Trop J Nat Prod Res, October 2025; 9(10): 5237 - 5244 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Performance And Blood Indices Of Broilers Fed Diets Containing Fermented *Citrus Sinensis* (Orange) Pulp Supplemented With Or Without Multi-Enzyme

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ARTICLE INFO

Article history:
Received 19 June 2025
Revised 08 August 2025
Accepted 11 August 2025
Published online 01 November 2025

ABSTRACT

Citrus sinensis (Orange) pulp is rich in energy and could serve as a feed ingredient for poultry. This study investigated the performance and blood indices of broilers fed diets containing fermented orange pulp (FOP) supplemented with or without multi-enzyme. Two hundred birds were utilized in a six-week daily feeding trial. The broilers were split up into five groups of four replicates having ten birds in a completely randomized design. The five groups were fed: (Control) diet without FOP meal or multi-enzyme (Natuzyme®); the FOP5 diet containing 5% FOP without multi-enzyme; the FOP10 diet containing 10% FOP without multi-enzyme; the FEOP5 diet containing 5% FOP with multi-enzyme; the FEOP10 diet containing 10% FOP with multi-enzyme. Performance and blood parameters were assessed. Blood was withdrawn via the brachial veins of the broilers on day 42. The FOP meal had higher ($p<0.05$) fibre content than maize meal. The FOP5 and FOP10 groups exhibited poor ($p<0.05$) average daily feed intake, average daily weight gain, final live weight, and feed conversion ratio compared to the control, FEOP5, and FEOP10 groups. Birds fed FOP5 and FOP10 diets had lower ($p<0.05$) packed cell volume (PCV) and mean corpuscular volume (MCV) compared to the control, FEOP5, and FEOP10 groups. Total serum protein, globulin, and glucose levels reduced ($p<0.05$) in FOP5 and FOP10 groups compared to the control group, while broilers on FEOP5 and FEOP10 diets showed improvements. This study indicates that multi-enzyme-supplemented FOP meal can be utilized in animal feed, as it enhances poultry performance and blood indices.

Keywords: Blood parameters, Broilers, Citrus pulp, Enzyme, Fermentation.

Introduction

The continuous increase in the global population has led to a corresponding rise in the demand for animal protein. This trend is projected to intensify future food insecurity if the supply of animal protein sources is not significantly improved.[1] Broiler production has emerged as one of the most practical means of meeting global animal protein needs.[2] However, a major setback hindering the utilization of broiler production in meeting global animal protein requirements is the issue of high feed costs.[3] The increasing cost of maize, a primary feed ingredient, can be partly attributed to the food-feed competition for available cereal grains among humans, animals, and industries.[4] This high cost has compelled many poultry farmers to seek cheaper and more readily available alternatives to conventional energy feedstuffs in broiler feed formulation.[3] Over the past few decades, numerous research efforts have focused on replacing maize with agro-industrial by-products.[5,6] One agro-by-product that has gained significant research interest as a potential energy feed ingredient is *Citrus sinensis* (orange) pulp.

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Citation: Ahiwe EU, Iwuji TC, Egenuka FC, Okehie UN, Ejifor I, Peter-Onoh CA, Ogbuewu IP, Emenalom OO. Performance And Blood Indices Of Broilers Fed Diets Containing Fermented *Citrus Sinensis* (Orange) Pulp Supplemented With Or Without Multi-Enzyme Trop J Nat Prod Res. 2025; 9(10): 5237 – 5244 https://doi.org/10.26538/tjnpr/v9i10.71

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Citrus sinensis (orange) pulp, the part of the fruit left after the exocarp is peeled off and the fruit juice is removed, is available throughout the year.[7] It belongs to the family *Rutaceae*, and is found in most tropical and sub-tropical regions/countries. *Citrus sinensis* (orange) pulp is composed of juice fibre-structured sacs or vesicles that are elongated and tabular in shape, and is divided into segments or wedges. [7] Because of its abundance, it is sometimes discarded, and this can lead to blockages, environmental hazards, and health risks when it decomposes.[8] Given its status as a potential environmental hazard, it becomes imperative to explore ways to convert orange pulp waste into useful products. The National Research Council (NRC) [9] states that *Citrus sinensis* (orange) pulp contains a high energy level comparable to maize, supporting its potential as an alternative energy source in broiler feed. Nevertheless, the utilization of orange pulp in poultry nutrition is constrained by the presence of soluble non-starch polysaccharide fibre and pectins, which can decrease its overall feeding value by increasing digesta viscosity, thereby negatively affecting nutrient digestion and absorption into the bloodstream. [5,10]

Nutritional trials examining the effects of dried orange pulp on broiler performance have yielded contradictory results. For example, a 2% inclusion of dried orange pulp improved broiler performance indices.[8] In contrast, Mourão et al. [10] observed that 5-10% sun-dried orange pulp compromised performance during both the starter and finisher phases. Similarly, inclusion levels of 7.5-10% dried *Citrus sinensis* pulp have been reported to depress body weight gain (BWG), feed conversion ratio (FCR), and certain blood parameters.[11,12] Likewise, Nisar [13] also concluded that sun-dried orange pulp included at 10% replacement of maize resulted in poor FCR and BWG of broilers. From the aforementioned studies, it can be inferred that broiler performance tends to decline as the level of orange pulp in their diet increases. Furthermore, several researchers observed that poor performance in

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

broilers fed sundried orange pulp could be ascribed to high soluble dietary fiber content. [10,11,12,13]
On a more positive note, the negative effects of high dietary fiber in broiler diets have been reported to be mitigated through employing appropriate processing methods. [5,14] The efficacy of fermentation in improving the nutritional composition and feeding value of high-fiber agro-by-products has been well documented.[5,14] Additionally, the use of exogenous enzymes (Roxazyme G®, Avizyme®) to enhance the nutritional value of high-fiber broiler diets is also thoroughly supported in the literature.[15,16,17] Based on the statement above, fermentation and/or multi-enzyme (Polyzyme®, Roxazyme G®) supplementation can be used to degrade fiber, and improve the nutrient content of orange pulp-based diet, whose effect can be observed and explained by the response of various performances and blood parameters of broilers. However, after an extensive literature review, it was observed that the effects of fermentation and multi-enzyme supplementation in degrading the high fiber content of *Citrus sinensis* (orange) pulp when included at 5% and 10% in broiler diets have not been tested on the performance and blood indices of broiler chickens, hence this study.

Materials and methods

Test material and feed ingredient
The sweet orange pulps were collected on 15th to 18th December, 2024, from citrus vendors in Owerri, Imo State, Nigeria, located between latitudes 05˚25' and 05˚32' N and longitudes 06˚57' and 07˚07'E with an average rainfall of 2190 mm.[18] The *Cirus sinensis* (orange) pulp (SM 42-2024) was identified by Mrs. Onoh (Plant Taxonomist) at the Department of Crop Science and Technology, School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri (FUTO). Multi-enzyme (Natuzyme®) containing α-amylase, protease, β-glucanase, cellulase, phytase, and xylanase was obtained from a reputable feed store in Owerri, Imo State. The multi-enzyme (Natuzyme®) was included in the broiler diet at 0.50 g/kg diet based on the manufacturer's (Bioproton Pty Ltd, Sunnybank, Australia) recommendation. Other feed ingredients utilized in this feeding trial were sourced from a reputable commercial feed mill situated in Owerri, Imo State, Nigeria.

Processing of the orange pulps
The orange pulps gathered were washed and put into synthetic sacks that were tied and kept for 48 hours to allow for fermentation process to occur before sun-drying.[5,14] After drying, the fermented dried orange pulp was ground to get the fermented orange pulp (FOP) meal that was used in this study.

Experimental diets and duration of the study
Five experimental starter and finisher broiler diets were formulated as follows: Diet1 contained maize-soybean diet (formulated according to ROSS-308 nutrient specification)[19] without orange pulp meal or multi-enzyme supplementation (control group); Diet 2 contained 5% fermented orange pulp without multi-enzyme supplementation (FOP5 group); Diet 3 contained 10% fermented orange pulp without multi-enzyme supplementation(FOP10 group); Diet 4 contained 5% fermented orange pulp with multi-enzyme supplementation(FEOP5 group); Diet 5 contained 10% fermented orange pulp with multi-enzyme supplementation (FEOP10 group). Diet compositions are detailed in Tables 1 and 2. The experimental diets were provided as mash during the initial and final growth stages. The birds had unrestricted access to feed and water. The feeding study was conducted over six weeks (42 days). In this experimental setup, the research groups were randomly assigned to one of five dietary treatments. The randomization process was strategically implemented to minimize potential bias by ensuring an even distribution of initial body weight and morphometric characteristics across different treatment and replicate groups.

Table 1: Composition of the starter experimental diets with determined nutrient compositions

Feed Ingredients (%)	CONTROL	FOP5	FOP10	FEOP5	FEOP10
Maize (9% CP)	56.00	51.00	46.00	51.00	46.00
Soybean meal (44 % CP)	32.00	32.00	32.00	32.00	32.00
Orange pulp meal	0.00	5.00	10.00	5.00	10.00
Palm kernel cake	4.50	4.50	4.50	4.45	4.45
Fish meal	3.00	3.00	3.00	3.00	3.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.00	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Vitamin/mineral premix[a]	0.25	0.25	0.25	0.25	0.25
Multi-enzyme	-	-	-	0.05	0.05
Total	100	100	100	100	100
Determined nutrient composition of the diets					
Crude protein	22.58	22.56	22.54	22.56	22.54
Crude fiber	4.90	5.02	5.08	5.02	5.08
Ether extract	3.39	3.25	3.21	3.25	3.21
Lysine	0.97	0.96	0.95	0.96	0.95
Methionine	0.45	0.42	0.41	0.42	0.41
Metabolizable energy (kcal/kg)	3012.00	2998.02	2984.00	2998.02	2984.00

Formulated according to Ross 308 nutrition specifications (Aviagen 2019).
[a]To provide the following per kilogram feed: vitamin A 12100 IU, vitamin D3 2500 IU, vitamin E 8 mg, vitamin K 2 mg, vitamin B1 3 mg, vitamin B2 5 mg, niacin 15 mg, pantothenic acid 6 mg, folic acid 4 mg, manganese 8 mg, zinc 0.05 mg, iron 29 mg, copper 3 mg, iodine 1.2 mg, selenium 0.16 mg and cobalt 2 mg. FOP5 = 5% fermented orange pulp without multi-enzyme supplementation; FOPM10 = 10% fermented orange pulp without multi-enzyme supplementation; FEOP5 = 5% fermented orange pulp with multi-enzyme supplementation; FEOP10 = 10% fermented orange pulp with multi-enzyme supplementation. CP = crude protein.

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Composition of the finisher experimental diets with determined nutrient compositions

Feed Ingredients (%)	CONTROL	FOP5	FOP10	FEOP5	FEOP10
Maize (9% CP)	60.00	55.00	50.00	55.00	50.00
Soybean meal (44 % CP)	28.00	28.00	28.00	28.00	28.00
Orange pulp meal	0.00	5.00	10.00	5.00	10.00
Palm kernel cake	5.00	5.00	5.00	4.95	4.95
Fish meal	3.00	3.00	3.00	3.00	3.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Oyster shell	2.00	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Vitamin/mineral premix[a]	0.25	0.25	0.25	0.25	0.25
Multi-enzyme	-	-	-	0.05	0.05
Total	100	100	100	100	100
Determined nutrient composition of the diets					
Crude protein	22.11	22.36	22.34	22.37	22.35
Crude fiber	4.17	5.13	5.19	5.13	5.19
Ether extract	3.5	3.42	3.39	3.42	3.39
Lysine	0.96	0.95	0.94	0.95	0.94
Methionine	0.42	0.41	0.40	0.41	0.40
Metabolizable energy (kcal/kg)	3208.10	3199.32	3187.90	3199.32	3187.90

Formulated according to Ross 308 nutrition specifications (Aviagen 2019).
[a]To provide the following per kilogram feed: vitamin A 12100 IU, vitamin D3 2500 IU, vitamin E 8 mg, vitamin K 2 mg, vitamin B1 3 mg, vitamin B2 5 mg, niacin 15 mg, pantothenic acid 6 mg, folic acid 4 mg, manganese 8 mg, zinc 0.05 mg, iron 29 mg, copper 3 mg, iodine 1.2 mg, selenium 0.16 mg and cobalt 2 mg. FOP5 = 5% fermented orange pulp without multi-enzyme supplementation; FOPM10 = 10% fermented orange pulp without multi-enzyme supplementation; FEOP5 = 5% fermented orange pulp with multi-enzyme supplementation; FEOP10 = 10% fermented orange pulp with multi-enzyme supplementation. CP = crude protein.

Experiment location

The research was conducted at the poultry unit of the School of Agriculture and Agricultural Technology (SAAT) Teaching and Research Farm, Federal University of Technology, Owerri (FUTO). The laboratory analysis was conducted at the Department of Animal Science and Technology, SAAT, Laboratory, FUTO.

Experimental birds and *design*

Two hundred (200) day-old Ross 308 broiler chicks of 42.0 ±0.12 g average weight sourced from Agrited Limited, Ibadan, Oyo State, Nigeria were used for the experiment. The birds were split into five experimental groups of 40 birds each, with each group having four replicates of ten birds each. Each group was allotted to one of the five diets in a completely randomized design. The ethical approval number (FUTO/2024/08) for the use of poultry was obtained from FUTO.

Housing and management of experimental birds

Each replicate was housed in a 2.0 × 2.0 m compartment within a half-open-sided poultry house. The pens were thoroughly washed and disinfected with Izal®, ensuring a clean environment. A layer of wood shavings, spread to a depth of 2 cm, was placed on the floor to enhance comfort and hygiene. The broilers across all treatment groups were managed and vaccinated following the guidelines outlined in the Ross 308 Broiler Management Guide. [20]

Composition, activities, and characterization of multi-enzyme used in this study

The enzyme Natuzyme® (Bioproton Pty Ltd, Sunnybank, Australia) used in this study is a multi-strain, commercially available, multi-enzyme product. The product contains α-amylase obtained from *Bacillus subtilis*, protease obtained from *Aspergillus niger*, β glucanase obtained from *Trichoderma reesei* and *Trichoderma longibrachiatum*, cellulase obtained from *Trichoderma reesei* and *Trichoderma longibrachiatum*, phytase obtained from *Aspergillus niger*, xylanase from *Trichoderma reesei* and *Trichoderma longibrachiatum*. The multi-enzyme preparation utilized in this study contained a comprehensive blend of digestive enzymes per kilogram of feed: α-amylase (1,800,000 units), protease (6,000,000 units), β-glucanase (1,000,000 units), cellulase (5,000,000 units), phytase (500,000 units), and xylanase (10,000,000 units). The multi-enzyme preparation was purposefully selected to enhance nutritional bioavailability by efficiently degrading complex carbohydrate and structural plant cell wall components, thus maximizing nutrient accessibility in high-fiber feed formulations. Consistent with the manufacturer's specifications, Natuzyme® was incorporated into the FEOP5 and FEOP10 dietary treatments at a standardized concentration of 0.50 g per kilogram of feed during the initial and final nutritional intervention period. Following the enzymatic activity determination methodology established by Jin et al.[21], the enzyme activity in the respective diet groups was quantified at 253 and 504 units per gram across starter and finisher feed stages. On December, 2024, biochemical analysis of the enzyme used in the present study was done at the Department of Animal Science and Technology Laboratory, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria, following the procedure described by Jin et al.[21], and the outcome unveiled the composite enzyme's maximal catalytic activity to be within a specific pH range of 4.7 and at a precise temperature of 53 °C, emphasizing its exceptional enzymatic capabilities in nutritional interventions.

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Data collection

Samples (4 replicates each) of the fermented orange pulp meal, maize meal, and the 5 diets were taken to the Department of Animal Science and Technology Laboratory, SAAT, FUTO, Imo State, Nigeria, for nutrient analysis. The fermented orange pulp meal, maize sample, and experimental diets were analysed to determine their dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), and ash contents according to AOAC. [22] The metabolizable energy (ME) concentrations of the fermented orange pulp meal, maize meal, and experimental diets used in the feeding trial were evaluated using the formula given by Wiseman [23] as:

ME (kcal/kg DM) = 3951 + 54.4 EE - 88.7 CF - 40.8 ash ……………….Equation 1

At the start of the feeding trial, the initial live weight of broiler chickens in each group was recorded, with subsequent weekly measurements taken. These weights were used to compute the average daily gain (ADG). The average daily feed intake (ADFI) was determined by subtracting the feed leftovers from the amount initially provided. The FCR was then calculated by dividing ADG by ADFI. On the 42nd day of the feeding trial, ten broiler chickens were randomly selected from each treatment group (2 birds per replicate). Using a sterile hypodermic syringe and needle, 5 mL of blood was withdrawn from their brachial veins. Of this, 2 mL was transferred into an ethylenediaminetetraacetic acid (EDTA)–treated collection tube for haematological analysis, while the remaining 3 mL was placed in bijou bottles without EDTA for serum biochemical evaluation. The EDTA concentration used in this study was 1.5 mg per mL of blood.[24] Blood samples were prepared for analysis according to the procedures outlined by the AGAPPE test kit (LiquiCHEK™). Haematological parameters were evaluated using the Beckman Coulter Ac-T 10 Laboratory Haematology Blood Analyzer, while serum biochemical assessments were conducted with a Semi-auto Chemistry Analyzer (BA-88A model, Mindray, Nansha, Shenzhen, China). The haematological parameters evaluated consists of haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cells (RBCs), RBC indices (mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean cell haemoglobin concentration (MCHC)), platelets, white blood cells (WBC), and differential WBC counts (lymphocytes and heterophils). The RBC indices were computed using Jain's [25] formulae: MCV = (10 × PCV) / RBC, MCH = Hb / RBC, and MCHC = (Hb × 100) / PCV. Serum biochemical parameters analyzed encompassed total protein, albumin, globulin, glucose, cholesterol, urea, creatinine, alkaline phosphatase (ALP), alanine transferase (ALT), and aspartate transferase (AST). These parameters were assessed by utilizing an AGAPPE kit, employing specific methodologies: urea (urease/GLDH method), creatinine (Jaffe's method), glucose (GOD-PAP method), AST (IFCC recommended procedure), ALT (IFCC recommended method), cholesterol (CHOD-PAP method), total protein (Biuret method), and albumin (Bromocresol Green method), with globulin derived as the difference between total protein and albumin values. Detection limits for the kits were as follows: urea (300 mg/dL), creatinine (24 mg/dL), glucose (600 mg/dL), AST (1000 U/L), ALT (1000 U/L), cholesterol (600 mg/dL), total protein (15 g/dL), and albumin (6 g/dL). Serum biochemical samples were analyzed using a Semi-auto Chemistry Analyzer (BA-88A model, Mindray, Nansha, Shenzhen, China) following the methodologies of Fischbach and Dunning [26] and Ruiz-Jimenez et al. [27]

Statistical analysis

Data on the proximate composition of the fermented *Citrus sinensis* (Orange) pulp meal and maize meal were analysed using Fisher's Student T-test as contained in Minitab 21 Statistical Software.[28] Data obtained on performance, haematological, and serum biochemical parameters were subjected to one-way analysis of variance (ANOVA) in the general linear model of the Minitab 21 Statistical Software.[28] The difference between the mean values was set to be significant at $p<0.05$. Mean values were separated using Tukey's range test contained in Minitab 21 software.[28]

Results and Discussion

Table 3 shows the proximate composition of the dietary fermented *Citrus sinensis* (orange) pulp and the maize meal used to compound the feed given to the broilers in the present study. There was no significant difference ($p>0.05$) in the dry matter, metabolizable energy, protein, ether extract, nitrogen-free extract, and ash, between the fermented orange pulp and maize meal analysed in this study. The fiber content of the fermented *Citrus sinensis* (Orange) pulp was significantly ($p<0.05$) higher than that obtained in the maize meal sample. The proximate composition values obtained for fermented orange pulp and maize meal in the present study are within the range reported by Sunmola et al. [17] for maize and sun-dried orange pulp. Furthermore, the similar values or non-significant differences obtained for dry matter, metabolizable energy, protein, fiber, ether extract, and ash for fermented orange pulp and maize samples indicate that fermented orange pulp may serve as a suitable alternative to maize in broiler diet. However, the high fiber content observed irrespective of the fermentation process employed on the *Citrus sinensis* (Orange) pulp meal compared to maize meal may limit the feeding value of the meal. In agreement with this observation, Oluremi et al. [5] and Oyewole et al. [14] observed a higher fiber content in sundried orange pulp compared to maize meal. The authors noted that the high fiber content in sundried orange pulp negatively affected broiler performance.

Table 3: Analyzed mean proximate composition of fermented C*itrus sinensis* pulp meal and maize meal

Parameters	FOPM	Maize	SEM	P-value
ME (kcal/kg)	3402.00	3440.00	43.30	2.01
Crude Protein (%)	7.90	10.00	13.40	1.30
Crude fiber (%)	9.25[a]	2.20[b]	10.20	0.02
Ether extract	2.12	4.50	6.87	0.09
Ash (%)	6.64	6.48	7.65	2.00
Dry matter	89.00	86.00	20.10	2.02

[a,b,] Means in the same row, not sharing a common superscript, are significantly different ($p<0.05$)
FOPM: Fermented orange pulp meal
ME: Metabolisable energy
SEM: standard error of the mean. Data presented the means based on 4 replicates per sample.

The growth performance parameters, including initial live weight (ILW), final live weight (FLW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and percentage mortality of broiler chickens fed a control diet and diets containing fermented orange pulp, with or without multi-enzyme supplementation, are presented in Table 4. Broilers in the FOP10 group, followed by those in the FOP5 group, showed a significant ($p<0.05$) reduction in FLW, WG, and ADG compared to the control group. In contrast, birds in the FEOP5 and FEOP10 groups exhibited FLW, WG, and ADG values similar to those in the control group. Compared to the control group, broilers in the FOP5 and FOP10 groups had poorer FCR ($p<0.05$), which was comparable to that observed in the FEOP10 group. Birds in the FEOP5 group had an FCR similar to the control group and significantly better ($p<0.05$) than those in the FOP5 and FOP10 groups.

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Meanwhile, broilers in the FEOP10 group had an FCR comparable to those in the FOP5, FOP10, and control groups. Regarding feed intake, broilers in the FOP10 group, followed by those in the FOP5 group, exhibited a significant (p<0.05) decrease in ADFI compared to birds in the control group. Birds in the FEOP5 group had an ADFI similar to those in the control group, while the FEOP10 group had an ADFI comparable to both the control and FEOP5 groups. No differences were observed in mortality rates across the various treatment groups. High dietary fiber content in certain feed ingredients can negatively impact poultry feed intake due to reduced digestion efficiency and increased retention time. [29] Orange pulp, known for its high soluble fiber content, has been linked to decreased feed intake. In this study, the decline in ADFI observed in broilers receiving FOP5 and FOP10 diets, irrespective of the fermentation process administered, may be attributed to the gut-filling effect caused by the fiber-rich orange pulp. This aligns with Mateos et al. [30], who reported that high dietary fiber can reduce feed intake in poultry due to its bulkiness. Conversely, the increased feed intake recorded in FEOP5 and FEOP10 diets could be associated with the effectiveness of the multi-enzyme supplement in breaking down fiber matrices, leading to faster gut emptying and reduced feed retention time, thereby enabling better feed intake. [31] Similarly, the reduction in final live weight (FLW) and average daily gain (ADG) observed in FOP5 and FOP10 diets compared to the control group likely stems from the negative impact of high-fiber diets on feed intake, which often results in diminished body weight in broilers.[30] The improved FLW and ADG in broilers fed FEOP5 and FEOP10 diets may be attributed to the multi-enzyme's role in enhancing fiber digestion, nutrient availability, and nutrient absorption, as reflected in the increased packed cell volume (PCV), total protein, globulin, and glucose values observed in this study (Tables 5 and 6). This finding aligns with Alefzadeh et al. [31], who reported that incorporating 0.50 g of multi-enzyme/kg diet improved the body weight of broilers fed a high-fiber orange peel-based diet. The poor feed conversion ratio (FCR) observed in broilers consuming FOP5 and FOP10 diets could be linked to the reduced ADFI and ADG recorded in this study. However, the improved FCR in broilers fed FEOP5 and FEOP10 diets may be associated with the multi-enzyme's ability to enhance nutrient digestion and utilization, resulting in better feed intake and body weight gain.[17] Mortality rates remained within the 5% threshold recommended by Aviagen [20], indicating that *Citrus sinensis* (orange) pulp, whether supplemented with multi-enzyme or not, had no harmful effects on broilers in this feeding trial.

Table 4: Performance of broilers fed diets containing fermented *Citrus sinensis* (orange) pulp meal supplemented with multi-enzyme

Parameters	Control	FOP5	FOP10	FEOP5	FEOP10	SEM	P -value
Initial live weight (g/bird)	42.10	42.12	42.10	42.11	42.10	0.95	0.17
Final live weight (g/bird)	2816.15^{a}	2601.00^{b}	2533.10^{c}	2793.10^{ab}	2776.20^{ab}	13.98	0.03
Average daily gain (g/bird/d)	67.05^{a}	61.93^{b}	60.31^{c}	66.50^{a}	66.10^{ab}	1.22	0.02
Average daily feed intake (g/bird/d)	101.23^{a}	98..47^{b}	96.24^{b}	101.14^{a}	101.06^{ab}	1.41	0.04
FCR (g/g)	1.51^{b}	1.59^{a}	1.60^{a}	1.52^{b}	1.53^{b}	0.02	0.03
Mortality (%)	2	1	1	1	1	-	-

$^{a,b,c.}$ Means in the same row not sharing a common superscript are significantly different (p<0.05). Data presented the means based on 4 replicate pens per treatment and 10 broilers per replicate., FOP5 = 5% fermented orange pulp without multi-enzyme supplementation; FOPM10 = 10% fermented orange pulp without multi-enzyme supplementation; FEOP5 = 5% fermented orange pulp with multi-enzyme supplementation; FEOP10 = 10% fermented orange pulp with multi-enzyme supplementation.
SEM: standard error of the mean.

Table 5 presents the haematological parameters of finisher broilers fed a control diet and diets containing fermented orange pulp, either with or without multi-enzyme supplementation. Across the different dietary treatment groups, no significant (p>0.05) differences were observed in haemoglobin (Hb) concentration, red blood cells (RBC), mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelets, white blood cells (WBC), lymphocytes, heterophils, and the heterophil-to-lymphocyte (H/L) ratio among broilers fed the experimental diets. Compared to birds in the control group, those in the FOP5 and FOP10 groups exhibited significantly (p<0.05) lower PCV values. Meanwhile, birds in the FEOP5 group recorded a PCV value that was comparable to that obtained by the control group. Similarly, broilers in the FEOP10 group had PCV values that were comparable to those of the FEOP5 and control groups. A significant (p<0.05) reduction in mean corpuscular volume (MCV) was noted in the FOP5 and FOP10 groups compared to the control group. However, the FEOP5 and FEOP10 groups exhibited MCV values comparable to those of the control group. Haematological parameters are essential for assessing poultry health and nutritional status. [32]

Table 5: Haematological characteristics of broilers fed fermented *Citrus sinensis* (orange) meal pulp meal supplemented with or without multi-enzyme

Parameters	Units	Control	FOP5	FOP10	FEOP5	FEOP10	SEM	P -value
Hb	(g/dl)	11.56	11.41	11.35	11.45	11.43	0.22	0.14
PCV	(%)	23.10^{a}	21.82^{b}	21.79^{b}	23.02^{a}	22.95^{ab}	0.34	0.03
RBC	(x10^{6}/µl)	2.29	2.26	2.24	2.28	2.27	0.07	0.12
MCV	(fl)	108.04^{a}	97.89^{b}	95.81^{b}	106.97^{ab}	105.96^{ab}	2.86	0.03
MCH	(pg)	51.81	52.34	52.37	51.99	52.01	0.39	0.25
MCHC	(g/dl)	51.51	52.86	53.02	51.93	52.22	0.64	0.28
Platelets	(× 10^{9}/L)	50.52	50.63	50.65	50.54	50.57	0.56	0.3
WBC	(x10^{3}µl)	13.69	13.72	13.74	13.76	13.79	0.96	0.12
Lymphocytes	(%)	50.19	50.32	50.35	50.39	50.41	0.32	0.14
Heterophils	(%)	24.72	25.55	25.59	24.68	24.64	0.48	0.08
H/L ratio		0.49	0.51	0.51	0.49	0.49	0.03	0.23

^{a,b}, Means in the same row not sharing a common superscript are significantly different (p<0.05). Data presented the means based on 4 replicate pens per treatment and 10 broilers per replicate. FOP5 = 5% fermented orange pulp without multi-enzyme supplementation; FOPM10 = 10% fermented orange pulp without multi-enzyme supplementation; FEOP5 = 5% fermented orange pulp with multi-enzyme supplementation; FEOP10 = 10% fermented orange pulp with multi-enzyme supplementation. Hb- haemoglobin; PVC-Pack cell volume; RBC- red blood cell count; MCV-mean corpuscular volume; MCH-mean corpuscular haemaglobin; MCHC-mean cell haemoglobin concentration; WBC-white blood count; H/L- heterophil/lymphocyte; fl – femtolitre; pg – pictogram.
SEM: standard error of the mean.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Changes in blood parameters can indicate the impact of various feed ingredients and supplements. [32] In this study, haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelets, white blood cells (WBC), lymphocytes, heterophils, and the heterophil-to-lymphocyte (H/L) ratio remained within normal ranges for broilers. [32,33] This suggests that fermented *Citrus sinensis* (Orange) pulp supplemented with or without multi-enzyme did not negatively affect the blood composition of broilers. Although PCV values remained within the standard range, the slight reduction observed in FOP5 and FOP10 diets could be linked to lower nutrient digestion, availability, and absorption, which could be attributed to the fiber-rich orange pulp. Abbasi et al. [8] similarly found that high-fiber diets led to decreased PCV values of broilers due to poor nutrient digestion and absorption. However, the combination of fermentation and multi-enzyme supplementation enhanced PCV levels in broilers receiving FEOP5 and FEOP10 diets, possibly due to improved digestion and nutrient uptake into the bloodstream. This observation is consistent with Behera et al. [34], who noted that multi-enzyme supplementation improved PCV in broilers fed sun-dried citrus pulp waste. MCV values in this study ranged from 95.81 to 108.04 fl, falling within the reported normal range (90–140 fl) for poultry. [32] The lower MCV recorded in FOP5 and FOP10 diets may be linked to reduced nutrient uptake due to the high fiber content. In contrast, the increased MCV observed in FEOP5 and FEOP10 diets could be attributed to the multi-enzyme's role in facilitating fiber digestion, improving nutrient absorption, and enhancing metabolic efficiency. The effect of the control and the FOP diets supplemented with or without multi-enzyme on serum biochemical parameters is presented in Table 6. There was no significant (p>0.05) effect of the dietary treatments on the Globulin, Urea, Creatinine, Cholesterol, Alkaline phosphate, Alanine transferase, and Aspartate transferase. Though comparable to birds in the FEOP5 and FEOP10 groups, broilers in the FOP5 and FOP10 groups had decreased (p<0.05) serum total protein and globulin values compared to birds in the control. The serum total protein and globulin values of broilers in the FEOP5 and FEOP10 groups were comparable (p>0.05) to those in the control group. Compared to birds in the control group, those in the FOP5 and FOP10 groups had lower (p<0.05) glucose values that were comparable to the glucose value of birds in the FEOP5 and FEOP10 groups. However, birds in the FEOP5 and FEOP10 groups had glucose values that were comparable (p>0.05) to those obtained in the control group. In this study, serum albumin, urea, creatinine, cholesterol, alkaline phosphatase (ALP), alanine transferase (ALT), and aspartate transferase (AST) remained unchanged across dietary treatments, indicating no nutritional deficiencies or health concerns among the broilers.[35] However, serum total protein and globulin levels were lower in FOP5 and FOP10 diets than in the control group, likely due to reduced protein availability resulting from the high fiber content. This is consistent with Oluremi [36] who observed a similar decline in serum protein levels when broilers were fed a diet containing sun-dried orange byproducts, attributing it to a systemic decrease in protein availability and utilization issues caused by the high fibre content inherent in the dried orange byproducts. Multi-enzyme supplementation in FEOP5 and FEOP10 diets improved serum total protein and globulin levels, potentially due to enhanced digestion of fiber-rich feed and greater availability of amino acids for protein synthesis. The reduced serum glucose levels in FOP5 and FOP10 diets compared to the control group suggest inadequate digestion of dietary fiber and reduced energy (glucose) availability and uptake. In contrast, the improvement observed in FEOP5 and FEOP10 groups may be linked to the multi-enzyme's capacity to enhance glucose synthesis and subsequent energy production for growth. Hu et al. [37] emphasized that glucose synthesis is closely associated with optimized energy metabolism in broilers. On the contrary, Behera et al. [34] found that supplementing a 10% sun-dried citrus waste diet with a cocktail of enzymes did not significantly affect serum glucose levels, which may be due to differences in enzyme type, dosage, dietary inclusion levels, or processing method (sun-dried versus fermented). Overall, the results suggest that while fermented *Citrus sinensis* (Orange) pulp diets may impair feed intake and nutrient absorption due to high fiber content, multi-enzyme supplementation can counteract these effects by improving digestion efficiency, enhancing nutrient availability, and optimizing growth performance in broilers.

Table 6: Serum biochemical parameters of broilers fed fermented *Citrus sinensis* (orange) pulp meal supplemented with or without multi-enzyme

Parameters	Units	Control	FOP5	FOP10	FEOP5	FEOP10	SEM	P-value
Total protein	(g/dl)	3.90[a]	3.41[b]	3.38[b]	3.85[ab]	3.83[ab]	0.16	0.03
Albumin	(g/dl)	1.40	1.39	1.38	1.39	1.39	0.02	0.07
Globulin	(g/dl)	2.50[a]	2.02[b]	2.00[b]	2.46[ab]	2.44[ab]	0.07	0.04
Glucose	(mg/dl)	162.39[a]	152.17[b]	152.03[b]	161.82[ab]	161.57[ab]	6.13	0.03
Urea	(mg/dl)	1.99	1.98	1.97	1.98	1.97	0.11	0.20
Creatinine	(mg/dl)	0.82	0.81	0.81	0.82	0.81	0.05	0.10
Cholesterol	(mg/dl)	130.12	130.06	130.04	130.07	130.05	5.68	0.20
Alkaline phosphatase	(IU/L)	42.82	42.89	42.90	42.87	42.88	3.10	0.12
Alanine transferase	(IU/L)	8.65	8.62	8.60	8.63	8.62	0.08	0.10
Aspartate transferase	(IU/L)	12.73	12.69	12.67	12.72	12.70	0.98	0.30

[a,b,] Means in the same row not sharing a common superscript are significantly different (p<0.05)
Data presented the means based on 4 replicate pens per treatment and 10 broilers per replicate.
FOP5 = 5% fermented orange pulp without multi-enzyme supplementation; FOPM10 = 10% fermented orange pulp without multi-enzyme supplementation; FEOP5 = 5% fermented orange pulp with multi-enzyme supplementation; FEOP10 = 10% fermented orange pulp with multi-enzyme supplementation.
SEM: standard error of the mean.

Conclusion

In conclusion, incorporating 5% and 10% fermented *Citrus sinensis* (orange) pulp with high fiber content into broiler diets depressed average daily feed intake (ADFI), average daily weight gain (ADG), final live weight (FLW), feed conversion ratio (FCR), packed cell volume (PCV), mean corpuscular volume (MCV), serum total protein, globulin, and glucose levels. However, supplementing 0.50 g/kg multi-enzyme alongside fermented orange pulp at these inclusion levels significantly enhanced ADG, FLW, FCR, PCV, serum total protein, globulin, and glucose concentrations, due to enhanced nutrient availability and uptake into the bloodstream. Based on these findings, 0.50 g/kg multi-enzyme supplementation is recommended for broiler diets containing 5–10% fermented *Citrus sinensis* (Orange) pulp to improve growth performance, PCV, MCV, serum total protein, globulin levels, and serum glucose concentration, while ensuring no adverse effects on other haematological or biochemical parameters examined in the present study.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

Acknowledgments

The authors are grateful for the TETFUND-IBR, grant number: REF: FUT/OVC/TETF/GEN.8/VOL 4 of 2023, sponsored by the Federal Republic of Nigeria and the Centre for Research Innovation and Development (CRID) of the Federal University of Technology, Owerri, Nigeria.

Funding

Funding was provided by the generous intervention of the TETFUND IBR 2023 Research Grant of the Federal Republic of Nigeria.

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ISSN 2616-0692 (Electronic)

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