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Broiler Chickens Fed Chromium Propionate Supplemented Diets in a Tropical Environment: Serum Biochemical and Intestinal Morphology

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Abstract— *Aims: The effects of dietary supplementation with Chromium Propionate (CrProp) on serum aspartate aminotransaminase, alanine aminotransaminase, and intestinal morphology in broiler chickens are investigated in this study. Study Design: The completely randomised design was used for this study. Methodology: A total of 644 Cobb 500 broiler chickens were randomly assigned to eight dietary treatments (10 birds per replication). Diets 1 to 4 were designed by dividing a base diet into eight equal halves. Diets 1 to 4 were supplemented with 0, 0.4, 0.8 and 1.2 mg/kg CrProp, respectively. For a 42-day trial period, the birds had unrestricted access to feed and water sources. The gross pathological alterations were documented during necropsy. The intestinal tissues were fixed in 10% buffered formalin for histological investigation. Results: Serum ALT and AST levels were measured to investigate Cr toxicity. There was a significant increase with CrProp supplementation, indicating that the Cr levels administered were hepatotoxic. Intestinal morphology was also enhanced by CrProp supplementation. Conclusion: In conclusion, serum metabolites such as ALT and AST were affected following 0.8 mg/kg of CrProp supplementation, and jejunal morphological qualities were improved.*

Keywords— ***Avian; chromium; aspartate aminotransaminase; alanine amino transaminase; intestinal morphology.***

I. INTRODUCTION

Poultry meat is the most accessible protein source for humans in most countries. However, the poultry sector may be limited by many problems, including high or low environmental temperature, high stocking density. Heat stress is one of the main problems encountered within the poultry sector (Meremikwu *et al.*,2013; Shakeri *et al.*,2020). The tropical regions with high ambient temperature and humidity were more susceptible to high heat stress than the polar or temperate regions (Zhao *et al.*, 2015). Notably, in Nigeria, the high environmental temperature may be responsible for reduced performance and increased mortality (Oguntunji and Alabi 2010; Yousaf *et a*l., 2019). In addition, heat stress has a negative impact on intestinal development, resulting in a decrease in nutrient utilisation (Makanjuola and Adebiyi, 2012).

Chromium propionate, as an organic form of Cr, was approved as a source of Cr in broiler diets, and 200 μg/kg Cr from chromium propionate was recommended in broiler chicken complete feed by the Food and Drug Administration (FDA, 2020), Dietary chromium (Cr) supplementation has been shown to increase growth performance (Huang *et al.*,2016) and immunological responses (Bahrami *et al.*,2012) in broiler chicks under heat-stressed conditions. Studies also showed that Cr (III) could induce histopathological changes and oxidative stress

in the liver and kidney in chicken (Fan *et al*.,2015; Liu *et al*.,2015). In low content, trivalent chromium can promote the growth and development of chickens, thereby improving the quality of their meat (Piva *et al*.,2003), which is the potential reason why Cr is added into animal feed. Studies of chromium propionate have focused on broilers (Brooks *et al.,* 2016; Xiao *et al.,* 2017; Luo *et al.*,2019). Its importance in stress situations in animals and birds is becoming more widely recognized, and it aids in reducing the adverse effects of environmental and nutritional stress. Besides the reported beneficial effects of Cr, there is also a need for studies on the potentially toxic impact of wrong or inappropriate dosage in poultry.

This research focused on how dietary supplemented chromium propionate (CrProp) affected broiler chickens' blood aspartate amino transaminase, alanine aminotransaminase, and intestinal morphology.

II. MATERIALS AND METHODS

This feeding trial was carried out at the Avian Unit of The Federal University of Technology, Akure (FUTA) Teaching and Research Farm (TRF), during the peak of the dry season (i.e. between January and February 2020). The experimental pen's daily temperature-humidity index (THI) was 34.08^{o}C±1.36. The THI was calculated (Tao and Xin, 2003) using the formula: $THI= 0.85*T_{db}+0.15*T_{wb}$ Where T_{db} = dry bulb temperature (^{o}C); T_{wb} = wet bulb temperature (^{o}C).

2.1 Chromium Propionate

he Chromium Propionate powder (purity level = 98%) was manufactured by Chemlock Nutrition Corporation (Cincinnati, OH, USA.), which provides 0.4% Cr.

2.2 Experimental Diets and Animals

A basal diet each was prepared for the starter (age 1-3 weeks) and the finisher (age 4-6 weeks) phases (Table 1) and analysed for proximate composition [AOAC.1995]. The basal diets were sundered equally into eight parts and labelled diets 1to 8 and supplemented as follows:

Table 1. Description of Experimental Diets/Treatments (T)

Treatment	Chromium source	Levels of Chromium
T1	Control	Basal diet+ Nil (Control)
T2	Chromium Propionate	Basal diet +0.4mg/kg
T3	Chromium Propionate	Basal diet + 0.8mg/kg
T4	Chromium Propionate	Basal diet + 1.2mg/kg

2.3 Blood Sample Collection and Analysis

Blood samples were collected from the jugular vein of three birds per treatment group randomly on day 42 of the experiment in plain tubes were immediately transferred to the laboratory. The blood samples in plain tubes was allowed to clot and was serum harvested and stored at −80°C to determine aspartate aminotransferase and alanine aminotransferase in serum biochemical analyzer (Dia-CHEM 240 Plus).

Table 2. Composition of the experimental diets

Ingredients (%)	Starter feed	Finisher diet
Maize	52.35	59.35
Rice bran	0.00	6.00
Maize bran	7.00	0.00
Soybean meal	30.00	24.00
Soy oil	3.00	3.00
Fish meal	3.00	3.00
Limestone	0.50	0.50
Bone meal	3.00	3.00
Salt	0.30	0.30
Premix	3.00	3.00
Methionine	0.30	0.30
Lysine	0.25	0.25
Nutrient composition (%)		
*Crude protein	22.18	20.03
Metabolizable energy (Kcal/kg)	3018.89	3108.10
Methionine	0.68	0.66
Lysine	1.36	1.24
Available phosphorus	0.45	0.33
Calcium	1.01	0.99

2.4 Intestinal Morphology

The intestinal mucosal morphometry was determined by analyzing the duodenum, jejunum and ileum villus height, crypt depth, villus surface area, and villus height to crypt depth ratio. Intestines, collected from birds (three birds from each group), were processed according to a conventional method of haematoxylin and eosin (Ashraf *et al.*, 2013). A light microscope was used to examine the slides (Olympus CX31, Olympus, Center Valley, Pennsylvania, USA) fitted with a digital imaging system (Olympus DP20, Olympus USA). Five villi with intact lamina propria and well orientation were used for observations. The villus height

was measured from the villus tip to the villus crypt junction, and the crypt depth was measured from the crypt base to the crypt-villus transition region. The surface area of the villus was calculated using the formula (2p) (villus width/2) (villus length).

2.5 Data Analysis

All data were subjected to analysis of variance from the General Linear Model stratagem for complete randomised design with 4 CrProp levels factorial setting of treatments. The data were checked for CrProp, and When the treatment out-turn was significant (P<0.05), means were differentiated using Duncan's multiple range test using SPSS version 28.

III. RESULTS

The AST concentration of control group, 0.4, 0.8, and 1.2 mg/kg CrProp was 169.83±1.12 U/L, 174.57±0.40U/L, 176.53±6.98 U/L, and 179.94±0.15 U/L, respectively. The concentration of AST in 0.4, 0.8, and 1.2 mg/kg CrProp supplemented diets tended to increase compared to control group. The AST concentrations in 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets showed significant difference from the control diet ($p < 0.00$). The ALT concentration of in control diet, 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets was 27.08±0.57U/L, 27.10±0.43 U/L, 28.55±0.46 U/L, and 29.15±1.20 U/L, respectively. The concentration of ALT in 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets increased compared to control group. also, ALT concentrations in 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets showed significant difference from the control group ($p < 0.05$) (Table 2).

Table 3: The concentration of AST and ALT in broiler chicken fed CrProp diet

parameters	0	0.4mg/kg	0.8mg/kg	1.2 mg/kg	P-value
AST (U/L)	169.83±1.12^{c}	174.57±0.40^{ab}	176.53±6.98^{b}	179.94±0.15^{a}	0.00
ALT (U/L)	27.08±0.57^{ab}	27.10±0.43^{ab}	28.55±0.46^{b}	29.15±1.20^{a}	0.05

Means with a different superscript in the same column are significantly (P<0.05) different; Cr Prop: Chromium Propionate

Table 3 shows the results of intestinal microarchitecture in various segments of the small intestine. With 0.8mg/kg of Chromium propionate supplementation, the villus height of the duodenum and jejunum was considerably raised in this study. The ileal villus height remained consistent when the food was supplemented with 1.2 mg/kg of Chromium propionates. The crypt depth of the duodenum was improved (P <0.001) by a chromium supplemented diet of 0.4 mg/kg. There was no effect in jejunal and ileal crypt depth with chromium propionate supplementation. The villus width, surface area, and height to crypt depth ratio in the duodenum and ileum were unaffected with Chromium propionate supplementation. However, compared to the control group, villus breadth and surface area in the jejunum of birds supplemented with 0.4mg/kg and 0.8mg/kg of chromium propionate were significant (P <0.001). In birds treated with 0.8 mg/kg of Chromium propionate, the villus height to crypt depth ratio of the jejunum was larger (<P0.05) than in the 1.2 mg/kg group.

Table 4. Effects of supplementation with chromium propionate on intestinal microarchitecture in broilers

Level of CrProp (mg/kg)	VH^3 (µm)	VW (µm)	CD (µm)	VSA (mm^2)	VH: CD
DUODENUM					
0	1223^{a}	75	115^{b}	0.24	8.65
0.4	950^{ab}	62	246^{a}	0.18	5.09
0.8	1221^{a}	46	148^{b}	0.14	5.82
1.2	619^{c}	66	140^{b}	0.15	5.36
SEM	64.02	3.20	10.79	0.01	0.44
P VALUE	0.00	0.06	<0.001	0.13	0.13
JEJUNUM					
0	462^{c}	47^{c}	98	0.06^{b}	4.79^{ab}
0.4	635^{ab}	82^{a}	117	0.16^{a}	5.62^{ab}
0.8	643^{a}	63^{b}	109	0.13^{ab}	6.47^{a}
1.2	472^{b}	60^{ab}	123	0.09^{c}	3.70^{b}
SEM	23.51	2.4	4.99	0.01	0.25
P VALUE	0.02	<0.001	0.35	<0.001	0.03
ILEUM					
0	533	76	146	1.3	3.9
0.4	534	77	155	1.33	3.62
0.8	500	73	130	1.19	3.91
1.2	449	80	144	1.21	3.16
SEM	12.86	2.12	3.87	0.05	0.1
P VALUE	0.37	0.65	0.19	0.17	0.31

$^{a-d}$ within the row different superscript indicates significantly different means at P <0.05. VH^3: villus height; VW villus width; CD: crypt depth; VSA: villus surface area; VH:CD: villus height to crypt depth ratio

IV. DISCUSSION

The AST enzyme is one of the indicators used to determine whether or not an individual has liver impairment. AST enzyme is found in cytosolic and mitochondrial isoenzymes of the liver, skeletal muscles, heart muscles, kidneys, brain, pancreas, lungs, leukocytes and red blood cells. On the other hand, the AST enzyme is less sensitive and specific for detecting liver disease (Zachariah *et al.*, 2017). There were significant differences in AST concentrations in the 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets compared to the control group. The increase was possible because of the increased Chromium concentration in the diet. However, the findings contradicted those of Liang *et al.* (2021), who found that Cr supplementation reduced aspartate transaminase (AST) activity considerably. Because the AST enzyme is specific to the liver and other body tissues, an increase in this enzyme does not always suggest liver damage. The ALT enzyme was observed to increase in the 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets compared to the control group, and the increase was statistically significant. Because this cytosolic enzyme was present in the highest amounts in the liver and was more selective in detecting liver function deterioration, ALT enzymes were a stronger predictor of liver damage than AST enzymes (Thapa and Walia, 2007). Because ALT enzyme activity in the liver is about 3000 times that in the serum, Kim et al. (2008) observed that ALT released from damaged liver cells would enhance the measured activity of the ALT enzyme in the serum in the case of hepatocellular injury or death. According to Ognik *et al.* (2020), the increase in the ALT enzyme caused oxidative liver damage, which resulted in histological abnormalities in the liver. As a result, reactive oxygen species (ROS) produce more free radicals, damaging effects on membrane phospholipids and causing a wide range of cell damage. However, Asbaghi *et al.* (2021) observed that serum AST and ALT were not

significantly influenced by chromium intakeSerum ALT and AST levels were measured to investigate Cr toxicity, and there was a significant change with Cr supplementation, revealing that supplemented Cr levels were hepatotoxic. The finding was inconsistent with Bakhiet *et al.* (2007), who observed no effect of Cr supplementation as $CrCl_3$ on blood AST and uric acid in broilers.

In birds, improvement in gut mucosal morphology is characterized as a health indicator and growth indicator (Awad et al., 2009). The intestinal mucosal barrier, which is made up of epithelial cells, allows only required nutrients to pass through while keeping harmful components like germs and toxins from entering the intestinal lumen (Lee *et al.*, 2015). Damage to intestinal cells breaks down the barrier, allowing dangerous chemicals to enter and cause villi to shorten and epithelial sloughing (Sikandar *et al.*, 2017). The villi and microvilli of the intestine promote nutrient absorption across the intestine (Awad *et al*., 2009). Intestinal health and integrity are associated with height, width, surface area, and villus height to crypt depth ratio, crucial for intestinal digestion and absorption (Li et al., 2018). There are few studies on the impact of chromium on intestinal histology in broilers. In this study, the villus height of the duodenum and jejunum was significantly higher in birds supplemented with 0.8mg/kg CrProp, while the ileum was unaffected. Crypt depth of duodenum in birds supplemented with 0.4 and 0.8mg/kg CrProp was higher ($P < 0.001$) than in other supplemented and control groups

The depth of the jejunal and ileal crypts was unchanged by CrProp supplementation compared to the control group. The duodenal and ileal villus surface area remained unaltered with CrProp supplementation. The surface area of the jejunal villus in birds supplemented with 0.4 and 0.8 mg/kg CrProp was significantly greater ($P< 0.001$) than in the control group. Compared to the control group, supplementation did not affect the villus height to crypt depth ratio of the duodenum and ileum. The 0.8mg/kg CrProp supplemented group had a more significant ($P< 0.001$) jejunal villus height to crypt depth ratio than the 1.2mg/kg CrProp supplemented group. Li *et al.* (2018) investigated the effects of Cr-picolinate on the villus height and crypt depth of the duodenum, jejunum, and ileum in ducks reared under heat stress and discovered that Cr-picolinate did not affect the villus height and crypt depth of the duodenum, jejunum, and ileum at days 14, 21, and 35. However, in the jejunum and ileum, Cr-picolinate treatment dramatically enhanced the villus height to crypt depth ratio.

V. CONCLUSION

Supplemental Chromium propionate CrProp significantly affects the serum metabolites such as ALT and AST and improves intestinal morphological qualities at 0.8mg/kg supplementation of CrProp of feed.

ETHICAL APPROVAL

This work was approved by the Research and Ethics Committee of the Animal Production and Health Department, The Federal University of Technology, Akure, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors OSO, OAA and CAC designed the study. Authors OAA and FBA performed the statistical analysis. Authors OSO, OAA and FBA wrote the protocol. Authors OSO, FBA and OTA wrote the first draft of the manuscript. All authors managed the analyses of the study. Authors OSO, OAA and FBA organised the literature searches. All authors read and approved the final manuscript

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