GLU (mg/dL)
1	0.000	0.000	4.26^{a}	1.54^{ab}	2.72^{a}	96.13^{ab}	32.1^{bc}	55.2	1.63	182^{a}
2	0.400	0.000	4.20^{a}	1.36^{cd}	2.83^{a}	101.97^{ab}	33.8^{bc}	67.2	2.10	167^{a}
3	0.800	0.000	4.09^{a}	1.32^{cd}	2.77^{a}	116.43^{a}	35.3^{bc}	69.0	2.13	164^{a}
4	1.20	0.000	3.67^{b}	1.33^{cd}	2.34^{b}	125.26^{a}	45.7^{a}	76.8	2.03	125^{b}
5	0.000	200	4.19^{a}	1.25^{d}	2.94^{a}	78.13^{b}	27.5^{c}	36.0	1.83	165^{a}
6	0.400	200	4.13^{a}	1.32^{cd}	2.81^{a}	79.66^{b}	29.6^{bc}	45.3	2.10	167^{a}
7	0.800	200	4.33^{a}	1.42^{bc}	2.91^{a}	79.03^{b}	29.1^{bc}	54.8	2.13	164^{a}
8	1.20	200	4.26^{a}	1.64^{a}	2.62^{ab}	80.37^{b}	30.6^{bc}	66.6	2.16	168^{a}
SEM			0.050	0.030	0.050	4.52	1.27	4.60	0.07	4.09
P-value			0.010	0.010	0.020	0.010	0.000	0.390	0.330	0.010
	0.000		4.23^{a}	1.39	2.83^{a}	87.1	29.8^{b}	45.6	1.73	173^{a}
	0.400		4.17^{ab}	1.34	2.82^{a}	90.8	31.7^{b}	56.3	2.10	167^{a}
	0.800		4.21^{a}	1.37	2.84^{a}	97.7	32.2^{b}	61.9	2.13	164^{ab}
	1.20		3.97^{b}	1.48	2.48^{b}	103	38.2^{a}	71.7	2.10	147^{b}
	SEM		0.070	0.04	0.070	6.52	26.8	9.02	6.16	6.16
	P-value		0.050	0.16	0.030	0.350	0.010	0.260	0.080	0.040
		0.000	4.06	1.39	2.66	110^{a}	36.7^{a}	67.03	1.98	160
		200	4.23	1.41	2.82	79.3^{b}	29.2^{b}	50.67	2.06	166
		SEM	0.050	0.030	0.050	4.61	1.01	6.38	0.080	4.36
		P-value	0.210	0.120	0.060	0.000	0.000	0.080	0.480	0.320
	Cr Pic$_3$ × vitamin C									
		SEM	0.090	0.050	0.110	9.22	2.03	12.76	0.170	8.72
		P-value	0.010	0.000	0.070	0.440	0.050	0.960	0.910	0.020

TP: total protein; ALB: albumin; GLO: globulin; AST: aspartate transaminase; ALT: alanine transaminase; CREA: creatinine; CHOL: cholesterol and GLU: glucose.
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.

In the broiler chickens fed a 1.2 mg/kg CrPic supplemented diet, the hepatocytes (H) appear polygonal and are disposed of in a sheet with a well-outlined nucleus. Section shows marked periportal hepatic inflammation (Star).

The histological examination of the liver from the broiler chickens fed diets supplemented with 200 mg/kg vitamin C, 0.4 mg/kg CrPic + 200 mg/kg vitamin C, 0.8 mg/kg CrPic + 200 mg/kg vitamin C, and 1.2 mg/kg CrPic + 200 mg/kg vitamin C that the liver hepatocytes (H) appear polygonal and are disposed of in sheet with a well-outlined nucleus. The hepatocytes are separated by the sinusoid (S) with a thin endothelial lining, free from collections and inflammatory cells. In addition, the 1.2 mg/kg CrPic + 200 mg/kg vitamin C supplementation caused moderate periportal hepatic inflammation (Star).

Figure 2 shows the histological effects of CrPic and vitamin C supplementation on broiler kidney. In the broiler chickens fed 0 mg/kg CrPic, 0.4 mg/kg CrPic, and 0.8 mg/kg CrPic dietary supplementations, the corpuscle is made up of the glomerulus (G) composed of podocytes and separated by a defined Bowman's space (BS). The renal tubules, (renal tubule (RT), proximal convoluted tubule (PCT), distal convoluted tubule (DCT)) are lined by columnar-cuboidal epithelium and are separated by the interstitium with mild inflammatory cells infiltration (star), the juxtaglomerular apparatus (circle). In the broiler chickens fed a 1.2 mg/kg CrPic supplemented diet, the corpuscle is made up of the Glomerulus (G) composed of podocytes and separated by a defined Bowman's space (BS). The renal tubules, RT (PCT, DCT), are lined by columnar-cuboidal epithelium. The section shows noticeable interstitial nephritis (star). The histological examination of the kidney from the broiler chickens fed diets supplemented with 200 mg/kg vitamin C, 0.4 mg/kg CrPic + 200 mg/kg vitamin C, 0.8 mg/kg CrPic + 200 mg/kg vitamin C, and 1.2 mg/kg CrPic + 200 mg/kg vitamin C that the corpuscle is made up of the Glomerulus (G) composed by podocytes and separated by a defined Bowman's space (BS). The renal tubules, RT (PCT, DCT), are lined by columnar-cuboidal epithelium and separated by the interstitium with mild inflammatory cells infiltration (star). The higher FBW and BWG in birds fed diets supplemented with 0.4 mg/kg CrPic, 0.8 mg/kg CrPic, 0.4 mg/kg CrPic + 200 mg/kg vitamin C, 0.8 mg/kg CrPic + 200 mg/kg vitamin C, and 1.2 mg/kg CrPic + 200 mg/kg vitamin C compared to those fed the control in this study indicates that Cr and vitamin C dietary enrichment improves the birds' FBW and BWG of the birds (Hajializadeh *et al.* 2017; Barrio *et al.* 2019). The improved FBW and BWG in broiler chickens by 0.4 and 0.8 mg/kg CrPic supplementation in this study agreed with previous studies by Naela *et al.* (2008) and Hajializadeh *et al.* (2017), which showed that dietary CrPic supplementation (200 to 4000 g/kg) could boost chicken growth efficiency.

One of Cr supplementation's beneficial aspects of biological activities may be the observed enhanced growth efficiency due to CrPic supplementation (Khan *et al.* 2014). Chromium's capacity to control glucose levels by inducing insulin secretion, in particular, promotes proper lipid, carbohydrate, and protein metabolism (Ognik *et al.* 2020). Since chromium is required for the activation of certain enzymes and the maintenance of protein and nucleic acids, a deficiency may disrupt carbohydrate, protein, and lipid metabolism (Sahin *et al.* 2010). In this study, the improvement of FBW and BWG by 200 mg/kg vitamin C dietary supplementation agreed with Barrio *et al.* (2019). Vitamin C supplementation has been shown to enhance poultry's growth output with a suppressed stress response, by lowering plasma corticosterone and adrenocorticotropic hormone levels (Lin *et al.* 2003; Lin *et al.* 2006).

The lower FBW and BWG observed in birds fed a CrPic supplemented diet at 1.2 mg/kg in this study indicates that CrPic supplementation at 1.2 mg/kg was excessive in feed and, therefore, toxic. When 1.2 mg/kg CrPic was added to the feed along with 200 mg/kg vitamin C in the diet, however, the birds' growth was not slowed. Vitamin C, by extension, has a beneficial biological function that may have mitigated the toxic effects of CrPic supplementation at 1.2 mg/kg. This helps to understand why CrPic and vitamin C have such a powerful interaction impact on the chickens' FBW and BWG in this research.

In this research, the decreased FI found in the birds at 1.2 mg/kg CrPic dietary supplementation may be a consequence of chromium's toxic impact on the gastrointestinal tracts and microflora, which are responsible for feed digestion and utilisation (Upreti *et al.* 2004). However, the significant increase in FI with 200 mg/kg vitamin C supplementation in this study further demonstrated vitamin C's ability to mitigate the adverse effects of chromium toxicity at high doses (Stanley *et al.* 2013). The significant interaction effects of CrPic and vitamin C supplementation, as reported in this study, indicate that these two supplements' activities can complement each other to boost the birds' feed intake. The improved FCR associated with CrPic and vitamin C supplementation and their interaction in this research highlight the importance of these supplements in improving animal performance, especially in stressful tropical environments (Ahmadu *et al.* 2015; Haq *et al.* 2016; Barrio *et al.* 2019).

Chromium, as an insulin cofactor, facilitates insulin activity and improves the absorption of amino acids into the muscular cell for protein synthesis (Ohba *et al.* 1986).

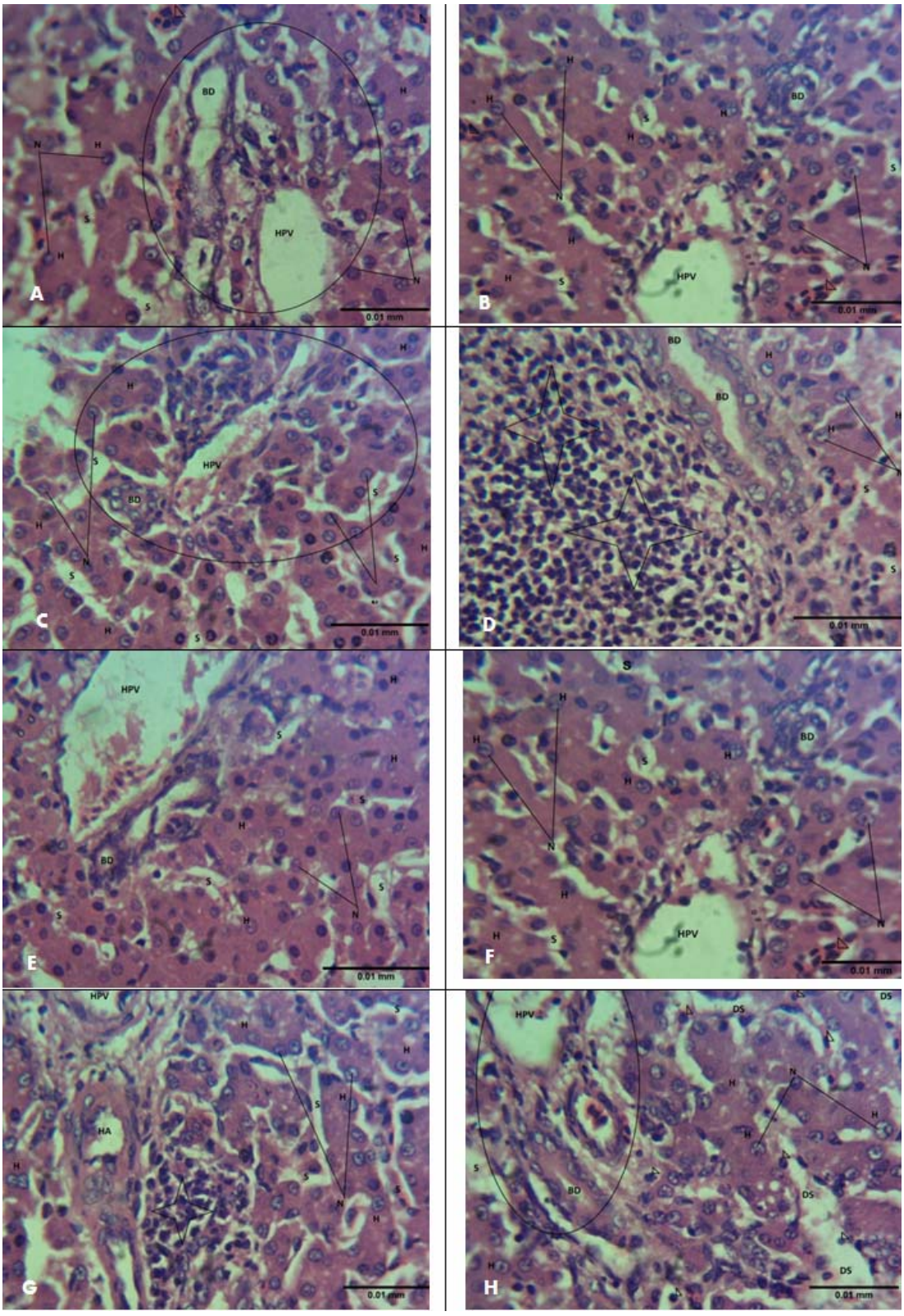

Figure 1 Histological effects of chromium picolinate **(**CrPic) and vitamin C supplementation on broiler liver (magnification X400)

Treatments: A: control group; B: 0.4 mg/kg CrPic; C: 0.8; D: 1.2 mg/kg CrPic; E: 200 mg/kg vitamin C; F: 0.4 mg/kg CrPic + 200 mg/kg vitamin C; G: 0.8 mg/kg CrPic + 200 mg/kg vitamin C and H: 1.2 mg/kg CrPic + 200 mg/kg vitamin C

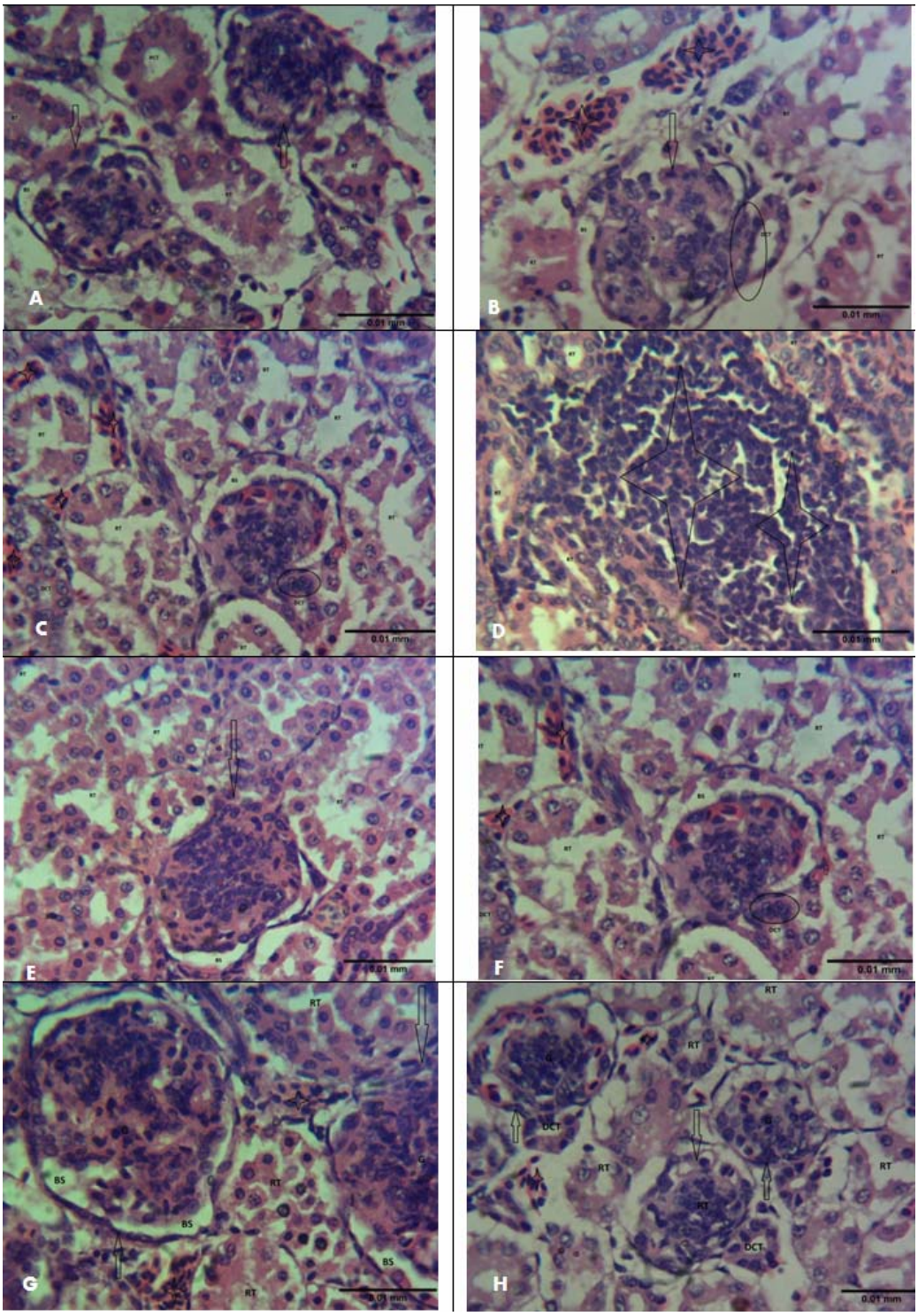

Figure 2 Histological effects of chromium picolinate **(**CrPic) and vitamin C supplementation on broiler kidney (magnification X400)
Treatments: A: control group; B: 0.4 mg/kg CrPic; C: 0.8; D: 1.2 mg/kg CrPic; E: 200 mg/kg vitamin C; F: 0.4 mg/kg CrPic + 200 mg/kg vitamin C; G: 0.8 mg/kg CrPic + 200 mg/kg vitamin C and H:1.2 mg/kg CrPic + 200 mg/kg vitamin C

Furthermore, dietary Cr supplementation increases carcass characteristics and meat quality in broiler chickens (Ward *et al*. 1993). In this research, the impact of CrPic and vitamin C supplementation on SW follows the same pattern as FLW and BWG. CrPic and vitamin C supplementation had similar effects on the FLW, BWG, and SW, we can deduce. This supported Oloruntola *et al*. (2018a) earlier finding that slaughtered and dressed weights are proportional to final live weight.

Since belly fat is related directly to total body fat content in avian species, it is a reliable criterion for assessing total body fat content (Chen *et al*. 2018). This study's findings of decreased belly fat caused by CrPic supplementation matched those of Toghyani *et al*. (2006) and Chen *et al*. (2018), who found significantly reduced abdominal fat in broiler chickens fed Cr supplemented diets. This indicates that CrPic reduced lipoprotein lipase activity (LPL) in experimental groups, resulting in a substantial decrease in LPL activity. LPL is a rate-limiting enzyme for the catalysis of triglycerides into glycerin and non-esterified fatty acids, which is essential for fat deposition in animal tissues. LPL catalysis products provide the raw material for fat synthesis and play a key role in fat metabolism and transportation (Chen *et al*. 2018). Furthermore, CrPic's role in decreasing the activity of fatty acid synthase, a rate-limiting enzyme in the final step of *de novo* synthesis of long-chain fatty acids in animals that also catalyses acetyl-CoA and maloney-CoA to synthesise the fatty acid, could be related to the observed decreased fat belly in this study (Smith *et al*. 2003; Chen *et al*. 2018).

Toxic effects can cause abnormally increased relatively internal organ weights (Ayodele *et al.* 2016), while these internal organs' stability indicates a stable health status (Oloruntola *et al.* 2018b). The enlargement of the internal organs (liver, lung, heart, kidney, spleen and gizzard) at 1.2 mg/kg CrPic supplementation level in this study may be the response of these organs to the toxic effects. While the reduction of these organs relative weights (except for the spleen) by 200 mg/kg vitamin C dietary supplementation further suggests that vitamin C could help ameliorate the toxic effects of CrPic dietary overdose in broiler chickens (Nosrati *et al*. 2017). This also depicts the positive effects of the CrPic and vitamin C interaction recorded in this study. Earlier, it was suggested that vitamin C (antioxidant) might produce a synergistic action to modify the carcass and lipid profile (Haq *et al.* 2016; Tavakoli *et al*. 2020). It is well established that exposure to the high level of transition metal ions(n+) represent a realistic *in vivo* production of reactive oxygen species (ROS) and free radicals due to intra-cellular reduction. Many studies have focused on metal-induced toxicity, emphasising their role in the development of ROS (Garcia-Rodriguez *et al*. 2017).

Low molecular weight antioxidants, such as vitamin C, are capable of chelating metal ions, reducing their catalytic activity, and reducing ROS formation. Since heavy metal genotoxicity is related to oxidative stress (Valko *et al.* 2006; Garcia-Rodriguez *et al*. 2017), vitamin C may effectively protect or reduce the induced toxic effects by suppressing oxidative stress (Garcia-Rodriguez *et al*. 2017).

Catalase, glutathione peroxidase, and superoxide dismutase are the principal enzymes that regulate free radicals' neutralisation (Jeeva *et al.* 2015; Ighodaro and Akinloye, 2018). The CrPic (1.2 mg/kg) and vitamin C (200 mg/kg) supplementation increased serum CAT and GPx concentrations in the experimental birds, suggesting that CrPic and vitamin C have the potential to improve antioxidant protection machinery (Khan *et al.* 2014; Higgins *et al*. 2020). Antioxidants such as chromium and vitamin Care are thought to defend against oxidative harm (Preuss *et al.* 1997; Haq *et al.* 2016). According to Onderci *et al*. (2005), chromium's antioxidant activity may be attributed to its insulinotropic effect, inhibiting epinephrine production. Furthermore, vitamin C has been shown to play a vital role in cellular antioxidant defences by forming dehydroascorbyl (an inert radical) and moving radical equivalents from lipid phases (Ahmadu *et al*. 2015). As a consequence, the increased serum GPx and CAT concentrations observed in this study as a result of CrPic and vitamin C supplementation are beneficial since GPx and CAT are among the enzymes responsible for constructing the cell's first level of antioxidant protection, and increased serum GPx and CAT concentration could have a positive impact on stress-prone performance characteristics.

Serum biochemical indices are crucial in diagnosing disease, especially when determining the health of animals fed experimental diets (Onasanya *et al.* 2015; Oloruntola *et al.* 2018a). Most enzymes, in particular, are tissue-specific, and an increase in blood concentration indicates tissue impairment (Ayodele *et al.* 2016; Oloruntola *et al.* 2020). The reduced serum total protein concentration found in this study due to 1.2 mg/kg CrPic supplementation relative to the control may be due to haemodilution, decreased protein production, or increased protein loss or catabolism (Lording and Friend, 1991). As a result, the 1.2 mg/kg CrPic supplementation could be unnecessary and potentially detrimental to the birds' health. However, adding 200 mg/kg of CrPic to the diet tends to minimise the harmful or adverse effects of CrPic at 1.2 mg/kg in the birds. This explains why total protein levels in birds fed the 1.2 mg/kg + 200 mg/kg vitamin C diet in this study were comparable to those in birds fed the control diet. The significant interaction of CrPic and vitamin C supplementation may explain the variation in serum albumin concentrations of

the birds fed the various experimental diets so that the globulin concentration of the birds fed diets supplemented with a combination of CrPic (0.8 mg/kg CrPic+200 mg/kg vitamin C and 1.2 mg/kg CrPic+200 mg/kg vitamin C) was similar to those fed the control diet. The lower globulin concentration observed after 1.2 mg/kg CrPic supplementation may be due to a failure of passive transfer, decreased globulin content, and increased globulin and albumin loss or catabolism (Lording and Friend, 1991). This is significant for health because immune-deficiency disorders are linked to lower globulin levels in animals (Lording and Friend, 1991).

The enzymes aspartate aminotransferase and alanine aminotransferase are commonly used to diagnose liver cell infarction and inflammation (Oloruntola *et al*. 2018; Oloruntola *et al*. 2018b). Vitamin C has been shown to have hepatoprotective effects related to its anti-oxidative properties by reducing chemical agent-induced hepatic damage in animals (Adikwu and Deo, 2013; Milosevic *et al*. 2018). Vitamin C's hepatoprotective properties illustrate why 200 mg/kg vitamin C supplementation resulted in lower AST levels. This also explains why the birds fed 1.2 mg/kg CrPic + vitamin C had lower AST levels than the birds fed 1.2 mg/kg CrPic in this report. The fact that 1.2 mg/kg CrPic causes a rise in ALT indicates toxicity or hepatotoxicity (Lording and Friend, 1991). However, in this study, 200 mg/kg vitamin C supplementation tended to avoid the pathological increase in ALT concentration in birds fed dietary 1.2 mg/kg CrPic + 200 mg/kg vitamin C supplementation. Bashandy and Alwasel (2011) found that vitamin C stabilised the levels of AST, ALT, and a variety of other serum biochemical indices in intoxicated rats. Furthermore, Grajeda-Cota *et al*. (2004) found that vitamin C maintained the AST and ALT integrity in cypermethrin-toxified rats. When compared to mammals, birds are thought to be less insulin-sensitive. In animals, however, chromium supplementation induces an increase in insulin sensitivity and a decrease in plasma glucose concentration (Brooks *et al.* 2016). The reduced blood glucose concentrations observed in this study as a result of 1.2 mg/kg CrPic dietary supplementation is consistent with previous findings (Jackson *et al*. 2008; El-Kholy *et al.* 2017), implying increased glucose removal due to excessive insulin, excessive glucose utilisation, and decreased glucose output (Lording and Friend, 1991). This may be attributed to chromium's metabolic role in accelerating insulin production through the presence of an organometallic compound called glucose tolerance factor (Sahin *et al*. 2003; Ngala *et al*. 2018). As a result, dietary supplementation with chromium can boost the activity of the glucose tolerance factor for insulin in broilers (Arif *et al*. 2019).

The broiler chickens fed dietary 1.2 mg/kg CrPic supplementation in this study had significant hepatic inflammation in the liver and significant interstitial nephritis of the kidney, indicating the possible toxic effect of an unsuitable higher CrPic dose. Asmatullah *et al*. (1999) and Haq *et al*. (2016) had previously reported similar findings. In broiler chickens, Ognik *et al*. (2020) recorded a significant degree of hyperaemia of hepatic tissue due to dietary chromium nanoparticle supplementation, while Liu *et al*. (2015) reported that excessive Cr (III) intake causes oxidative degradation and histopathological changes in organs.

CONCLUSION

Dietary supplements of 0.8 mg/kg CrPic and 200 mg vitamin C increased body weight gain, feed conversion ratio, and slaughtered weight, as well as antioxidant enzyme status. Dietary vitamin C supplementation (200 mg/kg) decreased aspartate and alanine aminotransferase levels, while dietary CrPic supplementation (1.2 mg/kg) reduced blood glucose levels. However, dietary supplementation with 0.4 mg/kg CrPic decreased abdominal fat, while supplementation with 1.2 mg/kg CrPic resulted in hepatic inflammation and interstitial nephritis. In broiler development under tropical conditions, a combination of 0.8 mg/kg CrPic and 200 mg vitamin C dietary supplementation is recommended.

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