Original Paper

Effect of *Rhizopus stolonifer* fermented cocoa pod husk meal supplemented with enzyme on growth performance, heamato-biochemical indices and sexual maturity of pullet chickens

Olayemi Aanuoluwapo Olugosi[1], Johnson Oluwasola Agbede[1]*, Oluwatosin Damilola Babarinde[1], Muyiwa Adegbenro[1], Taiwo Timothy Amos[2], Akinlolu Oluwafemi Ayeni[1], Muftau Kolawole Oladunmoye[3]
[1]*Federal University of Technology, Department of Animal Production and Health, Akure, Nigeria*
[2]*Federal University of Technology, Department of Agric Resource Economics, Akure, Nigeria*
[3]*Federal University of Technology, Department of Microbiology, Akure, Nigeria*

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A 25-weeks trial was carried out to determine the efficacy of feeding *Rhizopus stolonifer* fermented cocoa pod husk meal (FCPH) supplemented with Ronozyme multigrain enzyme (RME) at various stages of age of pullets. Two hundred and forty day old Isa Brown chickens were randomly assigned to six dietary treatments. Each treatment was replicated four times comprising of ten chickens per replicate and arranged in a 3 × 2 factorial in Completely Randomized Design. *Rhizopus stolonifer* FCPH meal was incorporated into the diet as test ingredient at varying inclusion level of 0%, 10% and 20% based on 100% diet. Each diet was then divided into two, one part void of enzyme and designated as diets I, III and V and the other parts supplemented with RME at 200 mg kg^{-1} of the diet and designated as diets II, IV and VI respectively. Weight gain, feed intake and feed conversion ratio were significantly (p <0.05) influenced by the dietary treatments with birds fed diet containing 10% FCPH meal without RME supplementation having highest weight gain across different stages of growth. The effect of enzyme and the interaction between diet and enzyme were not significant (p >0.05). Hematological and serum biochemical indices were also not significantly (p >0.05) influenced by the dietary treatments. Age at first lay and egg weight at first lay were also significantly (p <0.05) influenced by the dietary treatment. The use of *Rhizopus stolonifer* FCPH meal supplemented with RME in grower diets markedly increased age at sexual maturity and weight of first egg laid, with birds fed diets containing 10% FCPH meal fast coming to lay and birds fed 20% FCPH meal having higher weight of egg at first lay. Egg weight, egg width, shell surface area, shell thickness, yolk length and albumen length were also significantly (p <0.05) influenced by the diet treatment. *Rhizopus stolonifer* FCPH meal up to 10% without RME supplementation can effectively be used in pullet grower's diets without adversely affecting production performance or sexual maturity into lay.

Keywords: Isa Brown, fungi, solid state fermentation, first lay, enzyme supplementation

1 Introduction

The ISA Brown has proven to be the best brown layer in the world, known for its strong and reliable results. They convert feed exceptionally and are capable of laying 500 high quality eggs adapting well to different climates, management systems and housing systems. All this combined with an excellent feed conversion ratio makes them the choice of most for commercial egg producers. The conventional energy feed sources depending on the physiological age of the birds, constitute between 40 to 65% of formulated diets. These feed resources currently have high price tags as a result of their numerous alternative uses (Afolayan et al., 2012; Agbede, 2019). Among these energy sources, maize is the most widely employed for poultry feed formulation. However, owing to the various uses to which maize is put, the price of maize keeps increasing, making its continuous incorporation in livestock feed uneconomical. Also, the price tag for protein conventional feed resources is highly provocative because they are import-dependent items, especially in developing countries. In order to stem the problem of high and unstable price situation and save the collapse of poultry industry, there is need to broaden the energy/protein source base by assessing unconventional feedstuffs

***Corresponding Author:** Johnson Oluwasola Agbede, Federal University of Technology, Department of Animal Production and Health, Akure, Nigeria, phone: +2348064840143; e-mail: joagbede@futa.edu.ng

(Adegbenro et al., 2020). It is for this purpose that cocoa pod husk (CPH), an agro-industrial by-product, which is produced during cocoa processing is being evaluated in poultry diets. Improper disposal of these wastes by the farmers that produce them often lead to environmental pollution. Cocoa pod husk (CPH) produced in vast quantities from cocoa farmers could be a potential tropical feed in livestock feeds. If properly treated, its utilization in animal feeding will greatly reduce the disposal problem facing the cocoa processing factories (Aina, 1998).

Attempts to utilize raw cocoa waste materials as feed resources have shown that, when dietary concentrations exceed 10%, reproductive indices are negatively affected (Adeyina et al., 2012). Odunsi and longe (1998) reported poor performance following the ingestion of cocoa materials by chickens while egg production was adversely affected by the consumption of cocoa bean shell in a study conducted by Olubamiwa et al. (2006). Other researchers have attempted to use CPH for animal feeding especially in poultry diets (Hamzat and Babatunde, 2006; Adeyeye et al., 2019), but at minimal inclusion levels of 8% due to the low feed conversion ratio observed. In a recent study conducted by Olugosi et al. (2019), they reported an improvement in the nutritional quality of cocoa pod husk meal when taken through solid state fermentation using *Rhizopus stolonifer* has its starter culture, the proximate analysis of fermented cocoa pod husk meal is shown in Table 1. Documented information is still scanty on the use of microbial fermentation and enzyme supplementation to improve the nutritive quality of CPH in growing pullet diets. This study therefore, was conducted to assess the nutritive value of CPH meal when fermented with *Rhizopus stolonifer* as well supplemented with exogenous fibre degrading enzyme (Ronozyme® Multigrain) with a view to increasing its inclusion level in the diets of pullets. This might lead to the enhancement of the performance characteristics in respect to growth and age at sexual maturity. It is also expected that Ronozyme® Multigrain will aid fibre degradation in CPH meal when supplemented in diets of growing pullets.

2 Materials and methods

Animal ethics

The experimental design and methodology were approved by the Research Ethics Committee of the department of Animal Production and Health, The Federal University of Technology, Akure Ondo State Nigeria.

Experimental location

This study was carried out at the Department of Animal Production and Health of the Federal University of

Table 1 Nutrients, Anti-nutritional and Fibre fractions composition of raw and fermented CPH Meal at various days of fermentation with *Rhizopus stolonifer* (g 100 g^{-1} DM)

Proximate composition (g 100 g^{-1} DM)	Raw	3rd day	5th day	7th day	14th day
Crude protein	11.27	15.58	16.32	18.20	21.92
Ash	11.37	9.73	9.52	9.23	9.03
Crude Fiber	9.60	9.09	8.93	8.91	8.19
Crude Fat	7.15	6.96	6.65	6.39	5.54
NFE	60.61	58.64	58.58	57.27	55.32
M.E (MJ)	13.19	13.50	13.50	13.51	13.51
Anti-nutrients					
Theobromine	1.32	0.70	0.33	0.33	0.30
Tanin	0.50	0.37	0.10	0.10	0.03
Phytate	30.49	28.87	23.90	23.90	22.25
Fibre fractions					
NDF	91.89	81.59	73.89	69.59	65.89
ADF	61.29	54.17	49.95	48.45	45.25
ADL	23.33	21.81	20.15	17.91	10.79
HEMM	30.59	27.41	23.93	21.13	20.63
CELL	38.19	34.45	33.35	30.53	29.79

Source: Adapted from Olugosi et al. (2019), ME (Metabolizable energy) = (37 × %CP) + (81.8 × %FAT) + (35.5 × %NFE) (Pauzenga, 1985)
NFE – nitrogen free extract, NDF – neutral detergent fibre, ADF – acid detergent fibre, ADL – acid detergent lignin, HEMM – hemicellulose, CELL – cellulose

Technology, Akure, (FUTA) Nigeria. The feeding trial was carried out at the Poultry Unit of the Livestock Section, Teaching and Research Farm, FUTA. The University is geographically located between latitude 7° 5′ N and longitude 5° 15′ E at an altitude of 370 m above sea level (Oyinloye, 2013). The University is also located in the humid rain forest zone of Western Nigeria with tropical climate of two seasons: rainy season (April–October) and dry season (November–March) with a mean annual rainfall of 829 mm, an average annual temperature of 12.0 °C and an average relative humidity of 86% which characterize the climatic area (http://en.climate-data.org/location/674260/) and the experiment was carried out from June–November 2019.

Cocoa pod husk collection and Fermentation

Freshly discarded Cocoa pod husk was collected from cocoa plantation at Idanre, Akure South Local Government, Ondo state. It was cleaned, chopped, sun-dried to reduce the moisture content and finely milled to produce cocoa pod husk meal. The fermentation was done as earlier described by Olugosi et al. (2019). Cocoa pod husk meal (CPHM) was taken through solid state fermentation where 100 g of urea was dissolved in 100 l of water used to moisten 100 kg sterilized CPHM. One liter of the starter culture, *Rhizopus stolonifer* was used to inoculate the urea treated CPHM and kept in a tray incubating chamber covered with cellophane for 14 days after which it was sundried for 5 days to inactivate the microorganism. Fermented cocoa pod husk meal was analyzed for proximate composition (AOAC, 1995) and theobromine content (Bisto et al., 2002) and labelled as Fermented cocoa pod husk meal (FCPHM).

Experimental diets

The chick and grower phases of pullets were evaluated at this stage. Each phase lasted for 8weeks. One basal diet was formulated to meet the physiological needs of the chickens; FCPHM was included in the diet at 0%, 10% and 20% levels. Each of the three diets was mixed in one piece and later divided into two equal portions. One portion of each of the diet was supplemented with Ronozyme multigrain enzyme (RME) at 200 mg kg^{-1} of the feed while the other portion did not contain the enzyme.

Table 2 Ingredients composition and Analyzed nutrient composition of pullet chickens diets at starter and grower phases

	Starter phase % FCPHM			Grower phase % FCPHM		
Ingredient (%)	0	10	20	0	10	20
Maize	55.30	48.00	40.50	62.20	56.20	49.00
FCPHM	0.00	10.00	20.00	0.00	10.00	20.00
Soybean meal	17.00	12.00	7.00	15.00	16.00	17.00
Wheat Offal	19.90	22.20	24.70	18.00	13.50	10.00
Fish meal	2.00	2.00	2.00	0.00	0.00	0.00
Vegetable oil	2.00	2.00	2.00	1.00	1.00	1.00
Di-calcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	1.50	1.50	1.50	1.50	1.50	1.50
Premix	0.30	0.30	0.30	0.30	0.30	0.30
DL – methionine	0.10	0.10	0.10	0.10	0.10	0.10
L –lysine	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Analysed composition						
Crude protein, CP (%)	17.06	17.19	16.77	15.53	15.91	16.04
Crude fiber, CF (%)	3.99	5.10	5.88	4.03	4.11	4.24
Ash (%)	4.36	4.47	4.55	5.74	5.51	8.07
Ether extract (%)	10.38	11.62	11.27	9.33	8.49	7.23
Nitrogen-free extracts (%)	64.21	61.62	61.53	65.37	65.98	64.42
ME (MJ)	11.79	11.83	11.87	12.05	12.09	12.12

ME (metabolizable energy) of the FCPHM was calculated = (37 × %CP) + (81.8 × %FAT) + (35.5 × %NFE) (Pauzenga, 1985); FCPHM – fermented cocoa pod husk meal

In all, six diets were formulated and mixed for the trial and designated Diets I, II, III, IV, V, and VI. Diets II, IV and VI were those supplemented with enzyme. Also, six growers diets were formulated and mixed as described for pullet chickens. The gross compositions of the diets are presented in Table 2 for pullet chickens and growing pullets.

Experimental birds, layout and feeding trials

A batch of two hundred and seventy five day-old Isa brown chickens was procured from a reputable hatchery out of which two hundred and forty chickens were used for the trial and arranged in a 3 × 2 factorial in a Completely Randomized Design (3 levels of FCPHM inclusion: 0%, 10% and 20% and 2 levels of Ronozyme® Multigrain supplementation: 0 and 200 mg kg^{-1}). Forty day old chickens were allocated to each dietary treatment with 10 chickens per replicate of four replicates. The birds were housed in deep litter pens and reared based on the standard management guidelines provided by Teaching and Research farm, FUTA. The chickens were reared on their respective experimental diets from day old to 8 weeks of age for starter phase and 9 to 16 weeks of age at grower phase during which data on growth rate, dry matter intake and feed conversion ratio were the response criteria.

Management of experimental birds

Experimental birds were brooded in deep litter pens measuring 2 × 2 × 2 m. The birds were weighed at the start of the experiment and subsequently on a weekly basis. Birds in each replicate were given a known amount of feed on a daily basis. Feed leftover was weighed the next day prior to the day's feeding. The experimental birds had free access to feed and water. During the experiment, all medication and standard routine vaccination protocols were strictly adhered to. The birds were fed with the experimental starter diet for 8 weeks after which they were switched onto the grower diet. Growth and feeding records were recorded and summarized on a weekly basis. These included total feed intake (TFI), final body weight (FBW), weight gain (WG) and feed conversion ratio (FCR).

Haematological and Serum Parameters

At the end of the sixteen weeks feeding trial, feed was withdrawn from the pullets for a 12-hour period in order to considerably reduce the gut fill. Three pullets were randomly selected per treatment to determine the haematological and serum parameters. They were bled and blood was collected into Ethylenediamine tetraacetic acid (EDTA) bottles and plain bottles. The blood collected in the EDTA bottles was immediately capped and rocked gently for about a minute to mix the blood with the anticoagulant and the blood parameters determine using haematology analyzer. The blood in the plain bottle was centrifuged; thereafter, its serum was separated and frozen at -20 °C prior to analysis. The total protein, cholesterol, creatinine, urea, Aspartate amino transamase (AST) EC 2.6.1.1.1 and Alanine transamase (ALT) EC 2.6.1.1.2 were determined with a Reflectron^{R} Plus 8C79 (Roche Diagnostic, GobH Mahnheim, Germany) using commercial kits.

First lay production indices and age at sexual maturity and Egg quality evaluation

At 16 weeks of age, twenty seven pullets were carefully selected from each dietary treatment group, individually weighed and separated into three replicates of nine birds per replicate and transferred into cages to evaluate for performance at sexual maturity. Feed and water were given *ad libitum*. The performance indices accessed at this stage were age at which the first egg was laid, body weight of bird at first lay and weights of eggs at first lay. Egg quality parameters assessed included the external and internal qualities: egg length and width, egg shape index, shell thickness, shell weight, albumen height, albumen weight, percentage albumin weight, yolk weight, percentage yolk weight and Haugh unit.

Chemical analysis

Proximate analysis of experimental diets were carried out using the standard procedures. Dry matter was determined using AOAC method (method: 930.15; AOAC, 1995). Crude protein was determined according to method 942.05 (AOAC 1995). Crude fat was analyzed by the soxhlet method (method: 920.39; AOAC 1995). Ash was determined after ashing in a muffle furnace at 600 °C (method 965.17; AOAC 1995) while the nitrogen free extract was calculated by difference.

Statistical analysis

All data collected on growth performance, serum and haematology indices, egg production performance and egg quality assessment were subjected to One-Way ANOVA using SPSS version 20 and where there are significant differences, Duncan Multiple Range Test (DMRT) of the same package were used to compare the mean values. Below is the statistical model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + E_{ij}$$

where: factor A – 3 levels of FCPHM inclusion at 0%, 10% and 20%; factor B – 2 levels of Ronozyme® Multigrain supplementation at 0 and 200 mg kg^{-1}; Y_{ij} – the individual observation; μ – general mean; α_i – effect of factor A; β_j – effect of factor B; $\alpha\beta_{ij}$ – effect of interaction AB; E_{ijk} – experimental error

3 Results and discussion

Performance characteristics of pullet chicks fed diets containing varying levels of Rhizopus stolonifer fermented cocoa pod husk meal at starter phase (1– 8 weeks)

The growth of pullet chickens at age 1–56 days fed varying levels of *R. stolonifer* FCPHM with or without enzyme supplementation as reported in Table 3 shows a significant (p <0.05) effect among the dietary treatments. Highest final weight (423.75 g bird^{-1}) and Total weight gain (TWG) (390.23 g bird^{-1}) were recorded in pullet chickens fed diet containing 10% *R. stolonifer* FCPHM while lowest final weight (400.00 g bird^{-1}) and TWG (366.39 g bird^{-1}) were observed in chickens fed diet containing 20% *R. stolonifer* FCPHM. However, highest TFI (2,202.70 g chick^{-1}) with poorest FCR (6.01) was observed in chickens fed diet containing 20% *R. stolonifer* FCPHM while lowest TFI (2,069.50 g chick^{-1}) and best FCR (5.45) was observed in chickens fed diet containing 0% *R. stolonifer* FCPHM.

Enzyme supplementation and interaction between FCPHM × RME supplementation were not significant (p >0.05) for all the parameters measured.

Table 4 shows the growth performance parameters of pullet chickens of aged 1–84 days. All the parameters measured were significantly influenced by the FCPHM inclusion level. Highest final weight (842.50 g bird^{-1}) and TWG (808.98 g bird^{-1}) were recorded in pullet chickens fed diet containing 10% FCPHM (p <0.05) while lowest final weight (760.00 g bird^{-1}) and lowest TWG (726.39 g bird^{-1}) were observed in chickens fed 20% FCPHM based diet. The feed intake significantly (p <0.01) increased with increased inclusion of FCPHM in the diets with growers fed on diet containing 20% FCPHM having the highest TFI (4,485.50 g bird^{-1}). The feed conversion ratio of growers fed 0% and 10% FCPHM inclusion were similar but significantly (p <0.01) better than those fed diet containing 20% FCPHM diet. Generally, the

Table 3 Influence of *Rhizopus stolonifer* FCPHM inclusion in diets on the performance of pullet chickens (age: 1–56 days)
Growth indices

% FCPHM inclusion	Enzyme inclusion	Initial weight (g bird^{-1})	Final weight (g bird^{-1})	Total weight gain(g bird^{-1})	Total feed intake(g bird^{-1} week^{-1})	Feed conversion ratio
0		33.64	413.13^{ab}	379.49^{ab}	2,069.50^{b}	5.45^{a}
10		33.53	423.75^{a}	390.23^{a}	2,158.10^{a}	5.53^{b}
20		33.61	400.00^{b}	366.39^{b}	2,202.70^{a}	6.01^{c}
±SEM		0.59	5.57	5.62	24.40	0.11
P-value		0.99	0.05	0.04	0.04	0.02
Enzyme effect						
	with	33.58	417.08	383.50	2,147.50	5.59
	without	33.60	407.50	373.90	2,139.45	5.72
±SEM		0.47	5.40	5.36	25.43	0.11
P-value		0.98	0.20	0.20	0.79	0.38
FCPHM × enzyme inclusion						
0	with	33.63	412.50	378.88	2,093.80	5.52
0	without	33.65	413.75	380.10	2,045.10	5.38
10	with	33.53	432.50	398.98	2,144.90	5.37
10	without	33.53	415.00	381.48	2,171.40	5.69
20	with	33.60	406.25	372.65	2,203.80	5.91
20	without	33.63	393.75	360.13	2,201.60	6.11
±SEM		0.90	6.92	7.09	34.27	0.15
P-value		0.99	0.55	0.55	0.54	0.33

a–c – mean within rows having different superscripts are significantly different (P <0.05), FCPHM – fermented cocoa pod husk meal, RME – ronozyme multigrain enzyme

enzyme supplementation and the FCPHM × RME did not significantly ($p > 0.05$) affect the growth parameters measured.

Table 5 shows that the feed intake of birds fed 10% and 20% FCPHM based diets were similar but were significantly ($p < 0.01$) higher than those fed the control diet at age 112 days. While the TWG of birds fed the control diet (1,191.36 g bird^{-1}) and those fed 10% FCPHM based diet (1,184.47 g bird^{-1}) were similar ($p > 0.05$), they were however significantly ($p < 0.05$) higher than those fed 20% FCPHM based diet (1,085.14 g bird^{-1}). Also, the FCR significantly ($p < 0.01$) decreased beyond 10% inclusion level. The enzyme supplementation and the FCPHM × RME were not significant ($p > 0.05$) for all parameters measured.

Haematological indices and serum metabolites of pullets at 16 weeks fed diets containing varying levels of FCPHM

The haematological indices of pullets at 16 weeks fed diets containing varying inclusion levels of FCPHM based diets with or without enzyme supplementation in Table 6 shows that White Blood Cells (WBC), Neutrophils (NEU), Lymphocyte (Lym), Monocyte (Mono), Packed cell volume (PCV), Red blood count (RBC), Haemoglobin concentration (Hbc), Mean Cell Volume (MCV), Mean cell haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC) were all not significantly ($p > 0.05$) influenced by the dietary treatments and enzyme supplementation. Interaction between FCPHM × RME inclusion revealed level of significance ($p < 0.05$) for both RBC and MCV

Table 7 shows none of the metabolites of serum measured and the interactive effect was significantly ($p > 0.05$) affected.

First lay production indices of birds fed diets containing varying inclusion level of FCPHM

Table 8 reveals the age, weight and egg weight at first lay of birds fed varying inclusion levels of FCPHM based diets. Only the egg weight was significantly ($p < 0.001$) influenced by the dietary treatment. The egg weight had the highest value of 57.47 g in eggs laid by hens fed diet containing 20% FCPHM with enzyme supplementation

Table 4 Growth performance characteristics of pullet chickens at grower phase fed varying levels of FCPHM (age: 1–84 days)

% FCPHM Inclusion	Enzyme inclusion	Initial weight (g bird^{-1})	Final weight (g bird^{-1})	Total weight gain (g bird^{-1})	Total daily feed intake (g bird^{-1} day^{-1})	Feed conversion ratio
0		33.64	785.00^{b}	751.36^{b}	4,078.60^{b}	5.43^{a}
10		33.53	842.50^{a}	808.98^{a}	4,370.30^{a}	5.40^{b}
20		33.61	760.00^{b}	726.39^{b}	4,485.50^{a}	6.18^{c}
±SEM		0.59	16.25	16.42	58.72	0.14
P-value		0.99	0.03	0.03	0.01	0.01
Enzyme effect						
	with	33.58	798.33	764.75	4,360.90	5.70
	without	33.60	793.33	759.73	4,262.00	5.85
±SEM		0.47	16.62	16.73	68.35	0.16
P-Value		0.98	0.79	0.80	0.18	0.52
% FCPHM × RME						
0	with	33.63	807.50	773.88	4,135.20	5.34
0	without	33.65	762.50	728.85	4,022.00	5.52
10	with	33.53	845.00	811.48	4,397.00	5.42
10	without	33.53	840.00	806.48	4,343.50	5.39
20	with	33.60	742.50	708.90	4,550.40	6.42
20	without	33.63	777.50	743.88	4,420.50	5.94
±SEM		0.90	21.74	21.93	78.90	0.20
P-value		0.99	0.25	0.26	0.90	0.34

a-c – mean within rows having different superscripts are significantly different ($P < 0.05$), FCPHM – fermented cocoa pod husk meal, RME – ronozyme multigrain enzyme

Table 5 Growth performance characteristics of pullet chickens at grower phase fed varying levels of FCPHM: (age: 1–112 days)

% FCPHM inclusion	Enzyme inclusion	Initial weight (g bird^{-1})	Final weight (g bird^{-1})	Total weight gain (g bird^{-1})	Total daily feed intake (g bird^{-1} day^{-1})	Feed conversion ratio
0		33.64	1,225.00^{a}	1,191.36^{a}	9,170.80^{b}	7.72^{a}
10		33.53	1,220.00^{a}	1,184.47^{a}	9,722.60^{a}	8.21^{b}
20		33.61	1,118.75^{b}	1,085.14^{b}	9,987.70^{a}	9.23^{c}
±SEM		0.59	16.25	16.42	100.06	0.18
P-value		0.99	0.03	0.03	0.01	0.01
Enzyme effect						
	with	33.58	1,190.00	1,156.42	9,727.50	8.51
	without	33.60	1,185.83	1,152.23	9,526.50	8.26
±SEM		0.47	16.62	16.73	127.02	0.23
P-Value		0.98	0.79	0.80	0.10	0.22
FCPHM × RME						
0	with	33.63	1,232.50	1,198.87	9,369.00	7.95
0	without	33.65	1,217.50	1,183.85	8,972.70	7.49
10	with	33.53	1,195.00	1,161.47	9,709.90	8.04
10	without	33.53	1,245.00	1,211.47	9,735.20	8.38
20	with	33.60	1,142.50	1,108.90	10,104.00	9.54
20	without	33.63	1,095.00	1,061.37	9,871.60	8.91
±SEM		0.90	21.74	21.93	128.48	0.22
P-value		0.99	0.25	0.26	0.34	0.12

a-b – mean within rows having different superscripts are significantly different ($P < 0.05$), FCPHM – fermented cocoa pod husk meal, RME – ronozyme multigrain enzyme

and significantly ($p < 0.001$) higher than those fed control diet and 10% FCPHM with or without enzyme supplementation with the values 43.27 g, 44.13 g, 51.17 g and 47.67 g respectively. Although, not significantly ($p > 0.05$) influenced by the dietary factor, birds fed diet containing 10% FCPHM with enzyme supplementation had the earliest first egg laid (21 weeks) while hens fed diet containing 20% FCPHM with enzyme supplementation had the longest number of weeks before having the first egg laid (24.33 weeks). Also, the bird weight at first lay was not significantly ($p > 0.05$) influenced by the dietary compositions.

Egg Quality analysis for eggs first laid by birds fed varying inclusion level of FCPH meal

Tables 9 reveals the result of the egg quality analysis for the first egg laid by birds fed varying inclusion levels of FCPHM. All external egg parameters excluding egg length (EL), egg shape index (ESI) and shell weight (SW) were significantly ($p < 0.001$) influenced by the varying inclusion levels of FCPHM in the diets. The egg weight of birds fed on diet containing 20% FCPHM (54.59 g) was significantly ($p < 0.001$) higher than those fed the control diet (43.70 g) and 10% inclusion level (49.42 g). This same trend was observed in the egg width (EW) and shell surface area (SSA). However, the shell thickness (ST) of 0.29 and 0.30 mm for eggs of birds fed diets containing 10 and 20% FCPHM respectively were not significantly ($p > 0.01$) higher than those of birds fed the control diet (0.28 mm). Also, RME supplementation significantly ($p < 0.05$) influenced the egg width and the shell weight only. However, shell weight (SW) of eggs of birds fed diet with enzyme supplementation was higher (5.24 g) than those without enzyme supplementation (4.96 g). Only egg length, egg shape index and shell weight were not significantly ($p > 0.05$) influenced by the Diets X Enzyme interaction.

Table 10 shows that dietary treatments did not have significant ($p > 0.05$) effect on the yolk weight, % yolk weight, yolk width, yolk length, yolk height and yolk index while enzyme supplementation had significant effect on yolk length ($p < 0.01$), yolk weight ($p < 0.05$) and yolk length ($p < 0.01$). FCPHM X Enzyme was not significant ($p > 0.05$) for all parameters measured. Table 11 reveals that the albumen weight ($p < 0.001$), albumen length ($p < 0.05$)

Table 6 Haematological indices of growing pullets at 16 weeks fed diets containing FCPHM supplemented with or without RME
Blood parameters

% FCPHM inclusion	Enzyme	PCV (%)	HBC (g 100 ml^{-1})	RBC (× 10^{6} mm)	WBC (× 10^{6} mm)	MCV (%)	MCH (pg)	MCHC (u3)	NEU (%)	LYM (%)	MONO (%)
0		26.60	8.84	2.39	6.74	120.88	33.22	40.22	30.60	65.00	4.40
10		26.20	8.70	2.21	6.16	132.64	33.18	44.06	25.40	69.20	5.40
20		27.25	9.05	2.44	3.30	143.63	33.18	47.70	29.00	67.50	3.50
±SEM		1.32	0.44	0.47	1.46	28.01	0.05	9.32	3.66	4.05	1.00
P-value		0.84	0.84	0.90	0.35	0.73	0.74	0.84	0.63	0.76	0.53
Enzyme effect											
	with	27.50	9.13	2.29	6.03	142.20	33.18	47.25	29.00	66.00	5.00
	without	26.00	8.64	2.39	5.19	123.61	33.20	41.09	27.75	68.13	4.13
±SEM		1.01	0.33	0.36	1.34	22.30	0.04	7.42	3.04	3.22	0.94
P-Value		0.35	0.35	0.81	0.57	0.47	0.47	0.84	0.81	0.66	0.47
FCPHM × RME											
0	with	28.00	9.30	2.26^{b}	9.50	140.00^{c}	33.20	46.55	35.00	58.50	6.50
0	without	25.67	8.53	2.48^{b}	4.90	108.13^{b}	33.23	36.00	27.67	69.33	3.00
10	with	25.00	8.30	3.06^{a}	4.10	82.10^{d}	33.15	27.20	26.00	68.50	5.50
10	without	27.00	8.97	1.65^{c}	7.53	166.33^{c}	33.20	55.30	25.00	69.67	5.33
20	with	29.50	9.80	1.55^{c}	4.50	204.50^{a}	33.20	68.00	26.00	71.00	3.00
20	without	25.00	8.30	3.34^{a}	2.10	82.75^{d}	33.15	27.40	32.00	64.00	4.00
±SEM		1.50	0.49	0.53	1.33	26.77	0.08	8.85	5.20	5.26	1.57
P-value		0.24	0.23	0.05	0.25	0.02	0.07	0.84	0.54	0.37	0.40

a–d – means with different superscripts are significantly different ($P < 0.05$), SEM – standard error of mean, PCV – packed cell volume, RBC – red blood cells, HBC – haemoglobin concentration, MCV – mean cell volume, MCH – mean cell haemoglobin, MCHC – mean cell haemoglobin concentration, LYM – lymphocytes, NEU – neutrophils, MONO – monocytes, FCPHM – fermented cocoa pod husk meal, RME – ronozyme multigrain enzyme

and Haugh unit ($p < 0.05$) were significantly influenced by the varying dietary inclusion levels of FCPHM. Albumen weight was highest (34.42 g) in eggs laid by hens fed diet containing 20% FCPHM and it was significantly ($p < 0.001$) higher than the values 32.22 g and 31.82 g for hens fed diets containing 0 and 10% inclusion levels of FCPHM respectively. The albumen length increased significantly ($p < 0.05$) from 79.64 mm in eggs laid by hens fed the control diet to 82.94 mm in hens fed diet containing 20% FCPHM inclusion. However, the Haugh unit had the highest value recorded in egg laid by hen that consumed diet containing 10% FCPHM (91.60) but not significantly ($p > 0.05$) different from the value recorded in eggs laid by hen that consumed diet containing 20% FCPHM (89.61) while the control diet had the least value of 84.24. Also, the enzyme factor only significantly ($p < 0.05$) influenced the percentage albumen weight and albumen length. Percentage albumen weight was significantly ($p < 0.05$) higher (62.01%) for egg laid by hen fed diet without enzyme supplementation than 60.15% for egg laid by hen fed diet supplemented with enzyme. Conversely, the albumen length was statistically ($p < 0.001$) higher in egg laid by the hens fed with diet supplemented with enzyme (83.33 mm) than the one fed diet without enzyme (79.31 mm). % FCPHM inclusion level X Enzyme interaction was significant ($p < 0.05$) for all the albumen indexes except albumen height and Haugh unit.

Cocoa pod husk meal is seldomly used in poultry diets due to the presence of theobromine and high fibre in the meal. But results of the performance characteristics at each growth phase in this current study revealed that it could be a valuable feed ingredient if used at some certain levels. There was increased daily feed intake with increasing level of FCPHM in the diet. Results on feed intake at 10% and 20% inclusion levels of the FCPHM in the diets at each growing phase suggests its acceptance by the birds. The reduction in theobromine content as a result of solid state fermentation using *R. stolonifer* (Olugosi et al., 2019) might have led to

Table 7 Biochemical indices of pullet chickens at 16 weeks fed FCPH meal supplemented with or without enzymes

% FCPHM inclusion	Enzyme	Cholesterol (mmol L^{-1})	Creatinine (μmol L^{-1})	AST (μL L^{-1})	ALT (IU L^{-1})	Urea (mg dl^{-1})
0		4.19	29.43	91.90	36.70	2.48
10		3.07	19.63	93.25	37.08	2.82
20		3.06	30.18	111.50	36.53	2.60
±SEM		0.75	4.46	8.68	0.47	0.12
P value		0.58	0.37	0.25	0.77	0.25
Effect of enzyme						
	without	3.56	25.65	98.63	36.47	2.77
	with	3.45	27.17	99.13	37.07	2.49
±SEM		0.70	4.22	6.97	0.36	0.10
P-value		0.92	0.82	0.95	0.37	0.11
FCPHM × enzyme						
0	without	3.30	25.65	99.45	36.45	2.58
0	with	3.09	33.20	84.35	36.95	2.38
10	without	3.57	16.60	99.65	36.70	3.13
10	with	4.97	22.65	86.85	37.45	2.52
20	without	3.83	34.70	96.80	36.25	2.62
20	with	2.29	25.65	126.20	36.80	2.57
±SEM		1.11	4.11	8.37	0.72	0.11
P-value		0.58	0.53	0.18	0.99	0.37

a–b – means with different superscripts are significantly different ($P < 0.05$), SEM – standard error of mean different ($P < 0.05$), SEM – standard error of mean, FCPHM – fermented cocoa pod husk meal

Table 8 Age, weight and egg weight at first lay of birds fed *Rhizopus stolonifer* FCPH meal

% FCPHM Inclusion	Enzyme	Age (weeks)	Bird weight (kg)	Egg weight (g)
0% FCPHM	without	21.67	1.62	43.27^{d}
	with	23.00	1.59	44.13^{c}
10% FCPHM	without	23.67	1.63	51.17^{b}
	with	21.00	1.62	47.67^{bc}
20% FCPHM	without	23.33	1.70	51.70^{b}
	with	24.33	1.71	57.47^{a}
±SEM		0.79	0.03	1.78
P-value		0.088	0.085	0.001

a–d – means on the same column having different superscripts are significantly ($P < 0.001$) different, FCPHM – fermented cocoa pod husk meal

Table 9 External qualities of first laid eggs (20–24 weeks)

% FCPHM inclusion	Enzyme	Egg weight (g)	Egg length (mm)	Egg width (mm)	Egg shape index	Shell surface area	Shell weight (g)	Shell thickness (mm)
0%		43.70[b]	53.60	42.08[b]	77.13	65.71[b]	5.12	0.28[b]
10%		49.42[b]	54.13	41.65[b]	77.18	65.63[b]	5.00	0.29[a]
20%		54.59[a]	55.53	43.07[a]	79.01	68.08[a]	5.18	0.30[a]
±SEM		0.43	0.44	0.17	0.71	0.36	0.08	0.00
P-value		0.001	0.723	0.001	0.117	0.001	0.257	0.011
Enzyme								
	without	47.55	53.26	42.49[a]	78.38	66.48	4.96[b]	0.28
	with	50.92	54.58	42.05[b]	77.16	66.47	5.24[a]	0.29
±SEM		0.35	0.36	0.14	0.58	0.29	0.06	0.00
P-value		0.946	0.539	0.030	0.147	0.981	0.004	0.076
Diets X								
0 % FCPHM	without	43.27[d]	54.02	42.09[ab]	77.93	65.74[bc]	5.12	0.28[ab]
	with	44.13[c]	53.18	42.08[ab]	76.33	65.67[c]	5.11	0.27[b]
10% FCPHM	without	47.67[b]	54.70	42.74[ab]	78.33	67.61[b]	4.81	0.27[b]
	with	51.17[bc]	53.56	40.56[b]	76.03	63.65[d]	5.19	0.31[a]
20% FCPHM	without	51.70[b]	54.06	42.63[ab]	78.89	66.08[b]	4.96	0.30[a]
	with	57.47[a]	56.00	43.51[a]	79.14	70.08[a]	5.41	0.30[a]
±SEM		1.78	0.63	0.24	1.01	0.50	0.11	0.01
P-value		0.001	0.144	0.001	0.434	0.001	0.091	0.002

a–d – means on the same column having different superscripts are significantly (P <0.05, 0.01 and 0.001) different, FCPHM – *Rhizopus stolonifer* fermented cocoa pod husk meal

increase palatability hence, increased feed intake. Reduced feed intake is believed to be due to destruction of the intestinal lining and severe indigestion in the birds (Olubamiwa et al., 2001). This negates the previous reports by Sobamiwa (1998); Sobamiwa and Akinwale (1999); Odunsi et al. (1999) and Olubamiwa (2000) who reported reduced feed intake as the level of CPH increased in the diets of the birds but were consistent with the report of Olubamiwa et al. (2002). Although, this can also be attributed to reduced energy content in the diets containing *R. stolonifer* FCPHM. In an experiment to determine the effect of diets containing different levels of cocoa pod husk on broiler performance, Alemawor et al. (2010) observed that feeding higher levels of CPH resulted in higher average daily feed intake. This they attributed to a dilution in the energy content of the feed with increasing levels of CPH.

The addition of enzyme did not statistically improve the efficiency of the FCPHM by the birds and their total weight gain. Higher dosage of enzyme supplementation or a different brand is suggested. Exo-enzymes have been reported to increase the availability of nutrients by breaking down specific chemical structures that endogenous digestive bio-enzymes are not capable of breaking down with a resultant increase in the efficiency of feed utilization by the host animal (Rajendra et al., 2016).

Increased weight gain observed in birds fed diets containing 10% FCPHM suggest that 10% FCPHM inclusion might be the optimum inclusion level for this agro waste in pullet chicks diet. This was in accordance with observations of Olubamiwa et al. (2002). Teguia et al. (2004) also inferred that the birds fed with the diet of 10% substitution level of CPH showed significantly faster growth rate than birds placed on control diet whose growth rate were not significantly different from birds fed diet with 20% cocoa pod husk inclusion. At 16 weeks in this current study, weight gain of birds fed 10% FCPHM was at par with control but significantly higher than weight gain of birds fed 20% FCPHM. Feed conversion ratio (FCR) was significantly affected by the experimental diets and it decreased with the increased level of inclusion of FCPHM in the diets. However, birds on the control and 10% FCPHM were similar in the utilization of their diets. This is in line with the findings of Odunsi and Longe (2000) who reported that weight gain were depressed at 20% inclusion of cocoa bean cake and above when they fed six groups of day-old chickens

Table 10 Yolk qualities of first laid eggs (20–24 weeks)

% FCPHM inclusion	Enzyme	Yolk weight (g)	% yolk weight	Yolk width (mm)	Yolk length (mm)	Yolk height (g)	Yolk index
0%		13.12	24.94	39.56	41.56	16.28	0.41
10%		13.44	25.61	39.61	41.72	16.39	0.41
20%		13.63	24.57	40.35	41.56	16.39	0.41
±SEM		0.33	0.65	0.38	0.26	0.35	1.08
P-value		0.552	0.525	0.273	0.878	0.967	0.836
Enzyme							
	without	13.36	24.94	40.27	42.08[a]	16.3	0.41
	with	13.43	25.14	39.41	41.14[b]	16.41	0.42
±SEM		0.27	0.53	0.31	0.21	0.29	0.88
P-value		0.854	0.8	0.058	0.004	0.786	0.361
Diet X							
0 % FCPHM	without	12.97	24.58	40.44	41.78	16.06	0.40
	with	13.28	25.29	38.67	41.33	16.50	0.42
10 % FCPHM	without	13.79	25.13	40.33	42.67	16.56	0.41
	with	13.10	26.09	38.89	40.78	16.22	0.42
20 % FCPHM	without	13.33	25.12	40.03	41.80	16.28	0.41
	with	13.92	24.03	40.67	41.32	16.50	0.41
±SEM		0.47	0.92	0.54	0.37	0.50	1.53
P-value		0.364	0.483	0.067	0.099	0.726	0.590

a–b – means on the same column having different superscripts are significantly (P <0.05 and 0.01) different, FCPHM – *Rhizopus stolonifer* fermented cocoa pod husk meal

(Isa Brown pullet type) nitrogenous diets. The decrease in the FCR value at the inclusion rate of 20% FCPHM established that there was no proper utilization of the dietary treatment with increased inclusion level of FCPHM in the diet. The absence of mortality throughout the period of this experiment further attested to the suitability of FCPH meal as an alternative feed ingredient in growing pullet's diet. Also, the non-significance of FCPHM × RME for the performance indices suggest that the main factors are not interdependent on each other.

According to Togun and Oseni (2005), haematological indices such as RBC, WBC, PCV and Hbc have been found useful for disease prognosis and for therapeutic and feed stress monitoring. The present study suggests that the control and FCPHM based diets with or without enzyme supplementation supported similar haematopoietic activities in the experimental birds. This further confirms the wholesomeness of FCPHM as alternative feed ingredient in pullet chickens diets. As observed the use of RME might not be necessary as the effect elucidated was not statistically different. Nevertheless, the result in this study further revealed that the haematological indices of the chickens were within normal range except for MCV and MCHC which fell within the range reported by Ahamefule et al. (2006). The observed result was also similar to the findings of Ankrah et al. (2015) who reported that Hb, PCV, RBC and MCV were not significantly different among laying birds fed diet containing Pito mash treated cocoa pod husk. In the present study, the PCV value fell within the normal range of 25–45% for chickens as reported by Akinmutimi (2004) and Ahamefule et al. (2006). The PCV value below normal range is an indication of anaemia (Radostis et al., 1994) and poor quality of protein of the diets (Awoniyi et al., 2002). The present study also suggest that the values of MCV and RBC observed for the pullets are dependent on the two main factors been examined.

Blood biochemical components are feed toxicity elements sensitive (Onifade and Tewe, 1993). The present study reveals that all biochemical indices were not affected by the FCPH meal inclusion in the diet and therefore suggests that FCPHM might not pose any serious deleterious health challenges to the birds, especially as it relates to liver, as increased activities of these enzymes in the serum are well-known diagnostic

Table 11 Albumen qualities of first laid eggs (20–24 weeks)

% FCPHM inclusion	Enzyme	Albumen weight (g)	% albumen weight	Albumen length (mm)	Albumen height (mm)	Albumen width (mm)	Haugh unit
0%		32.22^{b}	61.07	79.64^{b}	7.08	63.80	84.24^{b}
10%		31.82^{b}	60.28	81.39^{ab}	8.33	63.65	91.60^{a}
20%		34.42^{a}	61.89	82.94^{a}	8.03	65.36	89.61^{ab}
±SEM		0.48	0.71	0.92	0.40	0.78	2.08
P-value		0.001	0.820	0.050	0.080	0.240	0.050
Enzyme							
	without	33.29	62.01^{a}	79.31^{b}	7.81	63.41	88.02
	with	32.35	60.15^{b}	83.33^{a}	7.81	65.13	88.94
±SEM		0.39	0.58	0.75	0.33	0.64	1.70
P-value		0.098	0.028	0.001	1.000	0.064	0.706
Diet X							
0 % FCPHM	without	31.99^{b}	60.61^{c}	80.94^{d}	6.50	65.88^{b}	79.95
	with	32.44^{ab}	61.52^{b}	78.33^{e}	7.67	61.72^{d}	88.53
10 % FCPHM	without	34.91^{a}	63.42^{a}	74.67^{f}	8.72	61.69^{d}	92.58
	with	28.72^{c}	57.15^{d}	88.11^{a}	7.94	65.61^{b}	90.61
20 % FCPHM	without	32.97^{ab}	62.02^{b}	82.33^{c}	8.22	62.67^{c}	91.54
	with	35.88^{a}	61.77^{b}	83.56^{b}	7.83	68.04^{a}	87.68
±SEM		0.68	1.00	1.30	0.57	1.10	2.94
P-value		0.001	0.002	0.001	0.206	0.001	0.089

a–f – means on the same column having different superscripts are significantly (P <0.05, 0.01 and 0.001) different, FCPHM – *Rhizopus stolonifer* fermented cocoa pod husk meal

indicators of liver injury (Agbede et al., 2011; Aro et al., 2012). Supplementation with enzyme and interaction between dietary inclusion level of FCPHM in the diet and enzyme inclusion did not affect all the biochemical indices of pullet chickens considered. The good health status of the experimental birds observed during the entire period of this present study suggests that the use of FCPHM up to 20% inclusion level can be tolerated by growing pullet.

The laying time for first-lay of the birds was not in tandem with Lala et al. (2016) who reported first lay within 20–22 weeks in Isa Brown laying birds as against 21–24 weeks observed in this current study. As expected, the eggs were small (43.27 g) in sizes. However, birds fed diets containing 20% FCPHM had their first lay at 24th week and the delay was compensated with bigger egg size (57.47 g). The bigger size could be said to have been as a result of the bulkiness of the test ingredient and the increasing age of poultry enhances fibre utilization which in turn improved sexual maturity. Farrel (1994) found that laying hens can tolerate higher levels of rice bran (a fibrous feed-stuff) than broiler chickens. It can thus be said that delay in first lay by birds fed 20% FCPH meal inclusion level was compensated with larger egg sizes. This corresponds with the results of Olumide et al. (2017) that recorded increase in egg weight as the percentage inclusion of fermented and enzyme-treated cocoa by-products increased in the diets.

Egg weight, egg width and shell surface area of birds fed 20% FCPHM with enzyme supplementation were the highest while those fed diet containing 10% inclusion levels were similar. The result from this study is in agreement with the report of Olumide et al. (2017) that the egg width and shell surface area were significantly influenced by the varying inclusion levels of cocoa by-products while other external egg qualities were not significant. Also, Nortey et al. (2015) reported that older birds were not significantly influenced by the varying inclusion levels of FCPHM and the inclusion of fibre degrading enzyme; which was consistent with the current result on egg weight, egg length, egg shape index, shell surface area and shell thickness. Nevertheless, enzyme supplemented diets enhanced shell weight but led to lower egg width. Also, in the present study, the egg weight, egg width, shell surface area and shell thickness values are dependent on the main two factors under study, implying their significance in these egg parameters when FCHPM is included.

The yolk qualities of eggs were not significantly influenced by the varying inclusion levels of FCPHM. However, the albumen weight and Haugh unit varied with albumen length and Haugh unit of eggs laid by hens fed diets containing FCPHM consistently having higher values than those fed the control diet. This correlates with the reports of Olumide et al. (2017) that cocoa by-products significantly influenced internal egg qualities. Also, the enzyme had no influence on the yolk indices except yolk length, but influenced the % albumen weight and albumen length. This further suggest that the influence of enzyme on the FCPHM utilization vis-à-vis internal egg qualities value might not be a major factor.

4 Conclusion

From the foregoing, *Rhizopus stolonifer* FCPHM can be included in the diets of pullet chicks up to 10% level without enzyme supplementation beyond which a decline in weight and feed utilization will decline. Higher value of 20% FCPHM in the diet might not precipitate adverse health challenge but could lead to delay in sexual maturity of the pullet chicks to about 24 weeks even with enzyme supplementation of the diets. Also 20% FCPHM though led to delayed sexual maturity, the first eggs laid had higher weight than those laid by pullets fed on control and 10% FCPHM based diet. Also, the egg weight, egg width, shell surface area, shell thickness, albumen weight, length and haugh unit were improved in pullets fed diets containing 20% FCPHM while enzyme supplementation appeared not to have significant effect on the egg parameters measured. Consequently, the use of FCPHM without enzyme supplementation is recommended as alternative energy/protein ingredient in pullet diets. The effects of feeding *Rhizopus stolonifer* FCPHM on laying performance and egg quality will be monitored in the subsequent trial.

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