Blood profile and liver histopathological analysis of broiler chickens fed dried cashew apples (*Anacardium occidentale* L.)

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Abstract

This study utilized a total of 210 one-day-old chicks to evaluate the blood profile and liver histopathology of Ross 308 broiler chickens fed diets containing Dried Cashew Apples (DCA). The chicks were allocated into seven treatment groups, each consisting of three replicates of 10 birds. The experimental design used a (2 x 3) +1 augmented factorial in a completely randomized arrangement. Diet 1 was the control (maize-soybean meal-based), while diets 2, 3, and 4 contained 10%, 20%, and 30% DCA without enzyme supplementation. Diets 5, 6, and 7 contained 10%, 20%, and 30% DCA with exogenous microbial enzyme supplementation. Data on haematological indices, serum biochemical indices, and liver histology were collected and analyzed using SPSS version 25, with means separated by Duncan's Multiple Range Test of the same statistical package at $p < 0.05$. The results indicated that DCA inclusion, with or without enzymes, significantly influenced all haematological parameters except MCV, showing lower PCV, Hb concentration, and RBC counts in DCA groups compared to the control. Enzyme supplementation at 20% DCA increased PCV, while at 10% and 30% DCA, it decreased Hb concentrations and increased WBC counts at 30% DCA, with varying effects on neutrophils and lymphocytes. Serum biochemistry showed that DCA inclusion affected all parameters except AST, with the highest ALT values in birds on 10% DCA without enzymes and the lowest in the control group. Enzyme supplementation significantly influenced AST, ALT, TP, globulin, albumin, urea, and creatinine levels, particularly at 20% DCA. Liver histology showed no lesions in control birds but varying degrees of necrosis, inflammation, atrophy, and congestion in DCA-fed birds, worsening with higher DCA and enzyme levels. In conclusion, broilers can tolerate 10% DCA in their diet, but higher levels lead to physiological stress, necessitating careful consideration of DCA inclusion levels to maintain bird health and welfare.

Keywords: broiler, cashew apples, blood profile, liver histology.

Perfil sanguíneo e análise histopatológica do fígado de frangos de corte alimentados com caju desidratado (*Anacardium occidentale* L.)

Resumo

Este estudo utilizou um total de 210 pintinhos de um dia de idade para avaliação do perfil sanguíneo e histopatologia hepática de frangos de corte Ross 308 alimentados com dietas contendo Caju Seco (DCA). Os pintinhos foram alocados em sete grupos de tratamento, cada um consistindo em três repetições de 10 aves. O delineamento experimental utilizado foi fatorial aumentado (2 x 3) +1 em arranjo inteiramente casualizado. A dieta 1 serviu como controle (à base de farinha de milho e soja), enquanto as dietas 2, 3 e 4 continham 10%, 20% e 30% de DCA sem suplementação enzimática. As dietas 5, 6 e 7 continham 10%, 20% e 30% de DCA com suplementação de enzimas microbianas exógenas. Os dados sobre índices hematológicos, índices bioquímicos séricos e histologia hepática foram coletados e analisados no SPSS versão 25, com médias separadas pelo Teste de Faixa Múltipla de Duncan do mesmo pacote estatístico em $p < 0,05$. Os resultados indicaram que a inclusão de DCA, com ou sem enzimas, influenciou significativamente todos os parâmetros hematológicos, exceto MCV, mostrando menor PCV, concentração de Hb e contagens de glóbulos vermelhos nos grupos de DCA em comparação com o controle. A suplementação enzimática a 20% de DCA aumentou o PCV, enquanto a 10% e 30% de DCA diminuiu as concentrações de Hb e aumentou a contagem de leucócitos a 30% de DCA, com efeitos variados em neutrófilos e linfócitos. A bioquímica sérica mostrou que a inclusão de DCA afetou todos os parâmetros, exceto AST, com os valores mais altos de ALT em aves com 10% de DCA sem enzimas e os mais baixos no grupo controle. A suplementação enzimática influenciou significativamente os níveis de AST, ALT, TP, globulina, albumina, ureia e creatinina, particularmente a 20% de DCA. A histologia hepática não mostrou lesões nas aves controle, mas graus variados de necrose, inflamação, atrofia e congestão nas aves alimentadas com DCA, piorando com níveis mais elevados de DCA e enzimas. Concluindo, os frangos podem tolerar 10% de DCA em sua dieta, mas níveis mais elevados levam ao estresse fisiológico, necessitando de uma consideração cuidadosa dos níveis de inclusão de DCA para manter a saúde e o bem-estar das aves.

Palavras-chave: frango, caju, perfil sanguíneo, histologia hepática.

1. Introduction

The broiler production industry faces significant challenges related to feed formulation, with feeding costs taking a substantial portion of the total cost of production (Kpomasse et al., 2021). Feed costs account for about 60-70% total cost of production in a broiler production enterprise. This hike in the prices of conventional feed ingredients, such as maize, wheat, barley, soya-bean-meal, full-fat soya, groundnut cake, etc, is a result of the stiff competition between humans, industry, and animals for the limited raw materials in most developing country (Thirumalaisamy et al., 2016).

This development has significantly reduced the profitability of broiler production enterprises. One of the objectives of a broiler production enterprise like any other business is profit maximization; to achieve this, researchers and farmers alike must search for cheaper alternatives to the expensive conventional feed ingredients (Alhotan, 2021). One promising alternative feed resource that has gained attention is Dried Cashew Apple (DCA). DCA a by-product in the cashew production industry presents a viable solution to the challenges confronting broiler production enterprises, offering potential benefits for both economic sustainability and animal nutrition (Oyekola et al., 2024).

The inclusion of DCA in broiler diets has the potential to decrease the overall cost of feed production by serving as a cost-effective alternative to traditional feed ingredients. Cashew apple is rich in energy, fibre, vitamins, and minerals, providing a nutritionally balanced feedstuff for broiler chickens (Akyereko et al., 2023). Its utilization in poultry diets can contribute to improved feed efficiency and growth performance while reducing reliance on expensive feed components, thereby increasing the profitability of broiler production enterprises (Swain et al., 2007).

Furthermore, DCA offers additional advantages beyond its nutritional value. Its high fibre content could be leveraged to support gastrointestinal health in broilers, thereby, promoting optimal digestion and nutrient absorption (Jha, and Mishra, 2021). Additionally, the bioactive compounds present in cashew apples may exert beneficial effects on immune function and disease resistance in broiler chickens, enhancing overall health and welfare (Cruz-Reina et al., 2022). By incorporating DCA into broiler diets, producers can not only achieve cost savings but also improve the sustainability and resilience of their production systems.

Despite the potential benefits of DCA as a feed ingredient for broiler chickens, there is a need for comprehensive scientific research to evaluate its impact on blood profile and overall health. Blood profile analysis is essential to assess the physiological responses of broilers to DCA inclusion and ensure the safety and efficacy of its utilization in commercial production settings. Through rigorous scientific investigation, this study was aimed at investigating the haematological, and serum biochemical analysis of broiler chickens (Starter and Finisher phases) fed DCA-based diet with or without fortification with exogenous microbial multi-enzymes cocktail, thereby contributing to the advancement of economically viable and environmentally responsible poultry production practices.

2. Materials and Methods

2.1 Experimental Site

The study was carried out in the Poultry Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, situated in Ogbomoso, Oyo State, Nigeria. The Ogbomoso is located at around 8°10'1" N latitude and 4°10'1" E longitude (Google Earth, 2023). The area is categorized as a derived savannah and has an average annual precipitation of 1070 mm. The average temperature in this region is 26.1°C.

2.2 Test Ingredient

Cashew apples were obtained from cashew farms in Ogbomoso and the surrounding areas after the separation of raw cashew nuts. This procedure occurred throughout the raw cashew nut harvest season, which spans from January to May. The harvested cashew apples were first put in plastic baskets and meticulously cleaned under flowing water to eliminate any extraneous substances. Afterward, the apples were placed in bags and subjected to mechanical pressure to extract the juice. The remaining pulp from the juice extraction procedure was then sun-dried to a consistent weight. The dried cashew apple (DCA) residue was processed using a hammer mill fitted with a 2 mm screen to acquire the appropriate particle size for inclusion into the experimental diets. A sample of the DCA was taken for proximate analysis to assess its dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), and total ash content. These analyses were done using the techniques defined by the AOAC (2005).

2.*3 Exogenous enzyme (Polyzyme ™)*

In this investigation, a multi-enzyme cocktail particularly formulated for poultry (Polyzyme™) was used. The enzyme mix includes Xylanase (750-1050 U/g), Phytase (75-150 U/g), Cellulase (525-700 U/g), Beta-glucanase (375-500 U/g), Pectinase (200-300 U/g), Alpha-Amylase (2700-3400 U/g), and Protease (< 1200 HUT/g). The purpose of introducing this multi-enzyme cocktail was to boost the nutritional content of the feed by enhancing nutrient availability and absorption. The enzyme combination, in a finely powdered form, was added to the diets at a rate of 1 kg per ton of feed, corresponding with the manufacturer's suggested inclusion range of 500 g to 2 kg per ton of feed.

2.4 Experimental diets

The feed materials for the experimental diets were purchased from a local feed mill in Ogbomoso, Oyo State. These diets were formulated to suit the dietary needs of broiler chickens as defined by the NRC (1994). Tables 1 and 2 offer the detailed formulas for the starter and finisher phases of the research, respectively.

Table 1. Gross composition of the starter experimental diets fed to the chickens.

Feed Ingredients (%)	Control (0%) DCA	D2 (10%) DCA	D3 (20%) DCA	D4 (30%) DCA	D5 (10%) DCA + Enzyme	D6 (20%) DCA + Enzyme	D7 (30%) DCA + Enzyme
Maize	55.00	49.50	44.00	38.50	49.50	44.00	38.50
Soybean Meal	32.00	32.00	32.00	32.00	32.00	32.00	32.00
Fish Meal (72%)	4.50	4.50	4.50	4.50	4.50	4.50	4.50
*DCA	0.00	5.50	11.00	16.50	5.50	11.00	16.50
Corn Bran	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vegetable Oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Bone Meal	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Oyster Shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit premix**	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Polyzyme***	-	-	-	-	+	+	+
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Composition							

Crude protein (%)	22.51	22.61	22.71	22.82	22.61	22.71	22.82
+ME (kcal/kg^{-1})	3093.10	3062.58	3032.06	3001.54	3062.58	3032.06	3001.54
Crude fibre (%)	3.58	4.27	4.95	5.63	4.27	4.95	5.63
Ether extract (%)	3.94	4.21	4.45	4.78	4.21	4.45	4.78
Methionine (%)	0.62	0.61	0.60	0.59	0.61	0.60	0.59
Lysine (%)	1.48	1.46	1.45	1.43	1.46	1.45	1.43
Available Phosphorous (%)	0.67	0.66	0.66	0.65	0.66	0.66	0.65
Calcium (%)	1.21	1.20	1.20	1.20	1.20	1.20	1.20

Note: *DCA: Dried cashew apple. ** Vitamin premix: Vitamin A 10,000,000.00 IU; Vitamin D3 2,000,000.00 IU; Vitamin E 23,000.00 mg; Vitamin K3 2,000.00 mg; Vitamin B1 1,800.00 mg; Vitamin B2 5,500.00 mg; Niacin 27,500.00 mg; Pantothenic acid 7,500.00 mg; Vitamin B6 3,000.00 mg; Vitamin B12 15.00 mg; Folic acid 750.00 mg; Biotin 60.00 mg; Choline chloride 300,000.00 mg; Cobalt 200.00 mg; Copper 3,000.00 mg; Iodine 1,000.00 mg; Iron 20,000.00 mg; Manganese 40,000.00 mg; Selenium 200.00 mg; Zinc 30,000.00 mg; Antioxidant 1,250.00 mg. *** Polyzyme composition: Xylanase 750-1050 U/g, Phytase 75-150 U/g, Cellulase 525-700 U/g, Beta-glucanase 375-500 U/g, Pectinase 200-300 U/g, Alpha-Amylase 2700-3400 U/g, Protease < 1200 HUT/g. +ME: Metabolizable energy. Source: Authors, 2024.

Table 2. Gross composition of the finisher experimental diets fed to the chickens.

Feed Ingredients (%)	Control (0%) DCA	D2 (10%) DCA	D3 (20%) DCA	D4 (30%) DCA	D5 (10%) DCA + Enzyme	D6 (20%) DCA + Enzyme	D7 (30%) DCA + Enzyme
Maize	56.00	50.40	44.80	39.20	50.40	44.80	39.20
Soybean Meal	25.00	25.00	25.00	25.00	25.00	25.00	25.00
*DCA	0.00	5.60	11.20	16.80	5.60	11.20	16.80
Wheat Offal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Corn Bran	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fish Meal (72%)	3.00	3.00	3.00	5.00	3.00	3.00	5.00
Vegetable Oil	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Bone Meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster Shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit Premix**	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Polyzyme***	-	-	-	-	+	+	+
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Composition							
Crude protein (%)	19.66	19.77	19.87	19.97	19.77	19.87	19.97
+ME (kcal/kg^{-1})	2997.34	2966.26	2935.19	2904.11	2966.26	2935.19	2904.11
Crude fibre (%)	3.80	4.49	5.19	5.88	4.49	5.19	5.88
Ether extract (%)	3.90	4.18	4.47	4.76	4.18	4.47	4.76
Methionine (%)	0.57	0.56	0.55	0.54	0.56	0.55	0.54
Lysine (%)	1.27	1.25	1.24	1.23	1.25	1.24	1.23

Available Phosphorus (%)	0.73	0.73	0.72	0.72	0.73	0.72	0.72
Calcium (%)	1.49	1.49	1.49	1.49	1.49	1.49	1.49

Note: *DCA: Dried Cashew Apple. ** Vitamin premix: Vitamin A 10,000,000.00 IU; Vitamin D3 2,000,000.00 IU; Vitamin E 23,000.00 mg; Vitamin K3 2,000.00 mg; Vitamin B1 1,800.00 mg; Vitamin B2 5,500.00 mg; Niacin 27,500.00 mg; Pantothenic acid 7,500.00 mg; Vitamin B6 3,000.00 mg; Vitamin B12 15.00 mg; Folic acid 750.00 mg; Biotin 60.00 mg; Choline chloride 300,000.00 mg; Cobalt 200.00 mg; Copper 3,000.00 mg; Iodine 1,000.00 mg; Iron 20,000.00 mg; Manganese 40,000.00 mg; Selenium 200.00 mg; Zinc 30,000.00 mg; Antioxidant 1,250.00 mg. *** Polyzyme Composition: Xylanase 750-1050 U/g, Phytase 75-150 U/g, Cellulase 525-700 U/g, Beta-glucanase 375-500 U/g, Pectinase 200-300 U/g, Alpha-Amylase 2700-3400 U/g, Protease <1200 HUT/g. +ME: Metabolizable energy. Source: Authors, 2024.

2.5 Experimental animals and management

A total of 210 one-day-old Ross strain broiler chicks were used for this study. On arrival, the chicks were weighed individually and randomly allocated into seven treatment groups, each consisting of 30 birds. These groups were subsequently separated into three replicates, with each replicate containing 10 birds. The birds were kept in a deep-litter, open-sided chicken house separated into compartments to guarantee segregation during the study.

Wood shavings were used as litter material, which was regularly turned and replaced as needed. Electric bulbs provided primary heat during the brooding phase, with charcoal pots and rechargeable lamps used as backup heat and lighting sources in the event of power outages.

Feed and water were offered to the birds ad libitum throughout the experiment. Routine vaccinations, medications, and prophylactic anti-coccidial treatments were administered to ensure the birds remained healthy all through the experimental period. These measures were implemented to maintain the welfare and well-being of the experimental birds during the study.

2.6 Experimental design

The experimental layout for this study is structured in a (2 x 3) + 1 augmented factorial arrangement in a completely randomized design.

The model of the experimental design is as expressed below:

$$Yijk = \mu + Di + Ej + DEij + \varepsilon ijk$$

Where:

Yijk = observation of the kth broiler chickens, from the ith Dried Cashew Apple and jth exogenous enzyme supplementation,

μ = common mean,

Di = fixed effect of ith Dried Cashew Apple,

Ej = fixed effect of jth exogenous enzyme supplementation,

DEij = interaction effect of ith Dried Cashew Apple and jth exogenous enzyme supplementation,

εijk = random error.

2.7 Data collection

2.7.1 Blood profile analysis

On day 42 of the study, two birds of median weight from each replicate were selected for blood sampling via the jugular vein. Two sets of 5 mL blood samples were collected; one for haematological analysis and the other for serum biochemical analysis. Haematological samples were placed in non-vacuum blood collection tubes containing Ethylene Diamine Tetra Acetic acid (EDTA) to prevent coagulation, while serum biochemical samples were collected in plain non-vacuum tubes without EDTA. The collected blood samples were stored in an icebox to reduce metabolic activity and minimize cell death before being promptly sent to the laboratory for analysis.

Haematological parameters, including Packed Cell Volume (PCV) and Haemoglobin concentration (Hb), were measured using the micro haematocrit method and the cyanmethemoglobin method, respectively, as outlined by Jain (1986). Red Blood Cell (RBC) and White Blood Cell (WBC) counts were determined using an improved Neubauer haemocytometer following appropriate dilution, as described by Dacie & Lewis (2017). Neutrophil and lymphocyte counts were derived from a differential smear of the WBC count stained with Wright stain and expressed as a percentage of the WBC count (Emiola et al., 2013). Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using PCV, haemoglobin concentration, and RBC count following the methods of Villatoro & To (2021).

Serum biochemical parameters such as Aspartate Transaminase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) were measured using spectrophotometric methods as described by Rej & Hoder (1983). Total Protein was determined using the biuret method, and albumin was measured using the Bromocresol green (BCG) method as described by Peter et al. (1982), with globulin concentration calculated by subtracting albumin from total protein. Urea and creatinine levels were determined following the methods outlined by Salazar (2014).

2.7.2 Liver histopathological analysis

On day 42 of the study, 21 birds (one per replicate) were randomly selected and humanely slaughtered by severing the jugular vein. The birds were hung upside down to ensure complete bleeding. A portion of the liver from each bird was collected, immersed in 1% formalin solution, fixed in *Bouin's* solution for 24 h, and embedded in paraffin wax. Sections were cut to 5 µm thickness using a microtome, stained with hematoxylin-eosin dye (H&E), and examined with a Nikon E100 light microscope (Tokyo, Japan).

2.8 Statistical analysis

The collected data were analyzed using a One-Way Analysis of Variance with the General Linear Model Procedure in IBM SPSS version 25, and treatment means were separated using *Duncan's* Multiple Range Test within the same software. Statistical significance was determined at $p < 0.05$.

3. Results and Discussion

3.1 Haematological indices (Starter)

Table 3 presents the effects of including DCA with or without an exogenous enzyme cocktail on selected haematological indices of broiler chicks. All measured haematological parameters were significantly ($p < 0.05$) affected by the experimental diet, except for MCV ($p > 0.05$). PCV and Hb concentrations were significantly ($p < 0.05$) lower in groups fed DCA-based diets compared to the control.

Among the DCA groups, the diet containing 20% DCA without exogenous enzymes showed significantly ($p < 0.05$) lower levels of these parameters, while other DCA groups had statistically similar values. Additionally, RBC counts were significantly ($p < 0.05$) lower in DCA-fed groups compared to the control. However, chicks fed 30% DCA diets with or without enzyme supplementation (D4 and D7) had statistically similar RBC counts, which were significantly ($p < 0.05$) higher compared to other DCA-based diet groups. This pattern was also observed in WBC counts, except for birds on 30% DCA with exogenous enzymes, which had significantly ($p < 0.05$) higher RBC counts compared to other DCA-based diet groups.

The interaction effects of varying DCA levels and exogenous enzyme supplementation on selected haematological indices in broiler chicks are shown in (Table 4). At 20% DCA, a significant ($p < 0.05$) interaction was observed, with chicks fed enzyme-supplemented diets having higher PCV compared to those on unsupplemented diets, while no interaction ($p > 0.05$) was noted at 10% and 30% DCA levels. Additionally, a significant ($p < 0.05$) combination effect on Hb concentration was seen at both 10% and 30% DCA levels, with higher Hb levels in chicks offered diets without enzyme supplementation.

This trend also appeared in RBC counts, but the interaction effect was significant ($p < 0.05$) only at 30% DCA, with no effects at 10% and 20% levels. Moreover, WBC counts were significantly ($p < 0.05$) higher in birds on 10% DCA without enzyme supplementation compared to those with supplementation, whereas the opposite was true at 30% DCA. Neutrophil levels were significantly ($p < 0.05$) higher in birds on 10% and 20% DCA with enzyme supplementation compared to those on unsupplemented diets. An inverse trend was observed in lymphocyte levels at these inclusion levels, with higher levels in birds on unsupplemented diets.

Table 3. Main effects of varying inclusion levels of DCA on the haematological indices of broiler chicks (0-21 days).

Parameters	Diets							SEM	*p*-Value
	1	2	3	4	5	6	7		
PCV (%)	34.33^{a}	32.33^{ab}	28.67^{b}	31.33^{ab}	32.67^{ab}	31.67^{ab}	31.33^{ab}	0.57	0.250
Hb concentration (g/L)	12.70^{a}	12.27^{ab}	11.13^{b}	12.63^{ab}	11.77^{ab}	11.90^{ab}	11.90^{ab}	0.15	0.067
RBC (10^{12}/L)	2.37^{a}	2.13^{bc}	2.00^{c}	2.33^{ab}	2.07^{c}	2.07^{c}	2.17^{abc}	0.03	0.007
WBC (10^{9}/L)	148.67^{a}	134.67^{c}	132.33^{c}	136.33^{bc}	127.33^{c}	135.33^{c}	145.67^{ab}	1.59	0.001
MCV (fl.)	123.33	128.33	124.33	124.33	126.33	127.33	123.67	0.64	0.240
MCH (pg.)	54.67^{b}	57.33^{a}	57.00^{a}	54.67^{b}	56.67^{a}	57.67^{a}	55.00^{b}	0.24	0.000
Neutrophils (%)	13.67^{bc}	15.33^{abc}	9.67^{c}	20.00^{ab}	23.33^{a}	20.00^{ab}	19.67^{ab}	2.14	0.009
Lymphocytes (%)	86.33^{ab}	84.67^{abc}	90.33^{a}	80.00^{bc}	76.67^{c}	80.00^{bc}	80.33^{bc}	1.11	0.009

Note: $^{a\ b\ c}$ Means within rows for different groups with different superscripts differ ($p < 0.05$). PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cells count; WBC: White Blood Cells count; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; SEM: Standard Error of Means; p-Value: Probability value. Diets: 1 = Control; 2 = 10% DCA – Enzymes; 3 = 20% DCA – Enzymes; 4 = 30% DCA – Enzymes; 5 = 10% DCA + Enzymes; 6 = 20% DCA + Enzymes; 7 = 30% DCA + Enzymes. Source: Authors, 2024.

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Table 4. Interaction effects of dietary inclusion of DCA and enzyme supplementation of the haematological indices of broiler starter chicks (0-21 days).

Parameters	Enzyme supplementation	Inclusion levels			SEM^{2}
		10%	20%	30%	
PCV (%)	–	32.33	28.67^{Y}	31.33	0.90
	+	32.67	31.67^{X}	31.33	0.90
	SEM^{1}	1.11	1.11	1.11	
Hb concentration (g/l)	–	12.27^{aX}	11.13^{b}	12.63^{aX}	0.20
	+	11.77^{Y}	11.90	11.90^{Y}	0.20
	SEM^{1}	0.25	0.25	0.25	
RBC (10^{12}/L)	–	2.13^{b}	2.00^{c}	2.33^{aX}	0.04
	+	2.07	2.07	2.17^{Y}	0.04
	SEM^{1}	0.05	0.05	0.05	
WBC (10^{9}/L)	–	134.67^{X}	132.33	136.33^{Y}	1.62
	+	127.33^{cY}	135.33^{b}	145.67^{aX}	1.62
	SEM^{1}	1.99	1.99	1.99	
MCV (fl.)	–	128.33	124.33	124.33	0.79
	+	126.33	127.33	123.67	0.79
	SEM^{1}	0.97	0.97	0.97	
MCH (pg.)	–	57.33^{a}	57.00^{a}	54.67^{b}	0.22

	+	56.67^{b}	57.67^{a}	55.00^{c}	0.22
	SEM^{1}	0.27	0.27	0.27	
	–	15.33^{bY}	9.67^{cY}	20.00^{a}	1.49
Neutrophils (%)	+	23.33^{X}	20.00^{X}	19.67	1.49
	SEM^{1}	1.83	1.83	1.83	
	–	84.67^{X}	90.33^{X}	80.00	1.49
Lymphocytes (%)	+	76.67^{Y}	80.00^{Y}	80.33	1.49
	SEM^{1}	1.83	1.83	1.83	

Note: $^{a\ b\ c}$ Means within rows for different groups with different superscripts differ (p < 0.05). XYZ Means within the column for different groups with different superscripts differ (p < 0.05). PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cells count; WBC: White Blood Cells count; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; SEM1: Standard Error of Means (Inclusion levels); SEM2: Standard Error of Means (Enzyme supplementation). Source: Authors, 2024.

3.2 Haematological indices (finisher)

The summary of the main effects of dietary inclusion of graded levels of DCA with or without microbial enzyme supplementation on some selected haematological indices of broiler finisher chickens fed the experimental diets is presented in (Table 5). All parameters measured in the study were significantly (p < 0.05) affected by the dietary treatment, except for WBC count, MCV, and MCH which were not influenced significantly (p > 0.05) by the treatment diets. Birds fed with a diet containing 20% DCA with exogenous enzyme fortification (D6) exhibited a significantly higher level of PCV over all other dietary groups, while a significant decrease was observed in the PCV of birds fed other DCA-based diets when compared with the control.

A significantly higher Hb concentration was also observed in birds fed diet 6, while birds fed diets 2, 3, 4, and 7 had a statistically similar Hb concentration with those fed the control diet; and the lowest Hb concentration was observed in birds fed diet 5. Additionally, birds fed diet 6 had the highest RBC count, while the lowest value for this parameter was observed in birds fed diets 2, and 3. A statistically similar RBC count was exhibited by bird fed diets 4, 5, 7, and the control diet.

Moreover, birds fed diet 5 had a significantly higher Neutrophils level, while those that were offered diets 2, 3, and 4 i.e. DCA-based diets without supplementation exhibited the lowest neutrophils level when compared with the control. However, birds fed un-supplemented DCA-based diets (2, 3, and 4) had a significantly higher lymphocyte level, while the lowest lymphocyte levels were observed in birds fed diet 5 and the control diet.

Table 5. Main effects of varying inclusion levels of DCA on the haematological indices of broiler finisher chickens (21-42 days).

Parameters	Diets							SEM	*p*-Value
	1	2	3	4	5	6	7		
PCV (%)	37.67^{ab}	32.67^{bc}	31.67^{c}	36.67^{abc}	33.00^{bc}	41.33^{a}	35.33^{bc}	0.75	0.002
Hb Concentration (g/L)	12.77^{ab}	12.53^{ab}	12.17^{ab}	12.63^{ab}	11.53^{b}	13.20^{a}	12.00^{ab}	0.18	0.272
RBC (10^{12}/L)	2.07^{ab}	1.86^{b}	1.90^{b}	2.20^{ab}	2.00^{ab}	2.53^{a}	2.20^{ab}	0.07	0.171
WBC (10^{9}/L)	106.33	121.00	112.00	120.33	99.67	120.67	117.00	3.39	0.554
MCV (fl.)	178.33	180.00	171.67	167.33	163.67	153.67	162.33	3.41	0.393
MCH (pg.)	66.67	71.00	66.67	57.67	57.33	56.00	55.67	1.98	0.179
Neutrophils (%)	34.67^{ab}	19.00^{c}	20.67^{c}	21.33^{c}	37.67^{a}	29.67^{abc}	26.00^{ab}	1.63	0.003
Lymphocytes (%)	65.33^{b}	81.00^{a}	79.33^{a}	78.67^{a}	65.33^{b}	70.33^{ab}	74.00^{ab}	1.54	0.007

Note: $^{a\ b\ c}$ Means within rows for different groups with different superscripts differ (p < 0.05). PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cells count; WBC: White Blood Cells count; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; SEM: Standard Error of Means; p-Value:

Probability value. Diets: 1 = Control; 2 = 10% DCA – Enzymes; 3 = 20% DCA – Enzymes; 4 = 30% DCA – Enzymes; 5 = 10% DCA + Enzymes; 6 = 20% DCA + Enzymes; 7 = 30% DCA + Enzymes. Source: Authors, 2024.

The interaction effects of dietary inclusion of graded levels of DCA and exogenous fortification on some selected haematological indices of broiler chickens are shown in (Table 6). Significant interaction effects were observed in all haematological parameters of interest measured in the current study. PCV was significantly higher in birds fed a diet containing 20% DCA fortified with exogenous enzymes compared to those fed the diet with the same inclusion level without enzyme fortification, while no interaction effect was observed at the dietary inclusion levels of 10, and 30% DCA with or without exogenous enzyme fortification. Interestingly, this same trend was observed in the RBC count of birds fed the experimental diets.

Moreover, the Hb concentration recorded in birds fed 10, and 30% DCA without enzyme supplementation was significantly higher than those recorded in bird-fed supplemented diets with the same inclusion levels, however, the Hb concentration was significantly higher in birds fed diet containing 20% DCA with exogenous enzyme supplementation compared with those fed diet with 20% DCA without exogenous supplementation. Additionally, a significant interaction effect was also observed in the WBC as birds fed 10% DCA with enzyme had a significantly higher WBC count over their counterpart fed the same diet without enzyme supplementation. Interestingly, the reverse was the case in birds fed a diet containing 20% DCA, as those fed the supplemented diet had a significantly higher WBC count compared to those fed the un-supplemented diet. However, there was no observable interaction effect in those fed diets containing 30% DCA. In addition, the MCV, and MCH levels in birds fed 10, and 20% DCA-based diet without supplementation were significantly higher than those fed diets with exogenous enzyme supplementation at the same inclusion levels.

Furthermore, neutrophil levels were significantly higher in birds fed DCA-based diets supplemented with exogenous microbial enzymes across all the inclusion levels compared to those fed diets without exogenous enzyme supplementation. However, an inverse relationship was observed between lymphocyte level and neutrophil level as the lymphocyte levels in bird fed diets without enzyme supplementation were significantly higher than when compared with those fed DCA-based diets with exogenous microbial enzyme supplementation.

Table 6. Interaction effects of dietary inclusion of DCA and enzyme supplementation of the haematological indices of broiler finisher chickens (21-42 days).

Parameters	Enzyme supplementation	Inclusion levels			SEM^2
		10%	20%	30%	
PCV (%)	–	32.67^{b}	31.67^{bY}	36.67^{a}	0.95
	+	33.00^{b}	41.33^{aX}	35.33^{b}	0.95
	SEM^1	1.16	1.16	1.16	
Hb concentration (g/l)	–	12.53^{X}	12.17^{Y}	12.63^{X}	0.18
	+	11.53^{bY}	13.20^{aX}	12.00^{bY}	0.18
	SEM^1	0.22	0.22	0.22	
RBC (10^{12}/L)	–	1.86^{b}	1.90^{bY}	2.20^{a}	0.08
	+	2.00^{b}	2.53^{aX}	2.20^{b}	0.08
	SEM^1	0.10	0.10	0.10	
WBC (10^{9}/L)	–	121.00^{aX}	112.00^{bY}	120.33^{a}	1.96
	+	99.67^{bY}	120.67^{aX}	117.00^{a}	1.96
	SEM^1	2.40	2.40	2.40	
MCV (fl.)	–	180.00^{X}	171.67^{X}	167.33	4.31
	+	163.67^{Y}	153.67^{Y}	162.33	4.31
	SEM^1	5.28	5.28	5.28	

MCH (pg.)	–	71.00^{X}	66.67^{X}	57.67	2.69
	+	53.33^{Y}	56.00^{Y}	55.67	2.69
	SEM^{1}	3.29	3.29	3.29	
Neutrophils (%)	–	19.00^{cY}	20.67^{cY}	21.33^{cY}	1.88
	+	37.67^{aX}	29.67^{bX}	26.00^{bX}	1.88
	SEM^{1}	2.30	2.30	2.30	
Lymphocytes (%)	–	81.00^{X}	79.33^{X}	78.67^{X}	1.78
	+	65.33^{Y}	70.33^{Y}	74.00^{Y}	1.78
	SEM^{1}	2.18	2.18	2.18	

Note: $^{a\ b\ c}$ Means within rows for different groups with different superscripts differ (p < 0.05). XYZ Means within the column for different groups with different superscripts differ (p < 0.05). PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cells count; WBC: White Blood Cells count; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; SEM1: Standard Error of Means (Inclusion levels); SEM2: Standard Error of Means (Enzyme supplementation). Source: Authors, 2024.

3.3 Serum biochemistry (Starter phase)

The serum biochemical indices of starter broiler chicks fed diets with different proportions of DCA, alongside varying levels of exogenous enzyme supplementation, were meticulously evaluated and are delineated in (Table 7). The findings of this study indicated that nearly all measured parameters were significantly (p < 0.05) influenced by the various dietary regimens, while Creatinine remained unaffected. Notably, AST level was significantly elevated in birds fed diet D6, while the lowest AST values were recorded in diets D2 and D3. In contrast, ALT was substantially higher in birds fed the control diet (D1), with the lowest value recorded in birds consuming diet D2. ALP level was more pronounced in diet D2 compared to the other diets in the study.

Total protein (TP) levels demonstrated a noteworthy increase in birds fed diets D2 and D6 compared to others. Additionally, the highest albumin levels were witnessed in birds fed diets D4, D6, and D7, while the lowest levels were observed in birds fed diet D3. Meanwhile, birds fed diet D2 had the highest levels of globulin, while diets D4 and D5 were associated with the lowest globulin levels. Furthermore, urea level was significantly higher in birds fed diet D4 compared to the control diet.

Subsequently, the interaction effects of dietary inclusion of graded levels of DCA and exogenous enzyme supplementation on these serum biochemical indices were also evident, as displayed in (Table 8). Enzyme supplementation triggered notable increases in AST levels in birds fed with diets containing 10% and 20% DCA. Moreover, a quadratic response was observed in TP levels with enzyme supplementation, showcasing significantly higher TP levels in birds fed with a diet containing 10% DCA with enzyme supplementation. This relationship reversed in diets containing 20% DCA, with no significant interaction noted in birds-fed diets containing 30% DCA. Enzyme supplementation also notably affected albumin, globulin, urea, and creatinine levels in birds consuming diets with varied DCA levels.

Table 7. Main effects of varying inclusion levels of DCA on the serum biochemical indices of broiler chicks (0-21 days).

Parameters	Diets							SEM	*p*-Value
	1	2	3	4	5	6	7		
AST (u/L)	110.67^{bc}	106.6^{c}	107.0^{c}	114.67^{b}	116.67^{ab}	123.33^{a}	116.67^{ab}	1.87	0.000
ALT (u/L)	7.00^{a}	4.00^{c}	5.00^{bc}	6.00^{ab}	5.67^{abcx}	6.00^{ab}	6.67^{ab}	0.26	0.022
ALP (u/L)	221.67^{b}	224.67^{a}	222.33^{b}	222.33^{b}	221.67^{b}	221.00^{b}	222.33^{b}	0.56	0.004
TP (g/L)	24.67^{ab}	25.33^{a}	23.00^{abc}	21.67^{bc}	21.33^{c}	25.67^{a}	22.67^{abc}	0.44	0.020
Albumin (g/L)	10.13^{ab}	10.27^{ab}	9.63^{b}	11.03^{a}	10.43^{ab}	11.07^{a}	10.83^{a}	0.91	0.047
Globulin (g/L)	14.53^{ab}	15.07^{a}	13.37^{abc}	10.63^{ab}	10.90^{c}	14.60^{ab}	11.83^{bc}	0.43	0.005

Urea (mg/dL)	3.03^{b}	2.47^{b}	2.83^{b}	4.47^{a}	2.80^{b}	3.23^{b}	2.30^{b}	0.17	0.006
Creatinine (mg/dL)	27.00	22.33	27.00	30.67	26.00	33.67	19.33	1.80	0.425

Note: $^{a\ b\ c}$ Means within rows for different groups with different superscripts differ (p < 0.05). AST: Aspartate aminotransferase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; TP: Total protein; SEM: Standard Error of Means; p-Value: Probability value. Diets: 1 = Control; 2 = 10% DCA – Enzymes; 3 = 20% DCA – Enzymes; 4 = 30% DCA – Enzymes; 5 = 10% DCA + Enzymes; 6 = 20% DCA + Enzymes; 7 = 30% DCA + Enzymes. Source: Authors, 2024.

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Table 8. Interaction effects of dietary inclusion of DCA and enzyme supplementation of the serum biochemical indices of broiler starter chicks (0-21 days).

Parameters	Enzyme supplementation	Inclusion levels			SEM^{2}
		10%	20%	30%	
AST (u/L)	–	106.67^{bY}	107.00^{bY}	114.67^{b}	1.64
	+	116.67^{bX}	123.33^{aX}	116.67^{b}	1.64
	SEM^{1}	1.42	1.42	1.42	
ALT (u/L)	–	4.00^{c}	5.00^{b}	6.00^{a}	0.47
	+	5.67^{ab}	6.00^{a}	6.67^{a}	0.47
	SEM^{1}	0.40	0.40	0.40	
ALP (u/L)	–	224.67^{aX}	222.33^{b}	222.33^{b}	0.42
	+	221.67^{bY}	221.00^{b}	222.33^{b}	0.42
	SEM^{1}	0.36	0.36	0.36	
Total protein (g/L)	–	25.33^{aX}	23.00^{bY}	21.67^{b}	0.88
	+	21.33^{bY}	25.67^{aX}	22.67^{b}	0.88
	SEM^{1}	0.76	0.76	0.76	
Albumin (g/L)	–	10.27^{a}	9.63^{bY}	11.03^{a}	0.29
	+	10.43^{a}	11.07^{aX}	10.83^{a}	0.29
	SEM^{1}	0.25	0.25	0.25	
Globulin (g/L)	–	15.07^{aX}	13.37^{a}	10.63^{b}	0.79
	+	10.90^{bY}	14.60^{a}	11.83^{b}	0.79
	SEM^{1}	0.69	0.69	0.69	
Urea (mg/dL)	–	2.47^{b}	2.83^{ab}	4.47^{aX}	0.27
	+	2.80^{ab}	3.23^{b}	2.30^{bY}	0.27
	SEM^{1}	0.23	0.23	0.23	
Creatinine (mg/dL)	–	22.33	27.00	30.67^{X}	2.72
	+	26.00	33.67	19.33^{Y}	2.72
	SEM^{1}	2.36	2.36	2.36	

Note: $^{a\ b\ c}$ Means within rows for different groups with different superscripts differ (p < 0.05). XYZ Means within the column for different groups with different superscripts differ (p < 0.05). AST: Aspartate aminotransferase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; Standard Error of Means (Inclusion levels); SEM2: Standard Error of Means (Enzyme supplementation). Source: Authors, 2024.

3.4. Serum biochemistry (Finisher phase)

The comprehensive analysis of serum biochemical indices in broiler chickens subjected to diets containing varied proportions of DCA with and without exogenous enzyme supplementation is meticulously outlined in (Table 9). The investigation revealed significant impacts (p < 0.05) of the dietary treatments on all measured parameters, except for AST, which exhibited no significant variation (p > 0.05) in response to the dietary treatments.

The distinctive dietary effects were vividly demonstrated in the ALT values. Birds on diet D2 (10% DCA without enzyme) displayed the highest (p < 0.05) ALT value of 3.67 u/L, while the lowest value of 1.00 u/L was observed in birds fed the control diet D1 (0% DCA without enzyme). Notably, ALT values from diets D3, D4, D5, D6, and D7 were statistically comparable to the ALT value obtained from birds on the control diet, D1 (0% DCA without enzyme). ALP levels were significantly (p < 0.05) influenced by the dietary treatments, with values from birds on DCA-based diets, with or without enzyme supplementation, being notably higher than the ALP value from birds on the control diet, D1 (0% DCA without enzyme). TP and Globulin levels exhibited significant (p < 0.05) variations due to the dietary treatments. Birds on the control diet (D1, 0% DCA without enzyme) displayed the highest values at 45.57 and 31.17, respectively. However, there was a significant reduction in these parameters with the dietary inclusion of DCA, with or without enzyme supplementation.

Urea and Creatinine levels were also significantly (p < 0.05) influenced by the dietary treatments, showing a quadratic response across the dietary treatments. Diet D6 (20% DCA with enzyme) recorded the highest urea value (1.80mg/dl), while the lowest value (0.30 mg/dL) was observed in birds on diet D3 (20% DCA without enzyme). Creatinine values displayed a similar pattern, with the highest values recorded in birds on diets D2, D6, and D7, and the lowest values in birds on the control diet and diet D5, while intermediate values were observed in birds on diets D3 and D4.

Table 10. elucidates the impact of exogenous enzyme supplementation on the serum biochemical indices of finisher broiler chickens fed diets with varied levels of DCA. Notably, a significant (p < 0.05) increase in AST level was observed in birds fed a diet containing 20% DCA supplemented with enzymes compared to their counterparts on the same diet without enzyme supplementation. ALT was also significantly (p < 0.05) affected by enzyme supplementation, with birds on diets containing 10% and 20% DCA without enzyme supplementation exhibiting significantly higher levels of serum ALT compared to birds on the alternate diets with enzyme supplementation.

Moreover, TP and Globulin levels in birds fed 20% DCA without enzyme supplementation were significantly higher than those of their counterparts fed the same diet with enzyme supplementation. However, albumin levels were found to be significantly higher in birds fed a diet containing 20% DCA with enzyme compared to those on the same diet without enzyme supplementation. Exogenous enzyme supplementation exerted a significant (p < 0.05) effect on the urea level of birds on diets containing 20% DCA, resulting in higher values in birds on the diet supplemented with enzymes. However, enzyme supplementation did not affect the urea level of birds on diets containing 10% and 30% DCA, with or without exogenous enzyme supplementation.

Finally, the Creatinine level in birds fed the diet containing 10% DCA without enzyme supplementation (D2) was significantly (p < 0.05) higher than that of birds on the alternate diet with enzyme supplementation (D5). However, the reverse was observed in birds fed the diet containing 30% DCA, with significantly higher creatinine levels in birds on the diet with enzyme supplementation (D7) and lower levels in birds on diet D4. No significant (p > 0.05) effect was observed as a result of exogenous enzyme supplementation on the creatinine level of birds fed 20% DCA.

Table 9. Main effects of varying inclusion levels of DCA on the serum biochemical indices of broiler finisher chickens (21-42 days).

Parameters	Diets*							SEM	*p*-Value
	D1	D2	D3	D4	D5	D6	D7		
AST (u/L)	57.67	69.00	56.67	79.00	62.67	87.33	69.33	3.98	0.015
ALT (u/L)	1.00^{b}	3.67^{a}	2.33^{b}	2.00^{b}	1.67^{b}	1.47^{b}	1.67^{b}	0.16	0.014
ALP (u/L)	246.67^{c}	253.33^{ab}	248.00^{bc}	255.33^{a}	254.67^{a}	255.33^{a}	253.67^{a}	0.70	0.006

Total Protein (g/L)	45.57^{a}	36.93^{c}	41.03^{b}	33.00^{c}	37.17^{c}	36.27^{c}	35.47^{c}	0.50	0.000
Albumin (g/L)	14.40^{ab}	13.93^{abc}	13.97^{abc}	11.20^{c}	13.00^{bc}	16.07^{a}	13.13^{bc}	0.34	0.024
Globulin (g/L)	31.17^{a}	23.00^{bc}	27.07^{ab}	21.80^{c}	24.17^{bc}	20.20^{c}	22.33^{bc}	0.61	0.001
Urea (mg/dL)	0.60^{b}	0.97^{ab}	0.30^{b}	0.40^{b}	0.73^{ab}	1.80^{a}	0.73^{ab}	0.13	0.007
Creatinine (mg/dL)	15.47^{b}	34.67^{a}	25.00^{ab}	23.67^{ab}	15.17^{b}	29.67^{a}	30.33^{a}	1.45	0.005

Note: $^{a\,b\,c}$ Means within rows for different groups with different superscripts differ (p < 0.05). AST: Aspartate aminotransferase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; SEM: Standard Error of Means; p-Value: Probability value. Diets: 1 = Control; 2 = 10% DCA – Enzymes; 3 = 20% DCA – Enzymes; 4 = 30% DCA – Enzymes; 5 = 10% DCA + Enzymes; 6 = 20% DCA + Enzymes; 7 = 30% DCA + Enzymes. Source: Authors, 2024.

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Table 10. Interaction effects of dietary inclusion of DCA and enzyme supplementation of the serum biochemical indices of broiler finisher chickens (21-42 days).

Parameters	Enzyme supplementation	Inclusion levels			SEM^{2}
		10%	20%	30%	
AST (u/L)	–	69.00	56.67^{Y}	79.00	6.21
	+	62.67	87.33^{X}	69.33	6.21
	SEM^{1}	7.61	7.61	7.61	
ALT (u/L)	–	3.67^{aX}	2.33^{bX}	2.00^{b}	0.26
	+	1.67^{Y}	1.47^{Y}	1.67	0.26
	SEM^{1}	0.32	0.32	0.32	
ALP (u/L)	–	253.33^{b}	248.00^{cY}	255.33^{a}	0.65
	+	254.67	255.33^{X}	253.67	0.65
	SEM^{1}	0.80	0.80	0.80	
Total protein (g/L)	–	36.93^{b}	41.03^{aX}	33.00^{cY}	0.69
	+	37.17	36.27^{Y}	35.47^{X}	0.69
	SEM^{1}	0.85	0.85	0.85	
Albumin (g/L)	–	13.93^{a}	13.97^{aY}	11.20^{bY}	0.52
	+	13.00^{b}	16.07^{aX}	13.13^{bX}	0.52
	SEM^{1}	0.63	0.63	0.63	
Globulin (g/L)	–	23.00^{b}	27.07^{aX}	21.80^{b}	0.92
	+	24.17	20.20^{Y}	22.33	0.92
	SEM^{1}	1.13	1.13	1.13	
Urea (mg/dL)	–	0.97	0.30^{Y}	0.40	0.22
	+	0.73^{b}	1.80^{aX}	0.73^{b}	0.22
	SEM^{1}	0.26	0.26	0.26	
Creatinine (mg/dL)	–	34.67^{aX}	25.00^{b}	23.67^{bY}	2.07

+	15.17^{bY}	29.67^{a}	30.33^{aX}	2.07
SEM[1]	2.54	2.54	2.54	

Note: $^{a\ b\ c}$ Means within rows for different groups with different superscripts differ ($p < 0.05$). XYZ Means within the column for different groups with different superscripts differ ($p < 0.05$). AST: Aspartate transaminase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase. SEM1: Standard error of Means (Inclusion levels); SEM2: Standard error of Means (Enzyme supplementation). Source: Authors, 2024.

3.5 Liver histopathological analysis

The photomicrographs showing the liver histological analysis of broiler chickens fed diets containing different proportions of DCA as a replacement for maize were shown in Plates 1–7 below.

As shown on Plate 1A, there is no observable lesion on the liver of broiler chickens fed the control diet D1 (0% DCA without enzyme) however, multifocal hepatocellular necrosis and inflammation were observable on Plate 1B; Mild to moderate atrophy was observed on the liver of birds fed diet D2 (10% DCA without enzyme) as shown in Plate 2 A and B; birds fed diet D3 (20% DCA without enzyme) had multifocal hepatocellular necrosis, inflammation, and moderate congestion of the central venule as observed on Plate 3 A and B respectively; however, there was no observable lesion on the liver of birds fed diet D4 (30% DCA without enzyme) as shown in Plate 4 A and B.

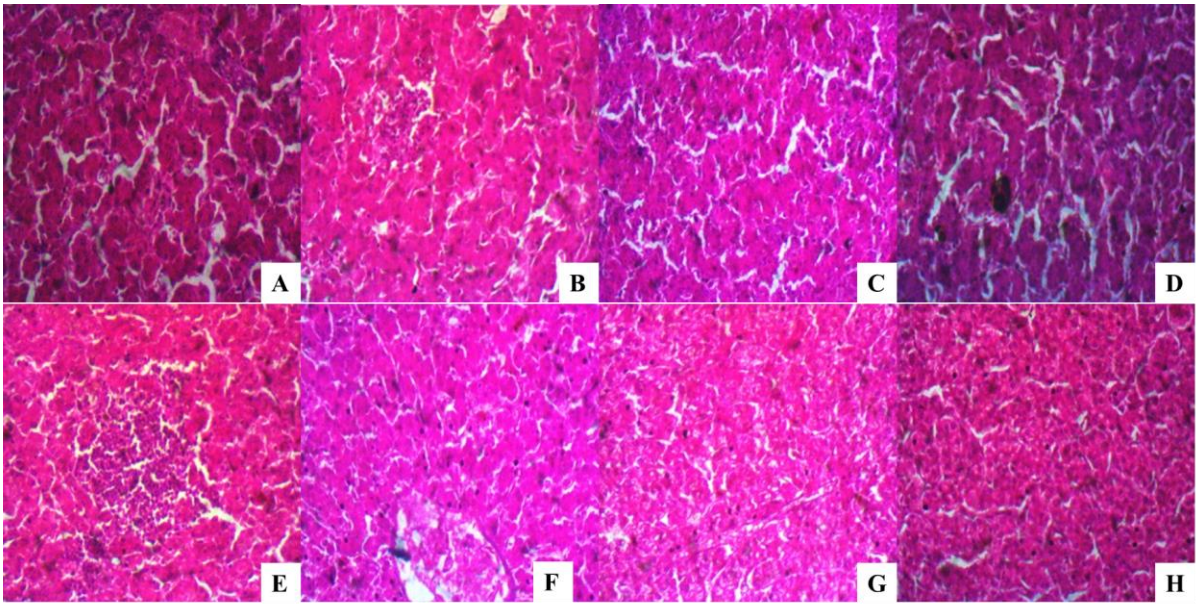

Figure 1. Diet 1. Plate 1. Photomicrographs of the liver of broiler chickens fed the control diet (D1 containing 0% DCA without enzyme supplementation). (A) Liver - There is no observable lesion. HE x400. (B) Liver- There is multifocal hepatocellular necrosis and inflammation. HE x400. Diet 2. Plate 2. Photomicrographs of the liver of broiler chickens fed diet D2 Containing 10% DCA without enzyme supplementation. (C) Liver - There is mild atrophy of hepatic plates (arrow). HE x400. (D) Liver - There is moderate hepatocellular atrophy (arrow). HE x400. Diet 3. Plate 3. Photomicrographs of the liver of broiler chickens fed diet D3 containing 20% DCA without enzyme supplementation. (E) Liver - There is multifocal hepatocellular necrosis and inflammation (arrow). HE x400. (F) Liver - There is moderate congestion of the central venule (arrow). HE x400. Diet 4. Plate 4. Photomicrographs of the liver of broiler chickens fed diet D4 containing 30% DCA without enzyme supplementation. (G) Liver - There is no observable lesion. HE x400. (H) Liver - There is no observable lesion. HE x400. Source: Authors, 2024.

Bird on diet D5 (10% DCA with enzyme) also had multifocal hepatocellular necrosis and inflammation, and severe congestion of the central venule was also observed as seen in Plate 5 A and B; focal hepatocellular coagulation necrosis and atrophy of the hepatic plate was observed on the liver of birds fed diet D6 (20% DCA with enzyme) as seen in Plate 6A and B. Finally, there is severe congestion, random hepatocellular necrosis, and inflammation in the liver of birds fed diet D7 (30% DCA with enzyme) as shown in Plate 7 A and B below.

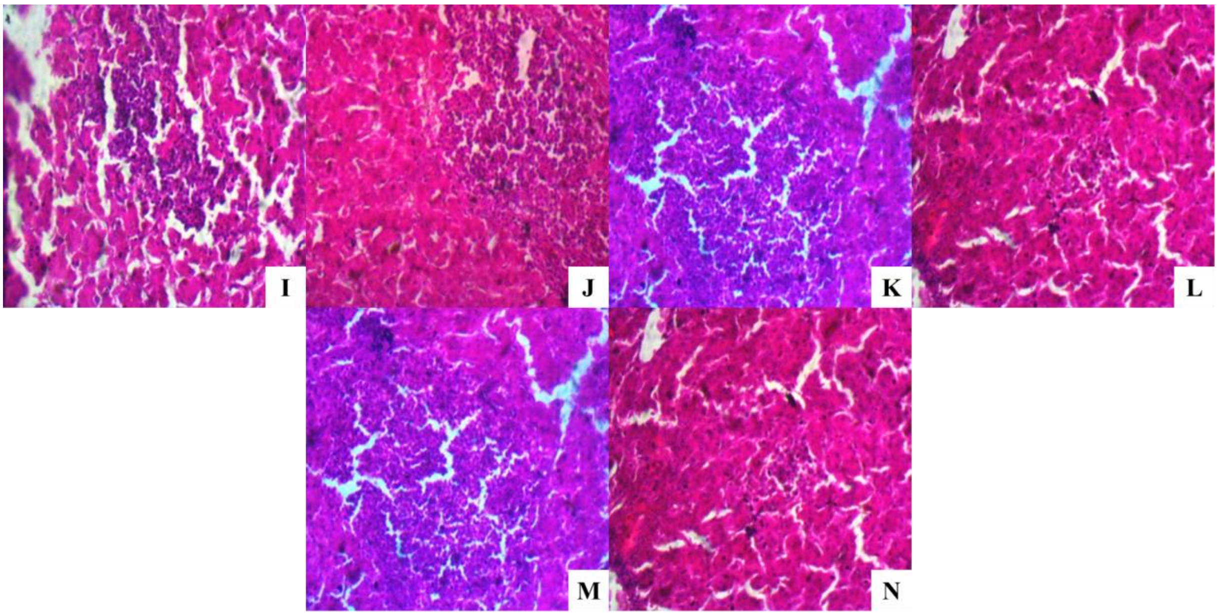

Figure 2. Diet 5. Plate 5. Photomicrographs of the liver of broiler chickens fed diet D5 containing 10% DCA with enzyme supplementation. (I) Liver - There is multifocal hepatocellular necrosis and inflammation (arrow). HE x400. (J) Liver - There is severe congestion of the central venule. HE x400, and Diet 6. Plate 6. Photomicrographs of the liver of broiler chickens fed diet D6 containing 20% DCA with enzyme supplementation. (K) Liver - There is focal hepatocellular coagulation necrosis (arrow). HE x400. (L) Liver - There is atrophy of hepatic plates (arrows) and Kupffer cell hyperplasia. HE x400. Diet 7. Plate 7. Photomicrographs of the liver of broiler chickens fed diet D7 containing 30% DCA with enzyme supplementation. (M) Liver - There is severe congestion (arrow). HE x400. (N) Liver - There is random hepatocellular necrosis and inflammation (arrow). HE x400Source: Authors, 2024.

4. Discussions

The current study evaluated the effects of dietary inclusion of DCA with or without exogenous enzyme supplementation on the blood profile of broiler chickens at both the starter and finisher phases. Our findings indicated significant changes in various haematological and serum biochemical parameters, which have important implications for broiler health and performance.

4.1 Haematological indices at the starter phase

The inclusion of DCA in the diets significantly influenced all measured haematological parameters except for MCV, which remained unaffected. This discovery contradicts the previously drawn conclusions by Yisa et al. (2018) who reported that dietary inclusion of cashew apple had no significant impact on the haematological indices of broiler chickens. In the current study, the PCV and Hb concentrations were notably lower in chicks fed DCA-based diets, particularly at the 20% inclusion level without enzyme supplementation. This reduction in PCV and Hb could indicate potential anaemia, likely due to anti-nutritional factors in the DCA (Dakuyo et al., 2023) that may interfere with nutrient absorption or metabolism (Obidah et al., 2020). Interestingly, RBC counts also followed this trend, being lower in DCA-fed groups compared to the control, with chicks on 30% DCA diets showing higher RBC counts when supplemented with enzymes. This suggests that enzyme supplementation may mitigate some negative effects of DCA on erythropoiesis, enhancing nutrient utilization and absorption (Beigh et al., 2018).

White blood cell (WBC) counts were also influenced, with chicks fed 30% DCA with enzyme supplementation showing higher counts compared to other DCA groups. Elevated WBC counts can be indicative of an enhanced immune response, potentially due to the immune-modulating effects of exogenous microbial enzymes (Liang et al., 2022). The observed increase in neutrophils and decrease in lymphocytes in enzyme-supplemented groups at 10% and 20% DCA levels suggest an acute inflammatory response, which could be a reaction to the dietary components or a stress response (Hannoodee and Nasuruddin, 2024).

4.2 Haematological indices at the finisher phase

In the finisher phase, the study revealed that PCV and Hb levels were significantly higher in birds fed 20% DCA with enzyme supplementation. This contrasts with the starter phase results, suggesting that older birds might better adapt to DCA diets, especially with enzyme supplementation enhancing nutrient availability and countering anti-nutritional factors (Ugwuanyi et al., 2016). The RBC count exhibited similar trends, reinforcing the notion that enzyme supplementation supports erythropoiesis under DCA inclusion.

However, WBC counts were not significantly affected by DCA inclusion at this phase, suggesting a stabilization in immune response as the birds aged and possibly adapted to the diet. This could be due to improved gut health and nutrient absorption facilitated by bioactive compounds in DCA and exogenous enzyme supplementation, thus, reducing the inflammatory stress observed in younger birds (Sugiharto, 2016). Additionally, the significant increase in neutrophils and decrease in lymphocytes in enzyme-supplemented groups across all inclusion levels implies a shift in immune cell dynamics, potentially indicative of enhanced resistance to infections (Malech et al., 2014; Orakpoghenor et al., 2019).

Moreover, findings from this study align with previous research indicating that dietary anti-nutritional factors can adversely affect haematological parameters (Onabanjo et al., 2021). For instance, Tijani et al. (2015) reported reduced PCV and Hb levels in broilers fed diets containing high tannin levels, which are known anti-nutritional components of cashew apples. Enzyme supplementation has been shown to improve nutrient digestibility and mitigate the adverse effects of anti-nutritional factors (Alagawany et al., 2017). This study corroborates these findings by demonstrating improved haematological outcomes with enzyme inclusion.

4.3 Serum biochemistry at the starter phase

In the starter phase, almost all measured parameters were significantly influenced by the dietary treatments, except for creatinine levels, which remained unaffected. AST level was significantly elevated in birds fed diet D6 (20% DCA with enzyme), while the lowest AST levels were observed in birds fed diets D2 (10% DCA without enzyme) and D3 (20% DCA without enzyme). The elevated AST level in diet D6 may indicate hepatic stress or damage, possibly due to the high inclusion level of DCA, which might contain anti-nutritional factors such as tannins that can exert hepatotoxic effects (Bacou, et al., 2021). ALT levels were higher in birds on the control diet, suggesting that the absence of DCA might lead to lower hepatic stress compared to DCA-based diets, this gives credence to Oyewole et al. (2017) who also reported significantly lower ALT in broiler chickens fed cashew apples-based diet.

Alkaline phosphatase (ALP) levels were most pronounced in diet D2, indicating possible bone growth or turnover linked to enzyme activity in low DCA diets (Vimalraj, 2020). TP levels also increased notably in birds fed diets D2 and D6, which might be due to enhanced protein synthesis and nutrient absorption facilitated by mild dietary inclusion levels of DCA (Adegoke et al., 2018). The highest albumin levels in birds fed diets D4, D6, and D7 are indicative of improved nutritional status and efficient protein metabolism as a result of dietary inclusion of DCA (Adriani et al., 2021). Additionally, globulin level was highest in birds fed diet D2, suggesting improved immune protein synthesis at lower DCA levels without enzyme supplementation (Adegoke et al., 2018). The significant increase in urea levels in birds fed diet D4 indicates higher protein catabolism, possibly due to the metabolic demands of digesting higher DCA content (Marín-García et al., 2022).

4.4 Serum biochemistry at the finisher phase

In the finisher phase, the dietary treatments significantly influenced all parameters except AST. The highest ALT value was observed in birds on diet D2, while the control diet exhibited the lowest. Elevated ALT levels in DCA-fed birds suggest potential liver strain due to metabolic adaptation to the anti-nutritional components of DCA (Tijani et al., 2015). ALP levels were significantly higher in all DCA-based diets, indicating enhanced bone turnover or growth, possibly due to enzyme-mediated nutrient release from DCA (de Souza Nakagi et al., 2013).

Furthermore, Total protein and globulin levels were highest in birds on the control diet, with significant reductions observed with DCA inclusion. This reduction could be due to the anti-nutritional effects of DCA inhibiting protein absorption or synthesis, despite enzyme supplementation (Ramteke et al., 2019). However, enzyme supplementation increased albumin levels in birds fed 20% DCA, suggesting improved hepatic protein synthesis (Alagawany et al., 2019).

Urea and creatinine levels showed a quadratic response to dietary treatments, with the highest urea levels in birds on diet D6. This indicates enhanced protein metabolism and excretion with enzyme supplementation at moderate DCA levels (Qaid; Al-Garadi, 2021). The highest creatinine values in birds on diets D2, D6, and D7 reflect increased muscle turnover or renal function stress due to higher metabolic demands (Majdeddin et al., 2023).

4.5 Liver histopathological analysis

The liver histological analysis of broiler chickens fed diets with varying proportions of DCA as a maize replacement revealed significant pathological changes, which varied depending on the inclusion level of DCA and the presence of exogenous enzymes. The control group (0% DCA without enzyme) exhibited no observable liver lesions, indicating that the standard maize-based diet maintains hepatic integrity (Plate 1A). Conversely, the introduction of DCA at 10% (D2) led to mild to moderate hepatic atrophy (Plate 2A and B). This atrophy might result from the presence of anti-nutritional factors in DCA, such as tannins and oxalates, which can impair nutrient absorption and liver function (Çalışlar, 2018).

Moreover, at 20% DCA (D3), the liver showed multifocal hepatocellular necrosis, inflammation, and moderate central venule congestion (Plate 3A and B). These lesions are indicative of severe hepatocellular damage and compromised hepatic blood flow, likely due to the higher concentration of anti-nutritional factors, which exacerbate oxidative stress and liver damage (Kang et al., 2023). However, at 30% DCA (D4), no observable lesions were noted (Plate 4A and B). This unexpected finding may suggest a possible adaptive response or a threshold effect where the liver adapts to higher levels of DCA, potentially through upregulation of detoxifying enzymes (Squirewell et al., 2020).

The addition of exogenous enzymes to DCA diets resulted in varied hepatic responses. At 10% DCA with enzymes (D5), multifocal hepatocellular necrosis, inflammation, and severe congestion of the central venule were observed (Plate 5A and B). This severe pathology might be attributed to the interaction between DCA constituents and enzyme action, potentially leading to the formation of toxic metabolites (Mega et al., 2021). Additionally, for birds fed 20% DCA with enzymes (D6), focal hepatocellular coagulation necrosis and atrophy of the hepatic plate were evident (Plate 6A and B).

The coagulative necrosis observed could be due to exacerbated oxidative stress or the release of reactive oxygen species during the enzyme-mediated breakdown of DCA components (Miller; Zachary, 2017). At 30% DCA with enzymes (D7), the liver exhibited severe congestion, random hepatocellular necrosis, and inflammation (Plate 7A and B). This severe pathology underscores the potential hepatotoxic effects of high DCA levels, even with enzyme supplementation. The enzyme's role in breaking down cell wall constituents might release bound anti-nutrients, leading to increased hepatic stress (Jamkhandea et al., 2013).

5. Conclusions

Based on the findings recorded in this study, it is therefore concluded that dietary inclusion of DCA resulted in lower PCV, Hb concentration, RBC, and WBC count in broiler chickens. However, it is important to note that though lower values were recorded for these parameters, the recorded values fall within the reference range reported for healthy domestic chickens.

Moreover, serum biochemical indices were also significantly influenced by the treatment diet, however, from the values recorded in this study dietary inclusion of DCA did not trigger an adverse response on the serum biochemical indices of broiler chickens. Additionally, the liver histopathology of birds fed DCA-based exhibited different levels of necrosis, inflammation, atrophy, and congestion. These abnormalities worsened as the inclusion levels of DCA in the diet increased. Hence, further research is required to optimize DCA inclusion levels and enzyme supplementation strategies to mitigate these adverse effects while harnessing the nutritional benefits of DCA.

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7. Authors' Contributions

Oluwatosin Solomon Oyekola: conceptualized and designed the study, performed primary data collection and analysis, and drafted the initial manuscript. *Favour Oluwasetemi Oyekanmi*: assisted with data collection and manuscript writing. *Olayemi Christianah Olagoke*: contributed to study design, data analysis, interpretation, and manuscript writing. *Taiwo Kayode Ojediran*: provided critical manuscript revisions. *Isiaka Adewale Emiola*: provided expertise in experimental methods, supervised the project, and reviewed the manuscript for intellectual content.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Yes, applicable. The study was approved by LAUTECH Animal Care and Use Committee (LAUACUC), this is the reference number for the ethical approval LAU/ANB/P/2000403.

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