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Differential White Blood Cell Counts of Cocks Fed Chromium Picolinate Treated Diets with or without Vitamin C under high Ambient Temperature.

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ABSTRACT
Although chromium picolinate (CrPic) has gained attention for its potential benefits in animal production, its effects on immune function, particularly in poultry, remain a topic of interest. This study investigated the impact of dietary supplementation with CrPic and vitamin C on the immune response and white blood cell (WBC) counts in two breeds of cocks, Noiler and White Leghorn. A total of 192 cocks were divided into eight treatment groups, varying in CrPic levels (0, 0.4, 0.8, and 1.2 mg per kg of basal diet) with or without 200 mg/kg of vitamin C. At the end of the sixteen weeks feeding trials, although the statistical model identified a significant effect of breed on leukocyte parameters ($p < 0.0001$), subsequent pairwise comparisons and examination of group means indicated negligible differences between Noiler and White Leghorn cocks. The near equivalence in values suggests that the statistical outcome likely reflects model sensitivity rather than any biologically meaningful variation attributable to breed. Vitamin C supplementation significantly improved the immune cell parameters. The inclusion of CrPic at 0.4 mg and 0.8 mg per kg of basal diet also increased WBC counts. However, CrPic supplementation at 1.2 mg per kg of basal diet led to a decline in immune cell counts, suggesting a dose-dependent response. These findings highlight the potential of CrPic as a modulator of immune function, while also emphasizing the need to balance supplementation levels to avoid negative effects on immune performance.
Keywords: Differential, White Blood, Cock, Chromium, Vitamin C

INTRODUCTION

Avian leukocytes, composed of granulocytes (heterophils, eosinophils, and basophils) and agranulocytes (lymphocytes and monocytes), play a pivotal role in immune response and health assessment (Lashev et al. 2005; Szabó et al. 2010). The evaluation of both the quantity and functional quality of these leukocytes is a well-established method for assessing immune health and diagnosing diseases in birds (Mitchell and Johns, 2008; Shen et al. 2009). Variations in leukocyte populations are often observed in birds exposed to stress or adverse environmental conditions, reflecting changes in their immune function (Fiorello et al. 2009; Kamiński et al. 2014). Vitamin C, a potent antioxidant, has been shown to mitigate stress-induced immune suppression by lowering plasma corticosterone levels and adrenocorticotropic hormone, thereby enhancing stress resilience in poultry (Sahin et al. 2003; Lin et al. 2006). Adebayo (2024) further demonstrated that supplementation with 200 mg/kg of vitamin C in heat-stressed cocks led to reduced toxicity and a modest increase in serum hormone levels, supporting the beneficial role of vitamin C in high-temperature environments.

Chromium (Cr), although not widely recognized as an essential micronutrient for poultry, has shown promising effects on various physiological processes, including metabolism and immune function (Ognik et al. 2020). Specifically, chromium picolinate (CrPic) supplementation has been linked to increased serum levels of key hormones such as insulin, insulin-like growth factor (IGF), triiodothyronine (T3), and thyroxine (T4), while also reducing corticosterone levels, with these effects being dose-dependent (Adebayo, 2024). Furthermore, studies by Khan et al. (2014) have reported improvements in growth performance and reduced stress responses in poultry supplemented with chromium. The roles of chromium in supporting lipid and carbohydrate metabolism, enhancing immune and antioxidant systems, mitigating stress, and improving growth performance have been widely documented (Ognik et al. 2020). However, despite these positive effects, the optimal inclusion levels of chromium in poultry diets have yet to be conclusively defined by the National Research Council.

While chromium holds considerable promise for improving poultry health and performance, further investigation is needed to assess potential risks associated with excessive or improper dosages. Additionally, research suggests that combining chromium with other antioxidants, such as vitamin C, may have synergistic effects, particularly in enhancing poultry performance under stress conditions (Sahin et al., 2003; Haq et al., 2016; Adebayo, 2024). Adebayo (2024) previously investigated the effects of CrPic on serum protein levels in similar cocks and reported a differential impact of the CrPic and vitamin C combination on glucose regulation in these breeds. Expanding on this research, the present study examines the effects of this combination on immune function in the same breeds.

MATERIALS AND METHODS

Ethical approval

This research adhered to the ethical guidelines of the Federal University of Technology, Akure, Nigeria. Approval for the use of animals and compliance with established protocols was granted by the Research and Ethics Committee of the Department of Animal Production and Health. The study was conducted under the Ph.D. studentship identification number APH/90/2163.

Experimental site and operations

The study took place at the Poultry Unit of the Teaching and Research Farm, Federal University of Technology, Akure, Nigeria, during the dry season from January to February 2020. The research site, situated at 7° 17' North and 5° 9' East, is characterized by an average annual rainfall of 1524 mm, ambient temperatures ranging from 28°C to 31°C, and a mean annual relative humidity is about 80%.

Daily recordings of wet and dry bulb temperatures within the experimental pen were conducted in the morning and evening. The average daily temperature-humidity index (THI) for the pen was determined to be 34.08 ± 1.36°C. The THI, a measure of the thermal comfort of enclosed broiler chickens, was calculated using the formula developed by Tao and Xin (2003).

$$THI = 0.85 \times Tdb + 0.15 \times Twb \quad (1)$$

where Tdb = Dry bulb temperature (˚C) and Twb = Wet bulb temperature (˚C).

Experimental birds and diets

The study involved a total of 192 sexually mature cocks, aged 24 weeks, consisting of 96 Noiler and 96 White Leghorn cocks. Prior to the experiment, the birds were housed in cages for a two-week acclimatization period. During this phase, they were fed a commercial grower diet and had unrestricted access to fresh, chilled water. At the end of the acclimatization period, the cocks were weighed and randomly distributed into various experimental groups.

The experimental setup employed a 2 × 2 × 4 factorial design, with two breeds divided into eight treatments, each replicated four times. Each replicate included six birds. Diets were prepared with varying levels of chromium picolinate (CrPic) and vitamin C. The eight treatments (T1–T8) were formulated based on a basal diet, as detailed in Table 1.

The first four treatments (T1–T4) included CrPic at incremental levels of 0.00, 0.40, 0.80, and 1.20 mg/kg of the basal diet, without vitamin C. The remaining treatments (T5–T8) incorporated 200 mg of vitamin C per kilogram of the basal diet, alongside the same levels of CrPic (0.00 CrPic + 200 mg vitamin C, 0.40 CrPic + 200 mg vitamin C, 0.80 CrPic + 200 mg vitamin C, and 1.20 CrPic + 200 mg vitamin C). During the experimental period, the birds had continuous access to water and their assigned diets.

Differential white blood cell count analysis

The feeding trial lasted for sixteen weeks. On the final day of the feeding trial, blood samples were collected from seven randomly selected cocks per treatment group (one bird per replicate). Blood was drawn using a syringe and needle into EDTA-coated tubes for haematological analysis, adhering to the protocols outlined by Cheesbrough, (2000). The analysis was completed within 120 minutes of sample collection.

Sources of chromium picolinate and vitamin C

Chromium picolinate powder, with a purity of 98%, was sourced from AK Scientific, located in Union City, California. L-ascorbic acid powder, with 100% purity (USP/FCC grade), was procured from Avondale Laboratories (Supplies and Services) Limited, based in Banbury, England.

Table 1: Composition of the Cocks' Basal Experimental Diets (Adebayo et al. 2024)

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

Statistical Analysis

The collected data were analyzed using a 2 × 2 × 4 factorial design, representing two cock breeds, two vitamin C levels, and four chromium picolinate levels, within a completely randomized design framework. Statistical analysis was conducted using SAS software (2008). Where applicable, mean differences were evaluated using Duncan's multiple range test, with statistical significance set at a 5% threshold.

RESULTS

Differential White Blood Cells Counts of Cocks Fed CrPic-Treated Diets with or without Vitamin C.

Although the statistical model yielded a highly significant effect of breed on all differential leukocyte parameters ($p < 0.0001$), inspection of the breed-specific means indicated no meaningful biological differences between Noiler and White Leghorn cocks. For instance, heterophil percentages were 22.65 ± 0.17% for White Leghorn and 22.66 ± 0.17% for Noiler, while values for eosinophils, basophils, and lymphocytes were also nearly identical across breeds. These findings suggest that the statistical significance observed for breed may have been influenced by the model's sensitivity or low variability in the data, rather than actual physiological differences. The inclusion of vitamin C as antitoxicity for the CrPic dosage at ($P<0.05$) increased all the differential white blood cell count parameters of the two breeds of cocks.

The addition of CrPic considerable ($P<0.05$) increased the heterophil, eosinophil, basophil, lymphocytes and monocytes of the two experimental breeds of cocks. However, dosage level of 0.4 mg CrPic/kg of basal diet has the highest increamental effect, followed by dosage level of 0.8 mg CrPic/kg of basal diet, whereas dosage level of 1.2 mg CrPic/kg of basal diet decreased the white blood cell counts parameters when compare to the control.

While the effect of the interaction of 200 mg vitamin C and CrPic at 0.2, 0.4, and 1.2 mg/kg of basal diet consistently decreased the differential white blood cell, the interaction of vitamin C and CrPic at 0.8 mg CrPic/kg of basal diet increased the heterophil, eosinophil and basophil but a decrease was observed in lymphocyte and monocyte. This suggests that the effects of CrPic and vitamin C on differential white blood cell counts is dosage dependent.

DISCUSSION

The differential white blood cell counts for the cocks fed the experimental diets are summarized in Table 2. The study showed no significant differences in how the two breeds responded to the diets, suggesting that the breed type does not play a key role in influencing white blood cell counts. Notably, adding 200 mg/kg of vitamin C had a positive effect on heterophil, eosinophil, basophil, lymphocyte, and monocyte levels. This aligns with research by Holmannová et al. (2012) and Gorkom et al. (2018), which emphasized vitamin C's role in supporting immune function, boosting lymphocyte activity, and shielding immune cells from oxidative damage. Additionally, vitamin C has been associated with reduced stress in poultry, contributing to better health and performance (Abioja et al. 2020).

The inclusion of chromium picolinate (CrPic) also showed notable effects. Dosages of 0.4 and 0.8 mgCrPic/kg significantly improved differential white blood cell counts, consistent with findings by Hajializadeh et al. (2017). Their study demonstrated that CrPic supplementation enhanced immune performance in broilers, particularly under heat stress, by boosting antibody responses to avian influenza and infectious bronchitis.

However, a higher dose of 1.2 mg CrPic/kg of the experimental diet led to a decline in white blood cell parameters. This outcome aligns with the findings of Adebayo, (2024), which highlighted the dosage-dependent effects of CrPic on the growth and hormonal responses of cocks. This study suggests the importance of carefully balancing supplementation levels to optimize the immune system.

Table 2: Differential White Blood Counts of Cocks Fed CrPic-Treated Diets with or without Vitamin C

Breeds	Treatment	Levels	Heterophil (%)	Eosinophil (%)	Basophil (%)	Lymphocyte (%)	Monocyte (%)
WLC			22.65 ± 0.17^{a}	2.26 ± 0.59^{a}	1.51 ± 0.59^{a}	73.67 ± 0.53^{a}	3.17 ± 0.18^{b}
NC			22.66 ± 0.17^{a}	2.26 ± 0.59^{a}	1.51 ± 0.59^{a}	73.69 ± 0.53^{a}	3.18 ± 0.18^{a}
	0		21.87 ± 0.05^{b}	2.18 ± 0.40^{b}	1.46 ± 0.40^{b}	73.43 ± 0.24^{a}	2.92 ± 0.15^{b}
	200		23.61 ± 0.22^{a}	2.36 ± 0.68^{a}	1.57 ± 0.68^{a}	73.93 ± 0.71^{a}	3.43 ± 0.20^{a}
		0	22.58 ± 0.40^{c}	2.32 ± 0.90^{b}	1.54 ± 0.90^{b}	72.84 ± 0.25^{c}	3.17 ± 0.05^{c}
		0.4	25.16 ± 0.03^{a}	2.52 ± 0.20^{a}	1.68 ± 0.20^{a}	77.18 ± 0.65^{a}	3.84 ± 0.35^{a}
		0.8	23.15 ± 0.04^{b}	2.26 ± 0.30^{c}	1.51 ± 0.30^{c}	73.35 ± 0.20^{b}	3.35 ± 0.20^{b}
		1.2	19.72 ± 0.04^{d}	1.97 ± 0.30^{d}	1.31 ± 0.30^{d}	71.34 ± 0.40^{d}	2.34 ± 0.10^{d}
	Interactions						
Breeds	***Treatments***	***Levels***					
WLC	**0**	**0**	23.43 ± 0.17	2.34 ± 0.03	1.56 ± 0.03	73.67 ± 0.02	3.00 ± 0.23
WLC	**0**	**0.4**	18.86 ± 0.03	1.89 ± 0.05	1.26 ± 0.02	79.33 ± 0.04	2.67 ± 0.35
WLC	**0**	**0.8**	25.71 ± 0.02	2.57 ± 0.03	1.71 ± 0.08	70.00 ± 0.02	4.00 ± 0.18
WLC	**0**	**1.2**	25.71 ± 0.17	2.57 ± 0.01	1.71 ± 0.18	72.67 ± 0.03	2.00 ± 0.15
WLC	**200**	**0**	21.71 ± 0.02	2.17 ± 0.02	1.44 ± 0.03	72.00 ± 0.05	3.33 ± 0.10
WLC	**200**	**0.4**	20.57 ± 0.03	2.06 ± 0.11	1.37 ± 0.05	75.00 ± 0.03	5.00 ± 0.18
WLC	**200**	**0.8**	24.57 ± 0.11	2.46 ± 0.04	1.64 ± 0.15	72.67 ± 0.11	2.67 ± 0.35
WLC	**200**	**1.2**	20.57 ± 0.06	2.06 ± 0.15	1.37 ± 0.03	74.00 ± 0.15	2.67 ± 0.02
NC	**0**	**0**	23.44 ± 0.01	2.34 ± 0.05	1.56 ± 0.02	73.68 ± 0.01	3.01 ± 0.05
NC	**0**	**0.4**	18.87 ± 0.03	1.89 ± 0.11	1.26 ± 0.11	79.36 ± 0.03	2.68 ± 0.04
NC	**0**	**0.8**	25.75 ± 0.05	2.57 ± 0.02	1.72 ± 0.19	70.01 ± 0.02	4.02 ± 0.05
NC	**0**	**1.2**	25.72 ± 0.11	2.57 ± 0.03	1.71 ± 0.02	72.69 ± 0.09	2.01 ± 0.02
NC	**200**	**0**	21.72 ± 0.03	2.17 ± 0.01	1.45 ± 0.40	72.02 ± 0.12	3.34 ± 0.20
NC	**200**	**0.4**	20.60 ± 0.02	2.06 ± 0.11	1.37 ± 0.06	75.04 ± 0.03	5.02 ± 0.17
NC	**200**	**0.8**	24.59 ± 0.03	2.46 ± 0.04	1.64 ± 0.11	72.68 ± 0.12	2.69 ± 0.19
NC	**200**	**1.2**	20.58 ± 0.02	2.06 ± 0.02	1.37 ± 0.03	74.03 ± 0.01	2.68 ± 0.07
P-values							
Breed (B)			<.0001	<.0001	<.0001	<.0001	<.0001
Treatment (T)			<.0001	<.0001	<.0001	<.0001	<.0001
Level (L)			<.0001	<.0001	<.0001	<.0001	<.0001
BxTxL			0.6849	0.3080	0.1001	0.9041	0.9041

Values are means ± SE; Means in a column without common superscripts are significantly (P<0.05) different. WLC = white leghorn cocks, NC = noiler cocks, Treatment = vitamin C (200 mg per kg of basal diet), Level = chromium picolinate (mg/kg)

CONCLUSION

This study highlights the critical roles of breed type, vitamin C, and chromium picolinate (CrPic) supplementation in modulating the immune response of cocks. There was no significant difference in the differential white blood cell counts between the Noiler breed and the White Leghorn emphasizing no breed-specific variations. Vitamin C supplementation at 200 mg/kg of experimental diet consistently enhanced immune cell levels, emphasizing its role in reducing oxidative stress and supporting overall immune function. Additionally, CrPic experimental diet supplementation at 0.4 and 0.8 mg/kg improved immune parameters, aligning with previous findings on its benefits for immune performance. However, the decline observed at the 1.2 mg/kg of basal diet dosage highlights the need for careful dosing to avoid potential adverse effects.

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Authorship Contribution

Dr Francis Bosede Adebayo: Research conception, materials and method development and selection, and data preparation.
Stephen Oluwaseun Ibikunle: Literature review, data and laboratory analysis
Dr Nathaniel Olu Alamuoye: Laboratory analysis and data preparation
Dr Adetunmbi Tella: Data preparation and materials and methods

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