

SERUM PROTEIN AND GLUCOSE OF COCKS FED CRPIC DIETS WITH OR WITHOUT VITAMIN C AT HIGH AMBIENT TEMPERATURE

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ABSTRACT. This study assessed the effect of chromium picolinate (CrPic) and vitamin C (Vit C) on the serum proteins and glucose of two breeds of cocks. A total of 192 sexually mature cocks, 24 weeks old, consisting of 96 Noiler cocks (NC) and 96 White Leghorn cocks (WLC), were utilised in the study. The two breeds were allocated to 8 treatments and reproduced four times, with six cocks per replication, in a $2 \times 2 \times 4$ factorial design. A total of 8 experimental diets were constituted with four diets containing CrPic at 0.00, 0.40, 0.80 and 1.20 mg CrPic/kg without Vit C and another four diets containing CrPic at 0.00, 0.40, 0.80 and 1.20 mg CrPic/kg with Vit C inclusion at 200 mg/kg diet in each of the treatments for 16 weeks. The data collected were subjected to a $2 \times 2 \times 4$ factorial arrangement using SAS (version 9.2). The serum proteins studied included total protein (TP) (g/dl), albumin (ALB) (g/dl), and globulin (GLB) (g/dl). Supplementation of CrPic significantly

($p < 0.05$) enhanced serum proteins up to 0.8 mg/kg diet. However, a 1.2 mg/kg diet significantly reduced serum protein concentrations. Serum glucose was not significantly ($p > 0.05$) different among cocks fed varied CrPic supplements compared to the control diet. The immunostimulant activity of vitamin C at a 200 mg/kg diet increased serum protein and decreased glucose levels compared to the control. Notably, interactions between 0.8 mg/kg CrPic and 200 mg/kg vitamin C revealed breed-specific effects: It increased serum protein in both breeds, decreased serum glucose in White Leghorns, but increased it in Noilers. This suggests a differential effect of the CrPic and vitamin C combination on glucose regulation in these breeds. This study therefore suggests that a 0.8 mg CrPic/kg diet with Vit C has the potential to significantly increase serum protein and enhance insulin sensitivity in cocks raised at high ambient temperatures.



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Keywords: chromium picolinate; glucose; protein; serum; vitamin C.

INTRODUCTION

Proteins, which are large molecules composed of one or more linear chains of amino acids linked by peptide bonds, function as the primary components of the blood serum or plasma and serve a wide range of functions. The biochemical composition of most serum proteins is not purely proteinaceous, given that they are often bound to various other molecules; for instance, they may be linked to carbohydrates and circulate as glycoproteins (Stockham and Scott, 2002). The regulation of protein levels in the serum is rigorously maintained to ensure the necessary balance for their physiological functions in immunity, coagulation, transportation of small molecules, and inflammatory responses. Any disruption or imbalance in the levels of serum proteins can either lead to or be a consequence of pathological conditions (Pieper *et al.*, 2003). The primary categorization of serum proteins involves the separation into two main fractions: albumin and globulin (Tothova *et al.*, 2016).

Albumin, the predominant protein in serum, serves crucial biological roles such as sustaining colloidal osmotic pressure, binding a wide array of substances, and contributing significantly to the antioxidant capacity of plasma (Levitt and Levitt, 2016). Globulins, on the other hand, act as carriers for sex hormones and are integral to immune responses and inflammatory processes (McPherson, 2006).

The metabolism of carbohydrates, proteins, and fats depends on chromium;

it also strengthens immune response in cattle. Derived from organic sources, chromium picolinate and chromium propionate have proved to have a great degree of bioavailability. Numerous studies conducted on animals have validated the positive consequences of chromium supplementation (Chandrasekar and Balakrishnan, 2019). Studies have shown that including chromium in cattle diets can help lower the negative effects of stress, thereby improving general health and performance (Amata, 2013). When chromium forms part of a biomolecule called chromodulin, it exhibits biological activity. In an insulin signalling system, chromodulin is involved and influences the metabolism of carbohydrates and fats by means of insulin (Vincent, 2000). It has been shown that insulin increases the absorption of glucose and amino acids into muscle cells, therefore regulating energy generation, muscle tissue development, and fat metabolism. Insufficient insulin causes the body cells to convert glucose into fat and store it in adipose cells. Furthermore, hampered is the process of muscle development in absence of enough amino acids entering the cells (Anderson, 1994). In mammals, chromium (Cr) has been demonstrated to enhance the body's response to insulin; adding Cr has been proven to reduce blood glucose levels in broiler chickens (Moeini *et al.*, 2011).

MATERIALS AND METHODS

Ethical approval

The study was carried out under permission to use animal and animal protocol granted by the Research and Ethics Committee of the Department of

Animal Production and Health, The Federal University of Technology, Akure, Nigeria with a Ph.D. Studentship identification number of FUTA/APH/90/2163

Experimental site and operations

The experiment was conducted at the Teaching and Research Farm's Poultry section at Federal University of Technology, Akure, Nigeria. The area's vegetation is of rainforest type, marked by two peaks of humidity and rainfall throughout the wet season. Mean yearly relative humidity is about 75%; mean rainfall is over 1500 mm and rain falls from March to November.

In the experimental house, the temperatures of the wet and dry bulbs were recorded twice a day (in the morning and evening). For the controlled enclosure, the mean daily temperature-humidity index (THI) was 34.08 ± 1.36 .

According to Tao and Xin (2003), the THI was calculated using the following formula to determine the thermal comfort of enclosed grill chickens (*Equation 1*).

$$THI = 0.85 \times T_{db} + 0.15 \times T_{wb} \quad (1)$$

where T_{db} = Dry bulb temperature ($^{\circ}C$) and T_{wb} = Wet bulb temperature ($^{\circ}C$).

Sources of chromium picolinate and vitamin C

Produced with a purity level of 98%, the chromium picolinate powder was obtained from AK Scientific, a Union City, California, company. Avondale Laboratories (Supplies and Services) Limited, Banbury, England-based company developed the L-ascorbic acid powder with a hundred percent pure (USP/FCC grade) purity level.

Experimental birds and diets

For the purpose of this research, a total of 192 sexually matured cocks aged twenty-four (24) weeks were utilized. They were evenly divided into groups of ninety-six (96) Noiler cocks and ninety-six (96) White Leghorn cocks. These two breeds were then further divided into eight treatments, each replicated four times in a $2 \times 2 \times 4$ factorial arrangement, with six cocks per replication. The cocks were provided with unrestricted access to supplemented diets and drinking water over a sixteen-week period. The meals varied depending on chromium picolinate and vitamin C levels. The eight treatments (T1 – T8) were formed using a basal diet as illustrated in *Table 1*.

Table 1 – Composition of the cocks' basal experimental diets (Adebayo *et al.*, 2022)

Ingredients	Quantity (kg)
Maize	39.00
Soya bean meal	3.00
Ground nut cake	4.00
Corn bran	2.60
Palm kernel meal	4.00
Wheat offal	44.00
Limestone	1.20
Bone meal	1.50
Lysine	0.08
Methionine	0.07
Salt	0.30
Layer premix*	0.25
Total	100
Calculated values	
Crude protein (%)	15.12
ME (kcal/kg)	2506.38
Ca (%)	1.05
Crude fibre (%)	5.54
Crude fat (%)	4.48
Methionine (%)	0.32

The basal diet was applied to the eight treatments replicated four times with 6 cocks per replicate

The initial four treatments (T1 – T4) were formulated by incorporating four different levels of CrPic (0.00, 0.40, 0.80, and 1.20 mg CrPic) without vitamin C per kilogram of basic diet. The subsequent four treatments (T5 – T8) were formulated by supplementing 200 mg/kg of vitamin C to each level of CrPic (0.00 CrPic + 200 mg vit C, 0.40 CrPic + 200 mg vit C, 0.80 CrPic + 200 mg vit C, and 1.20 CrPic + 200 mg vit C) per kilogram of basic diet. At the end of the study, four cocks per replication were selected and fasted overnight to evaluate their hormone and blood parameters.

Serum metabolites and lipid analyses

Blood samples were first put on a test tube rack in the laboratory at room temperature in a tilted posture for 15 minutes, then centrifuged for five minutes at 3000 rpm to get clear supernatant serum for serological analysis. The resultant serum samples were kept at -20 °C, followed by centrifugation at 3000 rpm for five minutes. The resulting serum samples were stored at -20 °C until the determination of various parameters, which include total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein (TP), urea, creatinine, albumin (ALB), globulin (GLB), and bilirubin.

Statistical and data analysis

With the use of the SAS (2008) statistical software, all the data were subjected to a $2 \times 2 \times 4$ factorial analysis in a completely randomised design. The factors included 2 breeds of cocks, 2

levels of vitamin C, and 4 levels of chromium picolinate. Duncan's multiple range test was used to separate the means when appropriate, and the significance level of 5.00% was acceptable.

RESULTS

Serum protein and glucose of cocks fed CrPic-treated diets with or without vitamin C

The serum protein and glucose levels of the experimental cocks are presented in *Table 2*. The levels of total protein, albumin, and globulin in the WLC group were considerably greater ($P < 0.05$) compared to those observed in the NC group. Conversely, the serum glucose level was found to be significantly higher ($P < 0.05$) in the NC group.

However, the addition of vitamin C to diets resulted in a considerable ($P < 0.05$) increase in serum protein concentrations, whereas serum glucose levels were significantly ($P < 0.05$) decreased. The addition of CrPic significantly increased serum protein levels, reaching a dosage of 0.8 mg/kg diet. At this dosage, the levels of serum proteins were significantly higher compared to the levels observed in cocks fed other diets ($P < 0.05$).

Nevertheless, the addition of 1.2 mg/kg of food was found to significantly ($P < 0.05$) decrease the levels of these parameters. The serum glucose levels did not show a significant difference ($P > 0.05$) between the cocks fed different amounts of CrPic compared to those on the control diet. However, a substantial drop ($P < 0.05$) was found in the cocks on 1.2 mg/kg CrPic compared to those on 0.4 and 0.8 mg/kg CrPic.

Serum protein and glucose of cocks fed CrPic diets with or without vitamin C at high ambient temperature

Furthermore, there were no statistically significant differences ($P>0.05$) in blood protein and glucose concentrations across the treatment diets

when considering the interactions between the breeds and amounts of CrPic with and without vitamin C.

Table 2 – Serum protein and glucose of cocks fed CrPic-treated diets with or without vitamin C

Breeds	Vitamin C	Levels	Total protein g/dl	Albumin g/dl	Globulin g/dl	Glucose (mmol/L)
WLC			4.91 ± 0.24 ^a	2.01 ± 0.11 ^a	2.91 ± 0.14 ^a	10.07 ± 0.47 ^b
NC			3.78 ± 0.23 ^b	1.61 ± 0.12 ^b	2.18 ± 0.12 ^b	10.70 ± 0.17 ^a
	0		4.02 ± 0.29 ^b	1.63 ± 0.13 ^b	2.39 ± 1.17 ^b	11.24 ± 0.15 ^a
	200		4.67 ± 0.21 ^a	1.97 ± 0.10 ^a	2.70 ± 0.12 ^a	9.53 ± 0.41 ^b
		0	4.19 ± 0.32 ^c	1.83 ± 0.14 ^b	2.42 ± 0.20 ^c	10.20 ± 0.22 ^{ab}
		0.4	4.39 ± 0.22 ^b	1.85 ± 0.14 ^b	2.56 ± 0.09 ^b	10.99 ± 0.36 ^a
		0.8	4.88 ± 0.49 ^a	2.02 ± 0.20 ^a	2.86 ± 0.31 ^a	11.13 ± 0.25 ^a
		1.2	3.93 ± 0.37 ^d	1.50 ± 0.18 ^c	2.43 ± 0.18 ^d	9.22 ± 0.79 ^b
Interactions						
Breeds	Vitamin C	Levels	Total protein g/dl	Albumin g/dl	Globulin g/dl	Glucose (mmol/L)
WLC	0	0	4.83 ± 0.02	2.02 ± 0.01	2.81 ± 0.01	11.53 ± 0.33
WLC	0	0.4	4.55 ± 0.04	2.10 ± 0.06	2.45 ± 0.08	9.50 ± 0.21
WLC	0	0.8	6.41 ± 0.05	2.30 ± 0.01	4.11 ± 0.02	4.80 ± 0.17
WLC	0	1.2	2.80 ± 0.12	1.04 ± 0.09	1.76 ± 0.01	9.07 ± 0.19
WLC	200	0	3.53 ± 0.01	1.18 ± 0.05	2.35 ± 0.05	12.00 ± 0.53
WLC	200	0.4	5.74 ± 0.14	2.48 ± 0.01	3.26 ± 0.01	11.27 ± 0.19
WLC	200	0.8	5.81 ± 0.18	2.41 ± 0.02	3.40 ± 0.01	10.93 ± 0.09
WLC	200	1.2	5.58 ± 0.01	2.43 ± 0.01	3.15 ± 0.03	11.47 ± 0.09
NC	0	0	5.34 ± 0.02	2.48 ± 0.11	2.86 ± 0.01	10.73 ± 0.09
NC	0	0.4	3.34 ± 0.01	1.29 ± 0.19	2.05 ± 0.02	9.70 ± 0.12
NC	0	0.8	2.21 ± 0.06	0.90 ± 0.01	1.31 ± 0.01	9.73 ± 0.03
NC	0	1.2	2.65 ± 0.01	0.88 ± 0.01	1.77 ± 0.19	11.17 ± 0.09
NC	200	0	3.86 ± 0.03	1.64 ± 0.02	2.22 ± 0.01	10.23 ± 0.03
NC	200	0.4	3.11 ± 0.01	1.51 ± 0.06	1.60 ± 0.02	10.30 ± 0.12
NC	200	0.8	5.09 ± 0.11	2.49 ± 0.11	2.60 ± 0.05	11.40 ± 0.16
NC	200	1.2	4.67 ± 0.01	1.66 ± 0.01	3.01 ± 0.01	12.27 ± 0.09
P-values						
Breed (B)			<0.0001	<0.0001	<0.0001	<0.0001
Vitamin C (V)			<0.0001	<0.0001	<0.0001	<0.0001
Level (L)			<0.0001	<0.0001	<0.0001	<0.0001
BxVxL			0.2301	0.9098	0.1111	0.2100

Values are means ± SE; Means in a column with different superscripts are significantly ($P<0.05$) different. WLC = white leghorn cocks, NC = noiler cocks, vitamin C (mg/kg), Level= chromium picolinate (mg/kg)

DISCUSSION

Serum protein and glucose of cocks fed CrPic-treated diets with or without vitamin C

The serum protein and glucose of cocks fed the experimental diets are in *Table 2*. This present experiment showed that breeds of cocks could play a main factor in serum protein and glucose variability. The addition of 200 mg/kg vitamin C would positively influence the serum TP, ALB and GLB concentrations of cocks.

The findings of El-Bahr *et al.* (2017) who recorded an increase in serum proteins of broiler chickens fed containing 100 mg/kg diet vitamin C agreed with the present study. The result of this study further indicated that vitamin C is a potent immunostimulant to be used in the diet of chickens raised under high ambient temperature conditions.

The claim of Abdel-Wahab *et al.* (2002) that vitamin C increases biological activity of insulin which hence lowers blood glucose could help to explain the notable decrease in serum glucose shown in this study. Kucuk *et al.* (2003) also recommended that vitamin C can lower plasma glucose in laying hens by raising insulin concentration.

Moreover, this work showed that high inclusion of CrPic at 1.2 mg/kg diet can adversely affect protein metabolism as shown by their much reduced quantities at this inclusion level. At this level, the notable drop in serum TP, ALB, and GLB pointed to liver or kidney problems (Quinlan *et al.*, 2010). Among the birds on the diet with 1.2 mg CrPic/kg, the lowest concentrations of TP, ALB and GLB detected point to a

negative effect of high dosage of CrPic on these organs. The lower serum glucose at 1.2 mg/kg diet CrPic supported other studies (Cefalu *et al.*, 2010), which found lower blood glucose in laboratory animals and diabetic patients treated with CrPic with increase in insulin production.

CONCLUSIONS

The study revealed no appreciable variation in blood protein or glucose levels between White Leghorn and Noiler cocks. While lowering serum glucose in both breeds, dietary supplements with 0.8 mg/kg chromium picolinate (CrPic) and 200 mg/kg vitamin C raised total serum protein, albumin, and globulin. On the other hand, 1.2 mg/kg CrPic's supplementation lowered all these values. Notably, interactions between 0.8 mg/kg CrPic and 200 mg/kg vitamin C revealed breed-specific effects. While it increased serum protein in both breeds, it decreased serum glucose in White Leghorns but increased it in Noilers. This suggests a differential effect of the CrPic and vitamin C combination on glucose regulation in these breeds. Therefore, a combination of 0.8 mg/kg CrPic and 200 mg/kg vitamin C is advised for cocks reared in tropical settings to boost serum protein levels and may be increase insulin sensitivity.

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Author contribution: I declare that I have read and approved the publication of the manuscript in this present form.

Conflicts of interest: The author declares no conflict of interest.

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