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Reproductive and hormonal responses of two breeds of roosters fed diets containing chromium picolinate with or without vitamin C at high ambient temperature

Francis Bosede Adebayo[1], Olumuyiwa Joseph Olarotimi[2*] and Olufemi Adesanya Adu[1]

[1]Animal Physiology Laboratory, Department of Animal Production and Health, School of Agriculture and Agricultural Technology, The Federal University of Technology Akure, Akure, Ondo, Nigeria. [2]Animal Physiology Unit, Department of Animal Science, Faculty of Agriculture, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Ondo, Nigeria. *Author for correspondence. E-mail: olumuyiwa.olarotimi@aaua.edu.ng

ABSTRACT. This study evaluated the effects of chromium picolinate (CrPic) with or without vitamin C on the reproductive potentials of cocks raised under high ambient temperature. Four diets contained CrPic at 0.00 to 1.20 mg kg^{-1} without vitamin C and another four diets contained CrPic with 200 mg vitamin C each. A total of 192 White Leghorn and Noiler cocks (96 cocks each) were randomly assigned to the 8 treatments and replicated 4 times with 6 cocks per replicate in a 2 x2 x 4 factorial experiment. At the end of the sixteen weeks study, twelve birds per treatment were randomly sacrificed. The testes were carefully sampled, weighed and processed for estimation of daily sperm production using the homogenate method. The results revealed that the interactions of 0.40 mg CrPic kg^{-1} with or without vitamin C significantly ($p < 0.05$) influenced the gonadal and extra-gonadal weights, semen characteristics among the cocks while the daily sperm production and reproductive hormones were not negatively affected. This study, therefore, concluded that 0.40 mg CrPic kg^{-1} diet with vitamin C could significantly reduce the effects of heat stress on cocks raised under high ambient temperature without negatively impacting on their normal reproductive functions.

Keywords: heat; hormones; reproduction; semen; testes.

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Introduction

The potential of cocks in meeting the protein needs of the teeming population in the sub-Saharan has been severally stressed due to their ability as efficient converters of the readily available phytogenic additives and non-conventional protein sources into meat which could be sold at cheaper prices to the consumers (Olarotimi, Adu, & Olarotimi, 2020). A major factor in the tropics militating against profitable poultry production is high ambient temperature. High temperature often has a negative effect on carbohydrate and protein metabolism in poultry. Persistent high temperature usually leads to heat stress as it is always the case in the tropical and subtropical regions. Heat stress brings along with it huge economic losses in the poultry sub-sector of the economy in the tropical regions. Significant decrease in hen-day production of laying hens has also been identified as an effect of heat stress (Ayo, Obidi, & Rekwot, 2010). Increased cost of production, high rate of mortality due to depressed immunity, and reproductive failure among several other physiological effects of heat stress have been reported (Obidi, Onyeanusi, Rekwot, Ayo, & Dzenda, 2008; Ayo et al., 2010). Other physiological responses accompanying heat stress in chickens are respiratory alkalosis from panting, reduced feed intake and efficiency, reduced absorption of calcium, decrease in secretion of thyroid hormones, estrogen, progesterone and testosterone, and increased secretion of glucocorticoids, luteinising hormone and follicle stimulating hormone (Igbokwe, 2018).

Manipulation of diets to combat heat stress will bring about a productive and profitable poultry production in the tropics. One of such feeding management tools is dietary supplementation of CrPic and vitamin (Vit C). Chromium, generally, is known to have an antioxidant effect which plays a major role in carbohydrate and protein metabolism (Ali, Akram, Fahim, Singh, & Imran, 2018) and its deficiency is capable of disrupting the metabolism of carbohydrates, proteins and lipids (Sahina et al., 2010). Mitochondria are the site where physiological and pathological cellular reactive oxygen species (ROS) are produced and the generation of ROS is responsible for oxidative stress in animals. The most reported causes

of ROS generation leading to oxidative stress in animal production are metabolic, inflammatory and environmental factors such as heat stress and nutrition. Oxidations of carbohydrates and proteins have been explained to be involved in pathological conditions, such as respiratory diseases and parasitic infection; important physiological functions, such as reproduction, nutrition, metabolism, lactation, gut health, and neonatal physiology are also influenced by carbohydrates and proteins oxidations (Longman & Xiaobo, 2018). When cellular ROS accumulation is increased, it would lead to the oxidative damage responsible for pathological conditions related to enhanced glucocorticoid expression, which plays an important role in regulating the biosynthesis and metabolism of carbohydrates, lipids, and proteins (Martin-Montañez et al., 2014). It is understandable that if mitochondria normal functionalities are impaired, ROS production will be further enhanced and this will in turn cause oxidative stress in mitochondria (Rachek, Musiyenko, LeDoux, & Wilson, 2007). Chromium picolinate and vitamin C are antioxidants that have the capacity to scavenge the ROS formed within the mitochondria and protects against oxidative stress induced by environmental heat stress. Chromium picolinate, like some other chromium organic compounds, has higher bioavailability and lower toxicity than inorganic chromium compounds (Piva et al., 2003). Also, dietary inclusion of vitamin C in poultry diets has been reported to be necessary in mitigating physiological responses arising from increase in ambient temperature (Asli, Hosseini, Lotfollahian, & Shariatmadari, 2007). Adverse physiological effects such as poor immunity, low feed intake, low weight gain, oxidative stress, increased rectal and body temperature, poor fertility and semen quality, carcass weight and mortality in chickens induced by heat stress were documented to have been well managed as a result of vitamin C supplementation in the diets during high temperature increase (Bidin & Khatoon, 2013). Vitamin C ability to scavenge free radicals during periods of heat stress was explained to have made it an important dietary supplement capable of achieving optimum feed conversion ratio and increased body weight gain when included at 200 mg kg^{-1} diet in the broiler rations (Attia, Hassan, Tag El-Din, & Abou-Shehema, 2011). In layers where decreased percentage hen day egg production was recorded due to heat stress, vitamin C supplementation was found to be effective in bringing about increased egg production, egg weight and egg mass (Asli et al., 2007). Furthermore, previous studies indicated that exposure to high ambient temperature had negative effects on sperm qualities as a result of generation of heat-stress-induced reactive oxygen species (ROS) (Wang et al., 2020), vitamin C, a water-soluble chain-breaking antioxidant, on the other hand, had been reported to alleviate oxidative challenges induced by high environmental temperature (Ambali et al., 2018). This was achieved due to its ability to reduce free radical generation and maintain thiols of proteins which built up antioxidant compounds (Akorede et al., 2020). Since the potentials of chromium picolinate and vitamin C as useful and efficient antioxidants for poultry feeding have been emphasized, its effect on male reproduction has been not fully studied. Hence, this study, therefore, aims to evaluate their possible effects on cocks' testicular and epididymal parameters, such as weight, length and volume as crucial factors in evaluating the breeding soundness of domestic animals (Olarotimi & Adu, 2020) as they usually have positive and direct correlations with the sperm production activities of the testes (Nosseir, Ali, & Ebaid, 2012), sperm characteristics, testicular sperm reserves and reproductive hormonal changes which are traits necessary and useful in assessing good quality sperm for breeding purposes.

Material and methods

Experimental site

The study was carried out at the Poultry Unit, Teaching and Research Farm, the Federal University of Technology Akure, Nigeria. It was conducted in accordance with the research ethics and guidelines of the Animal Production and Health Department of the institution (FUTA/APH/15/4750).

Experimental cocks and management

A total of 192 sexually matured cocks of twenty-four (24) weeks of age comprising of ninety-six (96) Noiler and White Leghorn cocks each were used for the study. They were caged for two weeks before the onset of the experiment for stabilization. They were fed commercial grower ration throughout the stabilization period with fresh and cool water given *ad libitum*. At the end of the stabilization period, the cocks were weighed and randomly allotted to the experimental treatments. The two breeds of cocks were distributed on the 8 treatments and replicated 4 times with 6 cocks per replicate in a 2 x 2 x 4 factorial arrangement. The experimental diets and drinking water were provided *ad libitum* throughout

the sixteen weeks experimental period. The experiment was carried out between the months of December 2020 to March 2021 when the temperature was high. The average ambient temperature recorded was 33.4°C. All required managerial practices, such as strict bio-security measures, were ensured as at and when due, appropriate vaccines, and prophylactic treatments were administered. The birds were housed in an open-sided building in a thoroughly cleaned, washed and disinfected three tier cage system of 32 x 38 x 42 cm dimension. Two (2) birds were conveniently housed in a unit. At the end of the experimental period, four (4) cocks per replicate were selected and fasted overnight for semen and hormonal evaluations.

Experimental diets

Eight (8) experimental diets were constituted from the basal diet (Table 1) as follows:

T1: Basal (Control)

T2: Basal + 0.4 mg CrPi kg^{-1} diet

T3: Basal + 0.8 mg CrPi kg^{-1} diet

T4: Basal + 1.2 mg CrPi kg^{-1} diet

T5: Basal + 200 mg Vit C kg^{-1} diet

T6: Basal + (0.4 mg CrPi + 200 mg Vit C) kg^{-1} diet

T7: Basal + (0.8 mg CrPi + 200 mg Vit C) kg^{-1} diet

T8: Basal + (1.2 mg CrPi + 200 mg Vit C) kg^{-1} diet

The diets met the nutrient requirements of adult cocks according to National Research Council (NRC, 1994) recommendations.

Table 1. Composition of the cocks' experimental basal diets.

Ingredients (kg)	Quantity
Maize	39.00
Soya Bean Meal	3.00
Ground Nut Cake	4.00
Corn Bran	2.60
Palm Kernel Cake	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^{-1})	2506.38
Ca (%)	1.05
Crude fibre (%)	5.54
Crude fat (%)	4.48
Lysine	0.84
Methionine	0.32
Phosphorus	0.42

Composition of premix (Nutrivitas®): 2.5 kg of premix contains: Vit. A (10,000,000 iu), Vit. D3 (2,500,000 iu), Vit. E (12,000 iu), Vit. B1 (2000 mg), Niacin (25000 mg), Vit. B6 (1500 mg), Vit. B12 (10 mg), Vit. K3 (2500 mg), Biotin (75 mg), Folic Acid (2000 mg), Panthothenic Acid (7000 mg), Chlorine Chloride (50 %) (200000 mg), Manganese (80000 mg), Iron (40000 mg), Copper (10,000 mg), Zinc (60000mg), Selenium (200 mg), Iodine (1500 mg), Magnesium (100 mg), Ethoxyquine (500 g), BHT (700 g), Cobalt (250 mg).

Semen collection

The cocks were already trained for semen collection prior to the last six weeks of the experiment. Semen collections were carried out between 6 to 8 am on weekly basis for the last 6 weeks of the experimental period. The cocks were handled with gently to avoid undue physical stress. Semen was collected in a labeled plain sample bottles using the manual massage technique as described by Udeh, Ugwu, and Ogagifo (2011). Labeled plain sample tubes were used for the collection of the semen.

Semen evaluation

Semen characteristics such as volume, gross motility, live-dead count and concentration were evaluated within 6 min. after collection as described by Ewuola and Akinyemi (2017). Semen volume was determined by drawing the semen with a graduated tuberculin syringe of 1.0 mL capacity and reading directly to the nearest 0.01 mL. Progressive motility was evaluated by diluting a drop of semen with two drops of normal saline on a clean pre-warmed (37ºC) glass slide and covered with a clean coverslip as described by Olarotimi and Adu (2020). The observation was done under a microscope at x 40 magnification and scored 0-100%.

A dilution of 0.1 mL of the ejaculate with 5 mL normal saline was prepared in a clean test tube for the evaluation of the sperm concentration. Sperm concentration was evaluated using a Neubauer Haemocytometer and a binocular microscope (Olympus CH-2 CHS Binocular Microscope, Olympus Corporation, Japan) at 100 x magnification, as described by Ewuola and Egbunike (2010). The mass activity was estimated as described by Ogunlade (2015). A drop of raw undiluted semen was examined on pre-warmed slides under a microscope at 10 x magnification. The mass activity was scored subjectively according to the intensity of the wave motion, from the absence of wave motion (0) to slow motion (+), rapid motion (+ +) or turbulent motion (+ + +) characterized by the appearance of dark prominent wave in a rapid motion. The ratio of live sperm cells to dead sperm cells was evaluated by eosin-nigrosin vital staining technique. A drop of the ejaculate was placed onto a clean pre-warmed glass slide and two drops of eosin-nigrosin stain were added. This was mixed and a smear was prepared from the mixture and then viewed under a microscope at × 40 magnification. Dead sperm cells absorbed the stain while live sperm cells repelled it. The dead sperm percentage was obtained by dividing the number of dead cells in a field by the total number of sperm cells counted in the same field multiplied by 100. Percentages of live sperm cells (Liveability/Viability) were obtained by subtracting the value of the dead cell percentage from 100. From the values obtained above, the following characteristics were determined:

Sperm Concentration (Sperm Cells mL L^{-1}) = N x C x D (Maina, Chaudhari, Mshelia, & Williams, 2006).

Where:

N = number of sperm cells counted; C = constant = 52000 (Ewuola, Jimoh, Bello, & Bolarinwa, 2014);

$$D = \text{dilution factor} = \frac{\text{Volume of normal saline}}{\text{Volume of semen}}$$

$$\text{Liveability} = \frac{\text{Total number of counted cells} - \text{Dead cells} \times 100}{\text{Total number of counted cells}}$$

Total sperm cells $Ejaculate^{-1}$ = Sperm concentration mL^{-1} x Volume of ejaculate

$$\text{Total live sperm } mL^{-1} = \frac{\text{Liveability} \times \text{Sperm concentration } mL^{-1}}{100}$$

$$\text{Total motile sperm cells } mL^{-1} = \frac{\text{Motility} \times \text{Sperm concentration } mL^{-1}}{100}$$

Assessment of Gonadal and Extragonadal Morphometric Indices

After semen collection, the cocks were slaughtered and the reproductive tracts were carefully harvested. Testicular weights were obtained using a sensitive laboratory scale. The testes and epididymides were separated free of adhering connective tissues and fats. The left and right testes and epididymides were measured separately

and their weights recorded. The volumes of the testes were measured volumetrically using Archimede's principle of water displacement in a measuring cylinder and the testes densities were calculated from the testicular weights and volumes and expressed as g mL^{-1} (Olarotimi, Sokunbi, Abdullah, & 2015).

Testis weight (g)

$$\text{Testis density} = \frac{\text{Testis volume (mL)}}{\text{Testis weight (g)}}$$

Estimation of testicular sperm reserves

Testicular spermatozoa reserves (TSR) were determined haemocytometrically as described by Orlu and Egbunike (2009). A sample of each testis was cut and weighed. The samples were homogenized separately with a pair of sharp scissors in physiological saline at the rate of 5 mL g^{-1} testis. The testicular homogenate sample was stored overnight at 4ºC to allow the spermatozoa ooze out of the organ. The suspensions were mixed and filtered through a double layer of sterile gauze into clean glass test tubes and the filtrate diluted with distilled water to the ratio 1:10 (Olarotimi et al., 2020). Some drops of the homogenate were placed into an improved Neubauer haemocytometer counting chamber. All the elongated spermatids and mature sperm cells in the four diagonal and the centre squares of the haemocytometer were counted in each diluted homogenate. The concentration of the sperm cells per gram of testis was calculated as described by Olarotimi et al. (2020):

RTSR/LTSR = N x C x D

Where:

N = number of sperm cells count

D = dilution factor = d1 x d2

d1 = Volume of normal saline for homogenization

Weight of sample homogenized

d2 = Volume of normal saline for filtrate dilution

The volume of filtrate diluted

C = constant = 52,000

RTSR = right testicular sperm reserve; LTSR = left testicular sperm reserve

$$\text{RTSR/LTSR/testis} = \frac{\text{RTSR/LTSR x Total weight of right/left testis}}{\text{Weight of homogenated right/left testis}}$$

Therefore, Paired TSR (PTSR)/testis = RTSR/testis + LTSR/testis

$$\text{RTSR/LTSR per gram testis} = \frac{\text{RTSR/testis}}{\text{Total weight of right testis}} \quad \text{OR} \quad \frac{\text{LTSR/testis}}{\text{Total weight of left testis}}$$

Therefore, Paired TSR/gram testis = RTSR/gram testis + LTSR/gram testis

Estimation of daily sperm production (DSP) and daily sperm efficiency (SPE)

The DSP for each cock was therefore calculated by dividing the TSR by the time divisor for chicken. The time divisor was obtained by multiplying the fraction of the cycle of seminiferous epithelium occupied by

these cells by the duration of a cycle. Orlu and Egbunike (2009) reported that 48.25% of the cycle of 4 days was documented by researchers to be occupied by these cells.

$$DSP = \frac{\text{Testicular Sperm Reserve (TSR)}}{\text{Time divisor (1.93)}}$$

The efficiency of sperm production also known as daily sperm production per gram (DSP g^{-1}) parenchyma (testis) was estimated as:

$$DSP\ g^{-1} = \frac{\text{Paired TSR}}{\text{Time divisor (1.93)}}$$

Reproductive hormonal assay

Blood samples were also collected from the jugular veins of the cocks into dry clean plain centrifuged glass tubes to prepare serum for evaluation of serum reproductive hormones. The blood samples were left for 15 minutes at room temperature, and then the tubes were centrifuged for 10 minutes at 3000 rpm to obtain clean supernatant serum. The serum samples collected were kept frozen at -20ºC until the determination of serum hormones. Serum reproductive hormone concentrations were determined by double antibody RIA using commercially available RIA kits (IBL International GMBH, Flughafenstrasse 52a, D-22335 Hamburg, Germany) as described by (Darras, Visser, Berghman, & Kuhn, 1992).

Statistical analysis

Statistical analysis Data were subjected to analysis of variance using the General Linear Models procedure of SAS® software (SAS Institute Inc., 2008) and, in case of statistically significant differences the means were compared by test of Duncan at 5% of probability.

Results and discussion

Epididymal and testicular characteristics of cocks fed CrPic-treated diets with or without vitamin C

The extra-gonadal weights and lengths of cocks fed diets containing varying levels of CrPic with or without vitamin C are as shown in Tables 2 and 3, respectively. For the epididymal and vas deferens weights, there were no breed differences ($p > 0.05$) in the right, left and paired epididymal and vas deferens weights of the two breeds of cocks fed the experimental diets in this study. The varying inclusion levels of CrPic did not also significantly ($p > 0.05$) influence the epididymal and vas deferens weights. The inclusion of vitamin C did not significantly affect ($p > 0.05$) the right, left and paired epididymal and vas deferens weights in the present experiment. However, the interactions of the breeds, treatments and levels had significant ($p < 0.05$) effects on the various parameters studied. The interaction of the breeds with the varied levels of CrPic without vitamin C brought about a significant ($p < 0.05$) increase in the left, right and paired epididymal weights (LEW, REW and PEW respectively) of both the White Leghorn cocks (WLC) and Noiler Cocks (NC) fed diets containing 0.40 mg CrPic kg^{-1} diet when compared with the birds fed diets containing 0.00 and 1.20 mg CrPic kg^{-1}.

However, a non-significant ($p > 0.05$) decrease was recorded in these parameters when CrPic inclusion level was increased from 0.40 to 0.80 mg kg^{-1} diet for the two breeds of cocks and these values were statistically ($p > 0.05$) similar with what were recorded among the cocks fed the control diets. A further increase in CrPic inclusion level from 0.80 to 1.20 mg kg^{-1} diet caused a further but significant ($p < 0.05$) decrease in the studied parameters among the cocks fed high level of CrPic when compared with cocks on other diets irrespective of their breeds. Furthermore, inclusion of 200 mg kg^{-1} vitamin C in diets containing varied inclusion levels of CrPic showed insignificant ($p > 0.05$) improvements in the right, left and paired epididymal weights of WLC and NC fed diets containing 0.00 to 0.80 mg kg^{-1} CrPic except among the cocks fed diets containing 1.20 mg kg^{-1} CrPic with vitamin C where significant ($p < 0.05$) increase in these parameters were observed when compared with the cocks fed same inclusion of CrPic without vitamin C.

Table 2. Epididymal and Vas deferens Weights of Cocks Fed CrPic-Treated Diets with or without Vitamin C.

Breeds	Treatment	Levels	LEW (g)	REW (g)	PEW (g)	LVW (g)	RVW (g)	PVW (g)
WLC			0.69 ± 0.02	0.50 ± 0.02	1.19 ± 0.03	0.44 ± 0.02	0.72 ± 0.03	1.16 ± 0.04
NC			0.72 ± 0.02	0.52 ± 0.02	1.24 ± 0.03	0.46 ± 0.02	0.75 ± 0.03	1.21 ± 0.04
	0		0.71 ± 0.02	0.52 ± 0.02	1.23 ± 0.04	0.40 ± 0.02	0.73 ± 0.04	1.13 ± 0.05
	200		0.70 ± 0.02	0.50 ± 0.01	1.20 ± 0.02	0.50 ± 0.01	0.74 ± 0.02	1.24 ± 0.03
		0	0.78 ± 0.01	0.45 ± 0.01	1.23 ± 0.03	0.50 ± 0.03	0.79 ± 0.01	1.29 ± 0.01
		0.4	0.65 ± 0.01	0.56 ± 0.01	1.21 ± 0.03	0.48 ± 0.01	0.73 ± 0.03	1.21 ± 0.04
		0.8	0.65 ± 0.02	0.44 ± 0.01	1.09 ± 0.06	0.42 ± 0.04	0.68 ± 0.05	1.10 ± 0.04
		1.2	0.75 ± 0.03	0.59 ± 0.01	1.34 ± 0.03	0.40 ± 0.02	0.75 ± 0.06	1.15 ± 0.09
	Interactions							
Breeds	Treatments	Levels						
WLC	0	0.00	0.66 ± 0.01^{b}	0.43 ± 0.01^{b}	1.09 ± 0.01^{b}	0.40 ± 0.01^{b}	0.43 ± 0.01^{b}	0.83 ± 0.01^{b}
WLC	0	0.40	0.80 ± 0.01^{a}	0.58 ± 0.01^{a}	1.38 ± 0.01^{a}	0.56 ± 0.01^{a}	0.58 ± 0.01^{a}	1.14 ± 0.01^{a}
WLC	0	0.80	0.70 ± 0.01^{ab}	0.47 ± 0.01^{ab}	1.17 ± 0.01^{ab}	0.33 ± 0.01^{bc}	0.39 ± 0.01^{bc}	0.72 ± 0.01^{bc}
WLC	0	1.20	0.57 ± 0.01^{c}	0.40 ± 0.01^{c}	0.93 ± 0.01^{c}	0.29 ± 0.01^{c}	0.33 ± 0.01^{c}	0.62 ± 0.01^{c}
WLC	200	0.00	0.67 ± 0.01^{b}	0.56 ± 0.01^{ab}	1.23 ± 0.01^{ab}	0.47 ± 0.01^{ab}	0.49 ± 0.01^{ab}	0.96 ± 0.01^{ab}
WLC	200	0.40	0.83 ± 0.01^{a}	0.60 ± 0.01^{a}	1.43 ± 0.01^{a}	0.58 ± 0.01^{a}	0.59 ± 0.01^{a}	1.17 ± 0.01^{a}
WLC	200	0.80	0.73 ± 0.01^{ab}	0.57 ± 0.01^{ab}	1.30 ± 0.01^{ab}	0.45 ± 0.01^{ab}	0.50 ± 0.01^{ab}	0.95 ± 0.01^{ab}
WLC	200	1.20	0.63 ± 0.01^{b}	0.53 ± 0.01^{b}	1.16 ± 0.01^{b}	0.43 ± 0.01^{ab}	0.48 ± 0.01^{ab}	0.91 ± 0.01^{b}
NC	0	0.00	0.65 ± 0.01^{b}	0.46 ± 0.01^{b}	1.11 ± 0.01^{b}	0.42 ± 0.01^{b}	0.44 ± 0.01^{b}	0.86 ± 0.01^{b}
NC	0	0.40	0.82 ± 0.01^{a}	0.58 ± 0.01^{a}	1.40 ± 0.01^{a}	0.54 ± 0.01^{a}	0.75 ± 0.01^{a}	1.29 ± 0.01^{a}
NC	0	0.80	0.75 ± 0.01^{ab}	0.48 ± 0.01^{ab}	1.23 ± 0.01^{ab}	0.36 ± 0.01^{bc}	0.38 ± 0.01^{bc}	0.74 ± 0.01^{bc}
NC	0	1.20	0.60 ± 0.01^{c}	0.41 ± 0.01^{c}	1.01 ± 0.01^{c}	0.31 ± 0.01^{c}	0.35 ± 0.01^{c}	0.66 ± 0.01^{c}
NC	200	0.00	0.68 ± 0.01^{b}	0.48 ± 0.01^{b}	1.16 ± 0.01^{b}	0.48 ± 0.01^{ab}	0.50 ± 0.01^{ab}	0.98 ± 0.01^{ab}
NC	200	0.40	0.84 ± 0.01^{a}	0.64 ± 0.01^{a}	1.48 ± 0.01^{a}	0.69 ± 0.01^{a}	0.78 ± 0.01^{a}	1.46 ± 0.01^{a}
NC	200	0.80	0.77 ± 0.01^{ab}	0.52 ± 0.01^{b}	1.29 ± 0.01^{ab}	0.45 ± 0.01^{ab}	0.46 ± 0.01^{ab}	0.91 ± 0.01^{b}
NC	200	1.20	0.69 ± 0.01^{b}	0.48 ± 0.01^{b}	1.17 ± 0.01^{b}	0.40 ± 0.01^{b}	0.45 ± 0.01^{ab}	0.85 ± 0.01^{b}
P-values								
Breed (B)			0.0616	0.5246	0.3104	0.6267	0.2098	0.1384
Treatment (T)			0.5031	0.7638	0.7909	0.6782	0.4210	0.3329
Level (L)			0.6923	0.6890	0.5132	0.5848	0.5827	0.3015
BxTxL			0.0414	0.0231	0.0001	0.0196	0.0023	0.0412

Values are means ± SE; Means in a column without common superscripts are significantly ($p < 0.05$) different. LEW = left epididymal weight, REW = right epididymal weight, PEW = paired epididymal weight, LVW = left vas deferens weight, RVW = right vas deferens weight, PVW = paired vas deferens weight, WLC = white leghorn cocks, NC = noiler cocks, Treatment = vitamin C (200 mg kg^{-1}), Level = chromium picolinate (mg kg^{-1})

For the left, right and paired vas deferens weights (LVW, RVW and PVW respectively) among the two breeds of cocks fed diets containing varied inclusion levels of CrPic without vitamin C, inclusion of 0.40 mg kg^{-1} CrPic significantly ($p < 0.05$) increased the weights of the studied parameters. A further increase in the inclusion of CrPic from 0.40 to 0.80 mg kg^{-1} diet caused a significant ($p < 0.05$) decrease in the weights of the left, right and paired vas deferens which were statistically ($p > 0.05$) comparable with what were recorded for the control cocks. Similarly, an increase from 0.80 to 1.20 mg kg^{-1} diet further caused a downward but non-significant ($p > 0.05$) decrease in these parameters. Addition of 200 mg kg^{-1} vitamin C to the diets containing 0.00 to 0.80 mg kg^{-1} CrPic showed non-significant ($p > 0.05$) increases in all the weights of vas deferens studied in the two breeds of cocks when compared with those fed same quantity of CrPic without vitamin C respectively. However, an inclusion of 200 mg kg^{-1} vitamin C in the diets of both WLC and NC fed diets containing 1.20 mg kg^{-1} CrPic positively ($p < 0.05$) enhanced the vas deferen weights of the cocks.

Just as it was observed in the epididymal and vas deferens weights, there were no significant ($p > 0.05$) breed, treatment and level effects observed in the epididymal and vas deferens lengths of both the WLC and NC fed diets containing varying inclusions of CrPic with or without vitamin C. The interaction of the breeds, treatments and levels did not significantly ($p > 0.05$) affect the right, left and paired epididymal and vas deferens lengths studied in the present experiment.

Furthermore, the effects of diets containing different levels of CrPic with or without vitamin C on testicular parameters such as left testicular weight (LTW), right testicular weight (RTW), left testicular

volume (LTV), right testicular volume (RTV), left testicular density (LTD) and right testicular density (RTD) are shown in Table 4. There were no significant ($p > 0.05$) effects of the breeds, treatments and levels observed on the studied testicular parameters. However, the interactions of the breeds, treatments and levels presented their significant effects on the various testicular characteristics studied. For the testicular weights, the WLC and NC fed diets containing 0.40 mg kg^{-1} CrPic with or without vitamin C was significantly ($p < 0.05$) higher in the values of RTW when compared with other cocks fed diets containing other levels of CrPic with or without vitamin C. However, cocks (WLC and NC) fed diets containing 0.00, 0.40 and 0.80 mg CrPic kg^{-1} without vitamin C had statistically ($p > 0.05$) LTW and PTW values which were significantly ($p < 0.05$) higher than what were recorded by cocks on the diet containing 1.20 mg CrPic kg^{-1}. Conversely, cocks on diets containing 0.00, 0.40 and 0.80 mg kg^{-1} CrPic with vitamin C were significantly lower in the values of LTW and PTW when compared with those on diet containing 1.20 mg CrPic kg^{-1} but were similar ($p > 0.05$) when compared among themselves.

Table 3. Epididymal and Vas deferens Lengths of Cocks Fed CrPic-Treated Diets with or without Vitamin C.

Breeds	Treatment	Levels	LEL (cm)	REL (cm)	PEL (cm)	LVL (cm)	RVL (cm)	PVL (cm)
WLC			2.74 ± 0.06	3.67 ± 0.22	6.41 ± 0.19	8.32 ± 0.08	9.12 ± 0.08	17.44 ± 0.22
NC			2.76 ± 0.06	3.70 ± 0.22	6.46 ± 0.20	8.37 ± 0.08	9.17 ± 0.08	17.54 ± 0.22
	0		2.91 ± 0.04	4.25 ± 0.20	7.16 ± 0.15	8.83 ± 0.10	9.15 ± 0.09	17.98 ± 0.25
	200		2.58 ± 0.04	3.12 ± 0.16	5.7 ± 0.22	7.86 ± 0.06	9.14 ± 0.08	17.00 ± 0.19
		0	2.81 ± 0.10	3.33 ± 0.31	6.14 ± 0.21	7.64 ± 0.06	8.65 ± 0.01	16.29 ± 0.10
		0.4	2.64 ± 0.08	3.44 ± 0.17	6.08 ± 0.11	8.19 ± 0.05	9.55 ± 0.23	17.74 ± 0.22
		0.8	2.80 ± 0.10	3.93 ± 0.48	6.73 ± 0.17	9.07 ± 0.11	8.77 ± 0.04	17.84 ± 0.09
		1.2	2.75 ± 0.08	4.02 ± 0.07	6.77 ± 0.13	8.49 ± 0.18	9.61 ± 0.06	18.10 ± 0.53
	Interactions							
Breeds	Treatments	Levels						
WLC	0	0	3.13 ± 0.01	4.33 ± 0.01	7.46 ± 0.01	8.33 ± 0.01	8.34 ± 0.01	16.67 ± 0.01
WLC	0	0.4	2.37 ± 0.01	2.87 ± 0.01	5.24 ± 0.01	8.20 ± 0.01	8.06 ± 0.01	16.26 ± 0.01
WLC	0	0.8	3.10 ± 0.01	5.50 ± 0.01	8.6 ± 0.01	10.20 ± 0.01	8.57 ± 0.01	18.77 ± 0.01
WLC	0	1.2	3.00 ± 0.01	4.23 ± 0.01	7.23 ± 0.01	8.50 ± 0.01	11.53 ± 0.01	20.03 ± 0.01
WLC	200	0	2.47 ± 0.01	2.30 ± 0.01	4.77 ± 0.01	6.90 ± 0.01	8.93 ± 0.01	15.83 ± 0.01
WLC	200	0.4	2.87 ± 0.01	4.00 ± 0.01	6.87 ± 0.01	8.14 ± 0.01	11.00 ± 0.01	19.14 ± 0.01
WLC	200	0.8	2.47 ± 0.01	2.33 ± 0.01	4.8 ± 0.01	7.87 ± 0.01	8.9 ± 0.01	16.77 ± 0.01
WLC	200	1.2	2.47 ± 0.01	3.77 ± 0.01	6.24 ± 0.01	8.43 ± 0.01	7.63 ± 0.01	16.06 ± 0.01
NC	0	0	3.14 ± 0.01	4.38 ± 0.01	7.52 ± 0.01	8.37 ± 0.01	8.36 ± 0.01	16.73 ± 0.01
NC	0	0.4	2.40 ± 0.01	2.88 ± 0.01	5.28 ± 0.01	8.25 ± 0.01	8.10 ± 0.01	16.35 ± 0.01
NC	0	0.8	3.13 ± 0.01	5.53 ± 0.01	8.66 ± 0.01	10.26 ± 0.01	8.65 ± 0.01	18.91 ± 0.01
NC	0	1.2	3.04 ± 0.01	4.26 ± 0.01	7.3 ± 0.01	8.55 ± 0.01	11.59 ± 0.01	20.14 ± 0.01
NC	200	0	2.49 ± 0.01	2.32 ± 0.01	4.81 ± 0.01	6.96 ± 0.01	8.97 ± 0.01	15.93 ± 0.01
NC	200	0.4	2.91 ± 0.01	4.01 ± 0.01	6.92 ± 0.01	8.17 ± 0.01	11.04 ± 0.01	19.21 ± 0.01
NC	200	0.8	2.50 ± 0.01	2.37 ± 0.01	4.87 ± 0.01	7.93 ± 0.01	8.95 ± 0.01	16.88 ± 0.01
NC	200	1.2	2.48 ± 0.01	3.82 ± 0.01	6.3 ± 0.01	8.47 ± 0.01	7.69 ± 0.01	16.16 ± 0.01
P-values								
Breed (B)			0.1216	0.4296	0.1115	0.5917	0.2691	0.4190
Treatment (T)			0.6235	0.1708	0.4991	0.4204	0.3411	0.2112
Level (L)			0.1904	0.0990	0.1192	0.6721	0.2902	0.3325
BxTxL			0.1414	0.1931	0.8101	0.2106	0.5623	0.2422

Values are means ± SE; Means in a column without common superscripts are significantly ($p < 0.05$) different. LEL = left epididymal length, REL = right epididymal length, PEL = paired epididymal length, LVL = left vas deferens length, RVL = right vas deferens length, PVL = paired vas deferens length, WLC = white leghorn cocks, NC = noiler cocks, Treatment = vitamin C (200 mg kg^{-1}), Level = chromium picolinate (mg kg^{-1}).

For the testicular volume, LTV, RTV and PTV among cocks fed varying inclusion levels of CrPic without vitamin C were similar ($p > 0.05$) for the two breeds of cocks with those fed diet containing 0.40 mg CrPic kg^{-1} without vitamin C recording the higher significant ($p < 0.05$) value. Similarly, cocks fed diets containing varying inclusions of CrPic with vitamin C presented no significant ($p > 0.05$) difference in their LTV, RTV and PTV means with cocks on diets containing 0.00, 0.80 and 1.20 mg CrPic kg^{-1} recording higher significant figures when compared with those on 0.40 mg CrPic kg^{-1} diet.

The densities recorded by the cocks irrespective of their breeds, treatments and levels interaction were not significantly ($p > 0.05$) different.

Table 4. Testicular Characteristics of Cocks Fed CrPic-Treated Diets with or without Vitamin C.

Breeds	Treatment	Levels	RTW (g)	LTW (g)	PTW (g)	RTV (mL)	LTV (mL)	PTV (mL)	LTD (g mL^{-1})	RTD (g mL^{-1})	PTD (g mL^{-1})
			15.17 ± 0.29	13.85 ± 0.14	29.02 ± 0.17	17.96 ± 0.34	17.12 ± 0.39	35.08 ± 0.34	0.81 ± 0.01	0.84 ± 0.01	1.65 ± 0.03
NC			15.20 ± 0.29	13.88 ± 0.14	29.08 ± 0.19	17.98 ± 0.34	17.15 ± 0.39	35.13 ± 0.31	0.81 ± 0.01	0.85 ± 0.01	1.66 ± 0.03
	0		15.37 ± 0.20	14.16 ± 0.10	29.53 ± 0.11	17.18 ± 0.40	16.35 ± 0.04	33.53 ± 0.01	0.87 ± 0.01	0.89 ± 0.01	1.76 ± 0.04
	200		15.01 ± 0.36	13.57 ± 0.15	28.58 ± 0.13	18.76 ± 0.15	17.93 ± 0.24	36.69 ± 0.27	0.76 ± 0.01	0.80 ± 0.01	1.56 ± 0.02
		0	13.55 ± 0.28	13.38 ± 0.20	26.93 ± 0.21	17.34 ± 0.10	17.34 ± 0.30	34.68 ± 0.31	0.77 ± 0.01	0.78± 0.01	1.55 ± 0.03
		0.4	17.10 ± 0.04	14.36 ± 0.09	31.46 ± 0.10	19.35 ± 0.10	17.36 ± 0.40	36.71 ± 0.36	0.83 ± 0.01	0.88 ± 0.01	1.71 ± 0.03
		0.8	14.58 ± 0.06	13.74 ± 0.23	28.32 ± 0.20	18.18 ± 0.15	17.84 ± 0.15	36.02 ± 0.17	0.77 ± 0.01	0.8 0 ± 0.01	1.57 ± 0.06
		1.2	15.52 ± 0.09	13.98 ± 0.16	29.50 ± 0.13	17.01 ± 0.81	16.01 ± 0.90	33.02 ± 0.82	0.87 ± 0.01	0.91 ± 0.01	1.78 ± 0.03
	Interactions										
Breeds	Treatments	Levels									
WLC	0	0	4.47 ± 0.01^{b}	14.00 ± 0.01^{a}	28.47 ± 0.01^{a}	17.00 ± 0.01^{b}	16.33 ± 0.01^{b}	33.33 ± 0.01^{b}	0.86 ± 0.01	0.85 ± 0.01	1.71 ± 0.01
WLC	0	0.4	6.97 ± 0.01^{a}	14.67 ± 0.01^{a}	31.64 ± 0.01^{a}	19.67 ± 0.01^{a}	18.67 ± 0.01^{a}	38.34 ± 0.01^{a}	0.79 ± 0.01	0.86 ± 0.01	1.65 ± 0.01
WLC	0	0.8	4.77 ± 0.01^{b}	14.50 ± 0.01^{a}	29.27 ± 0.01^{a}	17.67 ± 0.01^{b}	17.33 ± 0.01^{b}	35.00 ± 0.01^{b}	0.84 ± 0.01	0.84 ± 0.01	1.68 ± 0.01
WLC	0	1.2	5.20 ± 0.01^{b}	13.43 ± 0.01^{b}	28.63 ± 0.01^{b}	14.33 ± 0.01^{b}	13.00 ± 0.01^{b}	27.33 ± 0.01^{b}	1.03 ± 0.01	1.06 ± 0.01	2.09 ± 0.01
WLC	200	0	2.60 ± 0.01^{b}	12.70 ± 0.01^{b}	25.30 ± 0.01^{b}	17.67 ± 0.01^{a}	18.33 ± 0.01^{a}	36.00 ± 0.01^{a}	0.69 ± 0.01	0.71 ± 0.01	1.40 ± 0.01
WLC	200	0.4	7.20 ± 0.01^{a}	12.03 ± 0.01^{b}	29.23 ± 0.01^{b}	19.00 ± 0.01^{b}	16.00 ± 0.01^{b}	35.00 ± 0.01^{b}	0.88 ± 0.01	0.91 ± 0.01	1.79± 0.01
WLC	200	0.8	4.37 ± 0.01^{b}	12.97 ± 0.01^{b}	27.34 ± 0.01^{b}	18.67 ± 0.01^{a}	18.33 ± 0.01^{a}	37.00 ± 0.01^{a}	0.71 ± 0.01	0.77 ± 0.01	1.30 ± 0.01
WLC	200	1.2	5.80 ± 0.01^{b}	14.50 ± 0.01^{a}	30.30 ± 0.01^{a}	19.67 ± 0.01^{a}	19.00 ± 0.01^{a}	38.67 ± 0.01^{a}	0.76 ± 0.01	0.8 ± 0.01	1.56 ± 0.01
NC	0	0	5.50 ± 0.01^{b}	14.05 ± 0.01^{a}	29.55 ± 0.01^{a}	17.02 ± 0.01^{b}	16.36 ± 0.01^{b}	33.38 ± 0.01^{b}	0.86 ± 0.01	0.85 ± 0.01	1.25 ± 0.01
NC	0	0.4	7.00 ± 0.01^{a}	14.68 ± 0.01^{a}	31.68 ± 0.01^{a}	19.70 ± 0.01^{a}	18.70 ± 0.01^{a}	38.40 ± 0.01^{a}	0.77 ± 0.01	0.86 ± 0.01	1.63 ± 0.01
NC	0	0.8	4.78 ± 0.01^{b}	14.52 ± 0.01^{a}	29.30 ± 0.01^{a}	17.68 ± 0.01^{b}	17.38 ± 0.01^{b}	35.06 ± 0.01^{b}	0.84 ± 0.01	0.84 ± 0.01	1.68 ± 0.01
NC	0	1.2	4.24 ± 0.01^{b}	13.45 ± 0.01^{b}	27.69 ± 0.01^{b}	14.35 ± 0.01^{b}	13.02 ± 0.01^{b}	27.37 ± 0.01^{b}	1.03 ± 0.01	1.06 ± 0.01	2.09 ± 0.01
NC	200	0	2.63 ± 0.01^{b}	12.75 ± 0.01^{b}	25.38 ± 0.01^{b}	17.68 ± 0.01^{a}	18.35 ± 0.01^{a}	36.03 ± 0.01^{a}	0.69 ± 0.01	0.71 ± 0.01	1.40± 0.01
NC	200	0.4	7.24 ± 0.01^{a}	11.07 ± 0.01^{b}	28.31 ± 0.01^{b}	19.02 ± 0.01^{b}	16.05 ± 0.01^{b}	35.07 ± 0.01^{b}	0.88 ± 0.01	0.91 ± 0.01	1.21 ± 0.01
NC	200	0.8	4.41 ± 0.01^{b}	12.98 ± 0.01^{b}	27.39 ± 0.01^{b}	18.69 ± 0.01^{a}	18.34 ± 0.01^{a}	37.03 ± 0.01^{a}	0.71 ± 0.01	0.77 ± 0.01	1.79 ± 0.01
NC	200	1.2	5.82 ± 0.01^{b}	14.52 ± 0.01^{a}	30.34 ± 0.01^{a}	19.70 ± 0.01^{a}	19.02 ± 0.01^{a}	38.72 ± 0.01^{a}	0.76 ± 0.01	0.80 ± 0.01	1.56 ± 0.01
P-values											
Breed (B)			0.1242	0.3452	0.1903	0.3712	0.2456	0.0774	0.2118	0.2121	0.1673
Treatment (T)			0.1657	0.1453	0.3562	0.1616	0.1876	0.2987	0.1317	0.1876	0.2163
Level (L)			0.1564	0.7683	0.1151	0.4320	0.5221	0.2312	0.3412	0.2109	0.4219
BxTxL			0.0025	0.0367	0.0421	0.0182	0.0361	0.0018	0.1224	0.4316	0.6003

Values are means ± SE; Means in a column without common superscripts are significantly ($p < 0.05$) different. RTW = right testicular weight, LTW = left testicular weight, PTW = paired testicular weight, RTV = right testicular volume, LTV = left testicular volume, PTV = paired testicular volume, RTD = right testicular density, LTD = left testicular density, PTD = paired testicular density, WLC = white leghorn cocks, NC = noiler cocks, Treatment = vitamin C (200 mg kg^{-1}), Level = chromium picolinate (mg kg^{-1}).

Parameters, such as weight, length and volume of the testis and the epididymis are crucial in evaluating the breeding soundness of domestic animals (Olarotimi & Adu, 2020) because of their direct relationships with the sperm production abilities of the testes (Nosseir et al., 2012). The non-significant main effects of the experimental diets in the present study indicated that the testicular and epididymal characteristics of the cocks were not negatively affected. Whereas, the significant increase observed in the left, right and paired epididymal weights, testicular weights and volumes of both the White Leghorn cocks (WLC) and Noiler Cocks (NC) fed diets containing 0.40 mg CrPic kg^{-1} diet indicated that the interaction of the breeds and CrPic at 0.40 mg kg^{-1} diet had a positive effect on spermatogic activies and storage capacities of the two breeds of the cocks irrespective of the prevailing high ambient environmental temperature. However, the significant decrease observed in the these parameters at 1.20 mg CrPic kg^{-1} diet in the present study suggests that a structural toxic effect on the testes, epididymides and *vas deferens* at this inclusion is a possibility. The reduction in testicular and epididymal parameters observed in this study further indicated the tendency of high inclusion of CrPic in cocks' causing testicular atrophy and this may ultimately lead to reduced sperm count and motility in cocks. Our observation gave credence to the European Food Safety Authority (EFSA, 2010) opinion that reported carcinogenic activity of chromium picolinate in male rats administered high concentrations of chromium picolinate and thus concluded it might cause DNA damage when administered at high concentration for a prolonged period. The significant reduction in the

parameters weights at high dose could be the result of structural damage. In another development, exposure to Cr was reported to cause a reduction in weights of testes, epididymis, seminal vesicle and prostate glands (Abd Elhafeez, Halawa, Hamed, & Abouelmaged, 2019). The testes being the site of spermatogenesis are very sensitive to Cr. Therefore exposure of cocks to high dose of CrPic may lead to testicular damage and consequently cocks' infertility even though the paired testicular sperm reserve, daily sperm production and sperm production efficiency were not significantly affected. Over all, results of our study were in agreement with previous study where it was opined that exposure to high Cr is a risk factor capable of inducing dysfunction in male reproductive system (Aruldhas et al., 2005). The free radical scavenging ability of vitamin C as an antioxidant was clearly seen among the birds fed vitamin C supplemented diet. Significant increase in the studied parameters was an evidence of restorative effect of vitamin C on the cellular damage caused by exposure of cocks to high CrPic. This further strengthens the hypothesis that free radicals are responsible for chromium toxicity and that dietary inclusion of antioxidants is a veritable means of preventing this cellular damage.

Semen characteristics of cocks fed CrPic-treated diets with or without vitamin C

The effects of the breeds, treatments and levels were not significantly ($p > 0.05$) noticed on the various semen characteristics studied in the present experiment except on the mass activity grade (MAG) where the treatment and level effects conspicuously influenced this parameter (Table 5). The mass activity of the cocks on diets without vitamin C ranged from rapid wave motion to very turbulent motion observed among the cocks fed diets containing vitamin C. The MAG of the cocks on the control and diet containing 0.40 mg kg^{-1} CrPic were not significantly ($p > 0.05$) different as they recorded very turbulent motion. However, the cocks on diets containing 0.80 and 1.20 mg kg^{-1} CrPic recorded rapid and slow wave motions respectively.

For the interaction of the breeds, treatments and levels, there were significant ($p < 0.05$) effects on all the semen characteristics studied except for the total sperm cells per ejaculate where the interaction did not statistically ($p > 0.05$) have any influence. The ejaculate volume (EV), sperm motility (SM), sperm viability (SV), sperm concentration (SC), total live cells (TLC) and total motile cells (TMC) of WLC and NC fed diets containing varied inclusion levels of CrPic without vitamin C followed a similar trend. Inclusion of CrPic at 0.40 and 0.80 mg kg^{-1} without vitamin C enhanced sperm production in the cocks irrespective of breed difference as indicated by significantly ($p < 0.05$) higher EV, SM, SV, SC, TLC and TMC among the cocks on these two diets when compared with those on the diet containing 1.20 mg kg^{-1} CrPic and the control. Similarly, the cocks (WLC and NC) fed varied inclusion levels of CrPic with vitamin C also shared the same pattern. The diet containing vitamin C only without CrPic inclusion significantly ($p < 0.05$) increased the EV, SM, SV, SC, TLC and TMC among the cocks when compared with the control diet. Furthermore, the EV, SM, SV, TLC and TMC recorded among the cocks fed diet containing 1.20 mg kg^{-1} CrPic without vitamin C were significantly ($p < 0.05$) lower when compared with cocks on other diets without vitamin C. In the same vein, the EV, SM and SV among the cocks fed diets containing 0.40 and 1.20 mg kg^{-1} CrPic with vitamin C were statistically ($p > 0.05$) similar to those of the cocks on diets containing 0.00 and 1.20 mg kg^{-1} CrPic with vitamin C unlike what were recorded in the same diets without vitamin C. The inclusion of vitamin C in the diet containing 1.20 mg kg^{-1} CrPic significantly ($p < 0.05$) lowered the SC, TLC and TMC of the two breeds of cocks when compared with cocks on 0.00, 0.40 and 0.80 mg kg^{-1} CrPic with vitamin C but significantly ($p < 0.05$) enhanced the same parameters in the cocks fed diet containing 1.20 mg kg^{-1} CrPic with vitamin C when compared with cocks on the same diet without vitamin C. The MAG among the cocks fed diets containing 0.80 and 1.20 mg kg^{-1} CrPic without vitamin C were positively enhanced from rapid to very turbulent wave motions and from slow to rapid wave motions respectively when vitamin C was included in their diets.

The attendant significant reduction in the EV, SM, SV, TLC, TMC and LTSR/T of the cocks fed 1.2 mg CrPic kg^{-1} diet without vitamin C was a testimony to the fact that high inclusion of CrPic without vitamin C could be deleterious to the processes of both steroidogenesis and spermatogenesis since they both take place in the testes. To further support the fact that high inclusion of CrPic (1.2 mg kg^{-1} diet) actually had an interference with spermatogenesis, the reduction in testicular and epididymal weight in this study was evidence as decrease in epididymal weight directly relates to reduced sperm counts (Chandra, Chatterjee, Ghosh, & Sarkar, 2007). It was previously established that sperm motility decreased among men who were occupationally exposed to high levels of Cr. Exposure of rats to Cr was also reported to significantly increase the sperm abnormality (Li et al., 2001). They further explained that there was attendant disruption in germ cell architecture closer to the walls of the seminiferous

tubules in the exposed rats. The finding of Bassey, Essien, Isong, Udoh, and Agbara (2013) was in agreement with the result of our study as high chromium concentration in seminal plasma was reportedly recorded among occupationally chromium exposed men. They, thus, concluded that Cr has adverse effect on sperm production, motility and sperm count in oligospermic and azoospermic infertile men. Contrary to our finding, Horký, Jančíková, and Zeman (2012) opined that high level of CrPic in boars' rations did not significantly influence the sperm concentration, volume and motility while Biswas, Divya, Mandal, Majumdar, and Singh (2014) claimed that sperm concentration, progressive motility and fertility were reportedly higher in male turkeys fed 750 µg than the control groups that received lower doses. The respective non significant and increasing influences reported in these two observations may be partly due to the species difference. Semen analysis of rats in sub chronic exposure to Cr was equally reported to result in a significant decrease in total sperm count and viability with a significant increase in the percent of sperms with abnormal morphology (Abd Elhafeez et al., 2019). In another study, the relative weight of testis, the number of epididymal spermatozoa and sperm motility were reportedly reduced in chromium treated rats (Marouani et al., 2012). Vitamin C supplementation had prevented high CrPic-induced reduction in semen characteristics among the birds fed the diets.

Table 5. Semen Characteristics of Cocks Fed CrPic-Treated Diets with or without Vitamin C.

Breeds	Treatment	Levels	EV (mL)	SM (%)	SV (%)	SC (x10^9 mL^{-1})	TSC/E (x10^8)	TLC (x10^9 mL^{-1})	TMC (x10^9 mL^{-1})	MAG
WLC			0.43 ± 0.05	75.25 ± 4.47	89.72 ± 2.36	2.39 ± 0.30	2.72 ± 0.23	2.21±0.29	1.62 ±0.20	+++
NC			0.58 ± 0.05	77.21 ± 4.38	91.03 ± 2.28	2.40 ± 0.27	3.73 ± 0.24	2.23±0.16	1.65 ±0.19	+++
	0		0.58 ± 0.06	71.81 ± 5.98	85.05 ± 2.84	2.22 ± 0.22	3.71 ± 0.17	2.02±0.23	1.65±0.27	++
	200		0.43 ± 0.04	80.66 ± 1.34	95.07 ± 0.51	2.55 ± 0.36	2.74 ± 0.29	2.40±0.33	1.60±0.10	+++
		0	0.50 ± 0.04	90.19 ± 2.04	97.90 ± 0.27	2.42 ± 0.25	3.29 ± 0.27	2.35±0.24	2.27±0.26	+++
		0.4	0.35 ± 0.02	89.81 ± 2.56	95.74 ± 0.93	2.32 ± 0.19	1.9 ± 0.04	2.20±0.16	2.11±0.19	+++
		0.8	0.57 ± 0.04	52.97 ± 8.58	89.70 ± 1.09	1.75 ± 0.11	3.61 ± 0.15	1.56±0.10	0.73±0.12	++
		1.2	0.61 ± 0.12	71.95 ± 0.70	78.18 ± 4.67	3.06 ± 0.75	4.38 ± 0.47	2.73±0.73	1.38±0.30	+
	Interactions									
Breeds	Treatments	Levels								
WLC	0	0	0.33 ± 0.01^b	53.00 ± 0.01^b	64.44 ± 0.01^b	0.43 ± 0.01^b	2.44 ± 0.01	1.81 ± 0.01^b	1.36±0.01^b	+++
WLC	0	0.4	0.48 ± 0.01^a	87.00 ± 0.01^a	97.53 ± 0.01^a	3.25 ± 0.01^a	2.71 ± 0.01	3.14 ± 0.01^a	3.10±0.01^a	+++
WLC	0	0.8	0.50 ± 0.01^a	80.33 ± 0.01^a	91.99 ± 0.01^a	2.91 ± 0.01^a	2.26 ± 0.01	2.71 ± 0.01^a	2.62±0.01^a	++
WLC	0	1.2	0.22 ± 0.01^c	43.33 ± 0.01^c	51.88 ± 0.01^c	0.56 ± 0.01^b	1.58 ± 0.01	0.31 ± 0.01^c	0.42±0.01^c	+
WLC	200	0	0.45 ± 0.01^a	96.33 ± 0.01^a	97.01 ± 0.01^a	3.59 ± 0.01^a	2.40 ± 0.01	3.49 ± 0.01^a	3.40±0.01^a	+++
WLC	200	0.4	0.47 ± 0.01^a	91.30 ± 0.01^a	98.74 ± 0.01^a	3.67 ± 0.01^a	1.96 ± 0.01	3.60 ± 0.01^a	3.85±0.01^a	+++
WLC	200	0.8	0.45 ± 0.01^a	71.67 ± 0.01^a	92.91 ± 0.01^a	3.05 ± 0.01^a	2.74 ± 0.01	2.81 ± 0.01^a	2.85 ± 0.01^a	+++
WLC	200	1.2	0.41 ± 0.01^{ab}	69.00 ± 0.01^{ab}	73.28 ± 0.01^{ab}	1.51 ± 0.01^b	1.48 ± 0.01	1.61 ± 0.01^b	1.11 ± 0.01^b	++
NC	0	0	0.36 ± 0.01^b	53.88 ± 0.01^b	68.25 ± 0.01^b	0.55 ± 0.11^b	2.99 ± 0.01	1.88 ± 0.01^b	1.35 ± 0.01^b	+++
NC	0	0.4	0.41 ± 0.01^a	87.91 ± 0.01^a	99.42 ± 0.01^a	3.28 ± 0.21^a	2.31 ± 0.01	3.16 ± 0.01^a	3.14 ± 0.01^a	+++
NC	0	0.8	0.52 ± 0.01^a	85.76 ± 0.01^a	93.26 ± 0.01^a	2.97 ± 0.03^a	2.58 ± 0.01	2.74 ± 0.01^a	2.73 ± 0.01^a	++
NC	0	1.2	0.23 ± 0.01^c	41.66 ± 0.01^c	53.56 ± 0.01^c	0.57 ± 0.11^b	2.86 ± 0.01	0.40 ± 0.01^c	0.40 ± 0.01^c	+
NC	200	0	0.56 ± 0.01^a	97.55 ± 0.01^a	97.62 ± 0.01^a	3.10 ± 0.01^a	2.24 ± 0.01	3.55 ± 0.01^a	3.41 ± 0.01^a	+++
NC	200	0.4	0.59 ± 0.01^a	93.00 ± 0.01^a	98.85 ± 0.01^a	3.69 ± 0.01^a	2.07 ± 0.01	3.65 ± 0.01^a	3.52 ± 0.01^a	+++
NC	200	0.8	0.48 ± 0.01^a	82.47 ± 0.01^a	93.19 ± 0.01^a	2.87 ± 0.01^a	2.77 ± 0.01	2.84 ± 0.01^a	2.87 ± 0.01^a	+++
NC	200	1.2	0.40 ± 0.01^{ab}	75.48 ± 0.01^{ab}	74.01 ± 0.01^{ab}	1.56 ± 0.01^b	2.02 ± 0.01	1.44 ± 0.01^b	1.63 ± 0.01^b	++
P-values										
Breed (B)			0.4654	0.1690	0.2347	0.1978	0.3288	0.1419	0.0787	
Treatment (T)			0.3229	0.8362	0.5890	0.8090	0.9214	0.7819	0.1562	
Level (L)			0.3452	0.0876	0.0912	0.7564	0.5555	0.0891	0.4590	
BxTxL			0.0191	0.0001	0.0035	0.0327	0.1960	0.0200	0.0325	

EV = ejaculate volume, SM = sperm motility, SV = sperm viability, SC = sperm concentration, TSC/E = total sperm cells ejaculate^{-1}, TLC = total live cells, TMC = total motile cells, MAG = mass activity grade, Treatment = vitamin C (200 mg kg^{-1}), Level = chromium picolinate (mg kg^{-1}).

Testicular sperm reserve, reproductive hormones, daily sperm production and efficiency of cocks fed CrPic-treated diets with or without vitamin C

The effects of breeds, treatments and levels on the testicular sperm reserve, daily sperm production and sperm production efficiency were as shown in Table 6. There were no significant ($p > 0.05$) effects of the breeds on the studied parameters except for the paired testicular sperm reserve per testis (PTSR/T) and daily

sperm production (DSP) where significant ($p < 0.05$) effect of the breeds were noticeable. The Noiler cocks showed higher PTSR/T and DSP than the White Leghorn cocks. The treatment has significant ($p < 0.05$) effect only on the right testicular sperm reserve per gramme testis (RTSR/gT) where the highest and least values were recorded among the cocks fed diet containing 0.40 and 1.20 mg kg^{-1} CrPic respectively. For the interaction of the breeds, treatments and levels, the cocks (WLC and NC) fed diets containing 0.40 and 0.80 mg kg^{-1} CrPic with or without C presented significantly ($p < 0.05$) higher left testicular sperm reserve per testis (LTSR/T) than cocks on all other diets. The effects of the breeds, treatments and levels as well as their interactions on the reproductive hormones of cocks fed different levels of CrPic with or without vitamin C are in Table 7. From the results of the present study, breeds, treatments, levels and their interactions did not have any significant ($p > 0.05$) effects on all the studied parameters.

Table 6. Testicular Sperm Reserve, Daily Sperm Production and Efficiency of Cocks Fed CrPic-Treated Diets with or without Vitamin C.

Breeds	Treatment	Levels	RTSR/T($x10^9$)	RTSR/gT($x10^8$)	LTSR/T($x10^9$)	LTSR/gT($x10^8$)	PTSR/T($x10^9$)	PTSR/gT($x10^8$)	DSP ($x10^9$)	SPE ($x10^6$)
WLC			0.94 ± 0.05	0.69 ± 0.05	0.94 ± 0.07	0.75 ± 0.05	1.88 ± 0.06^b	1.44 ± 0.07	0.97± 0.04^b	74.49± 3.70
NC			1.09 ± 0.06	0.80 ± 0.06	1.10 ± 0.08	0.87 ± 0.06	2.18 ± 0.08^a	1.67 ± 0.08	1.13 ± 0.04^a	86.41 ± 4.28
	0		1.04 ± 0.06	0.77 ± 0.06	1.02 ± 0.08	0.78 ± 0.06	2.06 ± 0.07	1.55 ± 0.07	1.07 ± 0.04	80.14 ± 3.86
	200		0.98 ± 0.06	0.72 ± 0.05	1.02 ± 0.08	0.84 ± 0.06	2.01 ± 0.09	1.56 ± 0.09	1.04 ± 0.05	80.76 ± 4.50
		0	1.03 ± 0.07	0.75 ± 0.08^b	1.11 ± 0.14	0.85 ± 0.10	2.14 ± 0.13	1.60 ± 0.01	1.11 ± 0.07	83.15 ± 6.54
		0.4	1.11 ± 0.10	0.91 ± 0.07^a	0.86 ± 0.06	0.73 ± 0.07	1.97 ± 0.12	1.64 ± 0.12	1.02± 0.06	84.91 ± 6.13
		0.8	0.98 ± 0.08	0.74 ± 0.07^b	1.11 ± 0.09	0.86 ± 0.06	2.09 ± 0.09	1.60 ± 0.07	1.08 ± 0.05	82.90 ± 3.82
		1.2	0.92 ± 0.10	0.57 ± 0.06^c	1.01 ± 0.14	0.80 ± 0.10	1.93 ± 0.12	1.37 ± 0.12	1.00 ± 0.06	70.85 ± 6.37
	Interactions									
Breeds	Treatments	Levels								
WLC	0	0	1.12 ± 0.22	0.94 ± 0.15	0.70 ± 0.06^c	0.60 ± 0.14	1.87 ± 0.28	1.54 ± 0.29	0.97 ± 0.14	79.95 ± 5.10
WLC	0	0.4	0.83 ± 0.15	0.52 ± 0.11	1.23 ± 0.15^{ab}	0.83 ± 0.18	1.98 ± 0.27	1.35 ± 0.24	1.03 ± 0.14	69.93 ± 2.46
WLC	0	0.8	0.76 ± 0.11	0.57 ± 0.13	1.15 ± 0.25^{ab}	0.90 ± 0.13	1.99 ± 0.05	1.47 ± 0.06	1.03 ± 0.03	76.32 ± 3.24
WLC	0	1.2	1.10 ± 0.10	0.82 ± 0.16	0.68 ± 0.10^c	0.55 ± 0.03	1.78 ± 0.06	1.36 ± 0.19	0.92 ± 0.03	70.63 ± 9.83
WLC	200	0	0.88 ± 0.12	0.75 ± 0.11	1.00 ± 0.14^b	0.74 ± 0.12	1.77 ± 0.19	1.49 ± 0.18	0.92 ± 0.10	77.29 ± 9.45
WLC	200	0.4	0.88 ± 0.25	0.53 ± 0.15	1.57 ± 0.21^a	0.65 ± 0.24	1.60 ± 0.05	1.18 ± 0.26	0.83 ± 0.28	61.27 ± 3.55
WLC	200	0.8	1.06 ± 0.13	0.81 ± 0.14	1.43 ± 0.29^a	0.68 ± 0.05	1.88 ± 0.22	1.49 ± 0.19	0.97 ± 0.11	77.20 ± 9.71
WLC	200	1.2	0.81 ± 0.12	0.58 ± 0.13	0.81 ± 0.07^b	1.03± 0.18	2.18 ± 0.29	1.61 ± 0.29	1.13 ± 0.15	83.35 ± 5.23
NC	0	0	1.35 ± 0.26	1.09 ± 0.18	0.82 ± 0.09^c	0.70 ± 0.16	2.17 ± 0.32	1.79 ± 0.34	1.12 ± 0.17	92.74 ± 7.51
NC	0	0.4	0.97 ± 0.18	0.61 ± 0.13	1.58 ± 0.10^a	0.96 ± 0.21	2.30 ± 0.31	1.57 ± 0.28	1.19 ± 0.16	81.12 ± 5.52
NC	0	0.8	0.89 ± 0.13	0.66 ± 0.15	1.19± 0.16^{ab}	1.05 ± 0.15	2.31 ± 0.06	1.71 ± 0.07	1.20 ± 0.03	88.53 ± 3.78
NC	0	1.2	1.27 ± 0.12	0.95 ± 0.19	0.72 ± 0.25^c	0.63 ± 0.03	2.07 ± 0.07	1.58 ± 0.22	1.07 ± 0.04	81.93 ± 1.41
NC	200	0	1.02 ± 0.13	0.87 ± 0.13	0.95 ± 0.24^b	0.86 ± 0.14	2.06 ± 0.22	1.73 ± 0.21	1.07 ± 0.12	89.65 ± 6.97
NC	200	0.4	1.02 ± 0.29	0.62 ± 0.17	1.83 ± 0.29^a	0.75 ± 0.28	1.85 ± 0.06	1.37 ± 0.30	0.96 ± 0.03	71.07 ± 5.72
NC	200	0.8	1.23 ± 0.15	0.94 ± 0.16	1.53 ± 0.18^a	0.79 ± 0.06	2.18 ± 0.25	1.73 ± 0.22	1.13 ± 0.13	89.55 ± 1.26
NC	200	1.2	0.94 ± 0.14	0.67 ± 0.15	0.79 ± 0.11^c	1.20 ± 0.21	2.52 ± 0.34	1.87 ± 0.34	1.31 ± 0.18	96.67 ± 7.67
P-values										
Breed (B)			0.0906	0.1446	0.1094	0.1467	0.0098	0.0684	0.0018	0.1604
Treatment (T)			0.4821	0.5038	0.9329	0.4482	0.6297	0.9229	0.9547	0.8265
Level (L)			0.5009	0.0235	0.2033	0.6368	0.5067	0.3855	0.3023	0.4530
BxTxL			0.4964	0.6419	0.0091	0.1964	0.6548	0.9966	0.5428	0.1348

Values are means ± SE; Means in a column without common superscripts are significantly ($p < 0.05$) different. RTSR/T (Right Testicular Sperm Reserve per testis), LTSR/T (Left Testicular Sperm Reserve per testis), PTSR/T (Paired Testicular Sperm Reserve per testis), RTSR/g testis (Right Testicular Sperm Reserve per gram testis), LTSR/g testis (Left Testicular Sperm Reserve per gram testis), PTSR/g testis (Paired Testicular Sperm Reserve per gram testis), DSP (Daily Sperm Production), SPE (Sperm Production Efficiency) Treatment = vitamin C (200 mg kg^{-1}), Level = chromium picolinate (mg kg^{-1})

The significant breed effect observed in the paired testicular sperm reserve per testis (PTSR/T) and daily sperm production (DSP) where significant was indicative that genetic makeup could play a main role in the reproductive mechanisms involved with sperm production. This means the genetic makeup of the NC could possibly favour and enhances daily sperm production. Though the alteration in semen parameters occasioned by Cr- exposure in rabbits was previously justified to be as a result of decreased serum concentration of testosterone and increased serum concentration of FSH (Yousef, El-Demerdash, Kamil, & Elaswad, 2006), this cannot be said to the reason in the present study as breeds, treatments, levels and their interactions did not influence the hormonal responses of the cocks. The difference between this previous report and ours as far as effects of Cr on reproductive hormones are concerned could be related to the

variations in the class of animals used, prevailing environmental factors as well as the length of exposures of the animals to Cr in the two experiments. However, inclusion of vitamin C in the diet played a maintenance role, especially, among the cocks fed higher dose of CrPic. Vitamin C as an antioxidant capable of arresting the oxidative damage impact occasioned by high Crpic dose was evidently clear in the present study. Just as previous studies have established that generation of reactive oxygen species (ROS) and its interference with cellular antioxidant system is a major mechanism by which toxic effect of an oxidative agent is mediated (Gupta, Gupta, Dhakal, Thakur, & Ahnn, 2004), vitamin C is believed to be a potent water soluble antioxidant capable of scavenging reactive oxygen species and reactive nitrogen species (Rekha et al., 2011) .Vitamin C was described as an excellent source of electron which enables it to donate electrons to free radicals thereby offsetting the reactivity of free radicals such as hydroxyl and superoxide radicals (Rekha et al., 2011). The increase in weight of testes and epididymedes observed could be correlated to normal serum testosterone level, restored epididymal sperm counts and normal daily sperm production and efficiency in the seminiferous tubule.

Table 7. Reproductive Hormones of Cocks Fed CrPic-Treated Diets with or without Vitamin C.

Breeds	Treatment	Levels	LH (IU L^{-1})	Progesterone (ng mL^{-1})	Oesterogen (ng dL^{-1})	Testosterone (nmol L^{-1})	FSH (IU L^{-1})
WLC			3.60 ± 0.01	1.53 ± 0.00	457.08 ± 0.32	24.04 ± 0.10	4.36 ± 0.01
NC			3.64 ± 0.00	1.55 ± 0.00	457.10 ± 0.32	24.07 ± 0.09	4.39 ± 0.01
	0		3.61 ± 0.01	1.54 ± 0.01	456.70 ± 0.31	24.04 ± 0.13	4.37 ± 0.01
	200		3.63 ± 0.01	1.53 ± 0.00	457.48 ± 0.31	24.07 ± 0.03	4.39 ± 0.00
		0	3.63 ± 0.00	1.53 ± 0.00	456.65 ± 0.25	23.71 ± 0.07	4.35 ± 0.01
		0.4	3.64 ± 0.01	1.53 ± 0.00	457.03 ± 0.21	24.09 ± 0.04	4.39 ± 0.00
		0.8	3.63 ± 0.00	1.53 ± 0.00	457.31 ± 0.78	23.81 ± 0.10	4.37 ± 0.01
		1.2	3.58 ± 0.02	1.55 ± 0.01	457.37 ± 0.34	24.61 ± 0.12	4.39 ± 0.01
	Interactions						
Breeds	Treatments	Levels					
WLC	0	0	3.61 ± 0.01	1.52 ± 0.01	455.83 ± 0.01	23.44 ± 0.01	4.31 ± 0.01
WLC	0	0.4	3.61 ± 0.01	1.52 ± 0.01	457.71 ± 0.01	24.21 ± 0.01	4.38 ± 0.01
WLC	0	0.8	3.62 ± 0.01	1.52 ± 0.01	454.73 ± 0.01	23.45 ± 0.01	4.33 ± 0.01
WLC	0	1.2	3.57 ± 0.01	1.56 ± 0.01	458.47 ± 0.01	24.99 ± 0.01	4.38 ± 0.01
WLC	200	0	3.63 ± 0.01	1.52 ± 0.01	457.44 ± 0.01	23.94 ± 0.01	4.36 ± 0.01
WLC	200	0.4	3.64 ± 0.01	1.52 ± 0.01	456.31 ± 0.01	23.94 ± 0.01	4.38 ± 0.01
WLC	200	0.8	3.62 ± 0.01	1.52 ± 0.01	459.87 ± 0.01	24.13 ± 0.01	4.38 ± 0.01
WLC	200	1.2	3.52 ± 0.06	1.52 ± 0.01	456.25 ± 0.01	24.22 ± 0.01	4.37 ± 0.01
NC	0	0	3.63 ± 0.01	1.54 ± 0.01	455.84 ± 0.01	23.48 ± 0.01	4.34 ± 0.01
NC	0	0.4	3.64 ± 0.01	1.54 ± 0.01	457.74 ± 0.01	24.24 ± 0.01	4.41 ± 0.01
NC	0	0.8	3.63 ± 0.01	1.54 ± 0.01	454.75 ± 0.01	23.50 ± 0.01	4.35 ± 0.01
NC	0	1.2	3.60 ± 0.01	1.59 ± 0.01	458.50 ± 0.01	25.00 ± 0.01	4.42 ± 0.01
NC	200	0	3.65 ± 0.01	1.54 ± 0.01	457.48 ± 0.01	23.96 ± 0.01	4.38 ± 0.01
NC	200	0.4	3.68 ± 0.01	1.54 ± 0.01	456.35 ± 0.01	23.97 ± 0.01	4.40 ± 0.01
NC	200	0.8	3.65 ± 0.01	1.54 ± 0.01	459.90 ± 0.01	24.17 ± 0.01	4.42 ± 0.01
NC	200	1.2	3.62 ± 0.01	1.54 ± 0.01	456.27 ± 0.01	24.23 ± 0.01	4.40 ± 0.01
P-values							
Breed (B)			0.9808	0.3497	0.9032	0.5092	0.2079
Treatment (T)			0.2659	0.1987	0.1098	0.1409	0.4287
Level (L)			0.1287	0.6509	0.4209	0.2310	0.1980
BxTxL			0.4995	0.9041	0.1338	0.8732	0.1893

Values are means ± SE; Means in a column without common superscripts are significantly ($p < 0.05$) different. LH = Luteinizing hormone , FSH = follicle stimulating hormone.

Conclusion

From the results of this study, it could be concluded that supplementing cocks' diets with CrPic at 0.40 mg kg^{-1} did not compromise the epididymal and testicular characteristics, semen characteristics, gonadal sperm reserves, daily sperm production and efficiency in the treated birds. Addition of vitamin C at 200 mg kg^{-1} diet further played a restorative role on the negative effects of feeding diets containing 1.2 mg kg^{-1} CrPic on semen characteristics such as ejaculate volume, sperm motility, sperm viability, sperm concentration, total sperm cells/ejaculate, total live cells, total motile cells, and mass activity grade. Fortifying the cocks' diets with the combination of CrPic and vitamin C at 0.40 m kg^{-1} and 200 mg, respectively could be a nutritional approach in combating the heat stress during higher ambient

temperature. This study will help the farmers and feed millers to be well equipped against the unprepared loss of birds occasioned by heat stress.

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