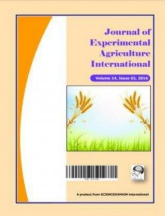

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Haematological Parameters, Serum Biochemistry and Gut Microbial Count of Broiler Chicks Fed Processed Dietary Fungi Treated *Jatropha curcas* Kernel Meals

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Authors' contributions

Author TKO designed the study, performed the statistical analysis and wrote the protocol. Author OEA wrote the first draft of the manuscript while author IAE managed the analyses of the study and managed the literature search. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This feeding trial evaluates the blood characteristics and gut microbial count of broilers chicks fed Processed Dietary Fungi Treated *Jatropha curcas* Kernel Meals.
Study Design: All data generated were subjected to analysis of variance in a complete randomized design.
Place and Duration of Study: The experiment was carried out at the Teaching and Research Farm of the Ladoke Akintola University of Technology, Ogbomoso, Nigeria, between November and December 2014.
Methodology: A total of one hundred and eighty Marshal strain Broiler chicks (n = 180) fed

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Aspergillus niger treated meals, namely: Raw Defatted Fermented Meal (RDFM), Toasted Defatted Fermented Meal (TDFM), Cooked Defatted Fermented Meal (CDFM), Lye treated Defatted Fermented Meal (LDFM) and Sand roasted Defatted Fermented Meal (ZDFM) were evaluated in a 21 day feeding trial. Six (6) dietary treatments were formulated such that Diet 1 contained 0% *Jatropha curcas* kernel Meal while diets 2, 3, 4, 5 and 6 contained 10.33% (one-third replacement of soybean meal) inclusion level of RDFM, TDFM, CDFM, LDFM and ZDFM.

Results: The red blood cell, eosinophyll, basophyll, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume, alanine transaminase, cholesterol, triglyceride and acid phosphatase were significantly influenced ($p<0.05$) by the dietary treatments while the values obtained for lactobacilli count was higher than that of *E. coli* within the treatments in the gut of the birds.

Conclusion: Prolong exposure might posed serious health challenge to the birds. It can also be concluded that *Lactobacillus* thrived in the gut of the broiler chicks than *E. coli*, although the populations of both were reduced.

Keywords: Broilers; blood; Aspergillus niger; microbial count; Jatropha curcas.

1. INTRODUCTION

Jatropha curcas and other *Euphorbiaceae like* Castor, *Ricinus communis* have gained interest as commercial seed trees for biofuel/biodiesel production [1-2]. Interest in *Jatropha curcas* as a source of oil for producing biodiesel has arisen as a consequence of its perceived draught resistance nature, ability to grow in semi-arid regions with low nutrient requirements. This means that the advent of eco-friendly fuel would mean an availability of surplus nutrient dense cake.

Jatropha curcas kernel meal has been suggested for use in livestock feed as additional source of plant protein [3], because the seeds had been reported to have about 35-50% crude protein [4-5], 60% oil and rich in essential amino acid and minerals [6]. Ojediran et al. [7] has reported a crude protein content of about 23.57% and 52.47% oil in the kernel. Makkar and Becker [8] observed that the presence of antinutritional factors such as lectin, saponin, tannin, phytate, trypsin inhibitors and phorbol esters in the seeds, cakes or its oil has limited the use in human or animal nutrition. Various procesing methods have been used but with unencouraging results [7]. Meanwhile, a combination of methods has been proposed by Emiola et al. [9], while Belewu et al. [3] has exploited the use of biodegraders and has fed resultant meals to goats. Therefore is need for further research into processing procedure that will ensure maximum utilization of the nutrients to prepare an acceptable product from *Jatropha curcas* kernel meals for livestock use.

Blood parameters are a good diagnostic tool to examine the influence of feedstuffs or antinutrients on the physiological well-being of livestocks. The commonly examined heamatological parameters in nutritional studies include Packed cell volume (PCV), Erythrocyte count (RBC), Leukocyte count (WBC), Haemoglobin (Hb), Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular volume (MCV) and clotting time [10]. It has been reported that serum biochemical constituents positively correlate with the quality of the diet [11]. Ojediran et al. [12] observed that differently processed *Jatropha curcas* kernel meals had antimicrobial properties which reacted against both gram positive and negative stain bacteria traceable to the residual antinutrients especilally the phorbol esters. The thrust of the study was to evaluate the effect of heat treated and fermented *Jatropha curcas* kernel meals on the blood characteristics and the microbial count of broiler chicks.

2. MATERIALS AND METHODS

2.1 Experimental Site

The research was conducted at the Poultry Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso.

2.2 Source of Test Material

Mature *J. curcas* seeds purchased locally were dehulled to remove the kernel. The kernel was later treated as follows after which all the resultant meals were subjected to fermentation for 7 days using *Aspergillus niger*:

i. **RDFM :-** A portion of the kernel was milled, deoiled using hydraulic press and fermented and was referred to as Raw Defatted Fermented Meal (RDFM).
ii. **TDFM :-** A portion of the milled kernel from (i) was roasted until it becomes crispy to touch and turn brown in a pan. It was stirred from time to time to maintain uniform heating while it lasted for 30 minutes, after which it was fermented as this was referred to as Toasted Defatted Fermented Meal (TDFM)
iii. **CDFM :-** A portion of the raw kernel was cooked at 120^0C ±5^0C for 30 minutes in a cooking pot, sun dried for 24 hours, after which they were oven dried at 85^0C for an hour before being milled, then deoiled using the hydraulic press and fermented as this was referred to as Cooked Defatted Fermented Meal (CDFM).
iv. **LDFM :-** The lye was prepared by putting wood ash in a muslin cloth and hot water (100^0C ± 5^0C) was poured on the ash and the filtrate (pH 9.5) was used to cook the kernel at 120^0C ± 5^0C and held for 30 minutes. The treated kernel was dried, milled, defatted and fermented as this was referred to as Lye Defatted Fermented Meal (LDFM).
v. **ZDFM :-** Raw whole seeds were roasted in sand (particle size of ¼–½ mm) at 115ºC ±5^0Cand held at this temperature for 30 minutes. The roasted seed was cooled, dehulled and kernels were milled, defatted and fermented to produce Sand Roasted-Defatted Fermented Meal (ZRDM).

All meals were at between 0.5-1.0mm mesh size.The defatted kernel meals were kept in polythene bags for autoclaving at 121°C for 30 min, so as to get rid of any microbes that could be present in the meals prior to fermentation. The preparation and sub-culturing of the fungi and innoculation of the substrates follows the procedure as described by Ojediran et al. [13].

2.3 Experimental Diets

Six (6) experimental diets were formulated: Diets 1 contained 0% *Jatropha curcas* Kernel Meals (JCKM) and served as the control diet, while diets 2, 3, 4, 5 and 6 contained 10.33% (one-third replacement of soybean meal) inclusion level of RDFM, TDFM, CDFM, LDFM and ZDFM respectively as shown in Table 1.

2.4 Experimental Birds and Management

One hundred and eighty (180) 1-day old Marshal Strain Broiler Chicks were used for this study. The birds were purchased from a reputable hatchery in Ibadan, Nigeria. All the birds were initially fed on commercial broiler starter mash for the first week to stabilize the chicks after which they were randomly distributed or divided without sexing into six dietary groups of thirty (30) birds each. Each treatmental group was further sub-divided into three replicates of ten (10) birds each. The birds were fed with their respective dietary treatment and water was served *ad-libitum* for three weeks.

Table 1. Gross composition of experimental diets for broiler starter (1-4 Weeks)

Ingredients %	Diet 1 (control)	Diet 2 (RDFM)	Diet 3 (TDFM)	Diet 4 (CDFM)	Diet 5 (LDFM)	Diet 6 (ZDFM)
Maize	54.30	53.50	52.50	53.00	53.50	52.00
Wheat offal	6.00	6.00	7.00	6.00	6.00	7.00
Soybean meal	31.00	20.67	20.67	20.67	20.67	20.67
JCKM	0.00	10.33	10.33	10.33	10.33	10.33
Fish meal	4.70	5.50	6.50	6.00	5.50	6.00
Fixed ingredients	4.00	4.00	4.00	4.00	4.00	4.00
Total(%)	100.00	100.00	100.00	100.00	100.00	100.00
Calculateded nutrient composition						
Crude protein (%)	22.85	22.96	23.07	22.94	22.96	22.78
M.E kcal/kg	2948.28	3057.60	3115.42	3079.82	3064.31	3089.02

*Fixed ingredients-Limesstone-1.35%, Dicalcium phosphate-2.00%, Salt-0.20%, *Vitamine premix-0.25%, Lysine-0.15%, Methionine-0.05%. *Vitamin premix contained the following vitamins and minerals in 1kg of broiler diet: 12500 IU Vit. A; 2500 IU Vit. D_3; 40mg Vit.E; 2mg Vit.K_3; 30mg Vit B_1; 55mg Vit.B_2;550mg Niacin; 115mg Calcium Pantothenate; 50mg Vit B_6; 0.25mg Vit B_{12}; 500mg Choline chloride; 10mg Folic acid; 0.08mg Biotin; 120mg Manganese; 1000mg Fe; 80mg Zn; 8.5mg Cu; 1.5mg I; 0.3mg Co; 0.12mg Se and 120mg Antioxidant*

JCKM= Jatropha curcas kernel cake meal, ME= Metabolizable Energy, RDFM= Raw defatted fermented meal, TDFM= Toasted defatted fermented meal, CDFM= Cooked defatted fermented meal, Lye defatted fermented meal, ZDFM= Sand-roasted defatted fermented meal

2.5 Experimental Design and Statistical Analysis

The experimental design is a Complete Randomised Design. All data generated and estimated were subjected to One-way Analysis of Variance using of SAS, [14] software package. Significant means were separated using Duncan's multiple range test of the same package.

2.6 Data Collection

2.6.1 Blood chemistry analysis

Three birds per treatment were randomly selected and slaughtered by cutting the jugular vein. 5ml of blood was collected into two sets of three sterilized glass bottles/tubes. For haematology, blood samples were collected into two sets of three sterilized bottles containing Ethylene Diamine Tetra-acetic Acid (EDTA). Blood samples for serum biochemical studies were collected into plain bottles (i.e without anticoagulant) for serum seperation. Serum was obtained by centrifugation and serum samples were stored in a deep freezer (at minus 10^0C) until required for analysis.

Blood parameters such as packed cell volume (PCV) and haemoglobin (Hb) were determined using the micro haematocrit method and cyanmethemoglobin methods respectively as described by Mitruka and Rawnsley [15]. Erythrocyte count (RBC) and Leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after the appropriate dilution [16]. Differential leukocyte counts were determined by scanning Giemsa's stained slides in the classic manner [16].

Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular volume (MCV) were calculated using the following formula:

MCH = Haemoglobin/RBC × 10; MCHC= Haemoglobin/Hematocrit x 100; MCV = Hematocrit/RBC x10.

Cholesterol was determined by spectrophotometric methods. Alanine Transaminase (ALT), Aspartate amino Transaminase (AST) and Alanine phosphatase (ALP) was determined manually by spectrophotometric method respectively as described by Schmidt [17]. Albumin was determined using the BCG (Bromocresol green) method as described by Peters et al. [18].

2.6.2 Microbial count

2.5 ml of jejunum digesta was collected into sterilized glass bottles/tubes for the Microbial evaluation from the birds slaughtered above. Mann Rogosa Sharpe (MRS) agar was used for of *Lactobacillus* growth colony count while MacConkey Agar no. 3 was used for the Isolation of *E. coli.* The composition of various media is as reported by Ojediran et al. [12].

3. RESULTS AND DISCUSSION

The heamatological parameters of birds fed the experimental diets is as shown in Table 2. RBC, eosinophyll, basophyll, MCV, MCH and MCHC were significantly influenced ($p<0.05$) by the dietary treatments. It is observable that values obtained for RBC and eosinophil had similar trend while this was similar for MCV and MCH. The PCV, Hb, RBC values obtained in this study were in accordance with the normal range (24.9-40.7%) described by Mitruka and Rawnsley [15] and [19] but were lower than the values reported when chicks were fed rubber seed meal [20]. Hb values were similar to the values reported by Diarra and Usman, [21] when broiler chicks were fed boiled mango kernel meal. The range obtained in this study showed that the birds were not anaemic. The high level of eosinophyll in birds fed LDFM and ZDFM could be attributted to destructive type of pulmonary dysfunction as reported by Chattopadhyay et al. [22]. Eosinophyll had been reported to play a primary role in detoxification. It was evident that the birds responded to allergic substances or foreign bodies which could be residual antinutrients as observed by Akande et al. [23]. MCV is an important trait which determines the cell size of erythrocytes and is therefore an important factor in determining the ability of birds to withstand prolonged oxygen starvation [15].

The results of the serum biochemical constituents are presented in Table 3. The results showed that alanine transaminase, cholesterol, triglyceride and acid phosphatase were significantly different ($P<0.05$) among birds on the dietary treatments. The values obtained for total protein, albumin, globulin, creatinine and aspartate transaminase (AST) were not affected by the treatments ($P>0.05$). A non significant increase ($P>0.05$) in creatinine and AST was observed among the birds fed processed

J. curcas kernel meals. All significant parameters followed no consistent pattern.

Various researchers have been reported that serum biochemical constituents are positively correlated with the quality of the diet [11,24]. Total serum protein is influenced by breed, age, physiological state, environment and antigen exposure and levels can be extremely variable [25]. When there is increase in level of globulin in the blood the following disease condition may be suspected; kidney dysfunction (decreased in nephrosis, a condition in which the kidney does not filter the protein from the blood and it leaks into the urine), liver dysfunction or damage, acute hemolytic anaemia and leukemia [26]. It was observed that the birds had oedema of feet especially in TDFM fed birds and this is an indication of kidney failure. Increase (P>0.05) in creatinine in birds fed processed-fermented JCKM than control and this is indicative of kidney failure as birds were found to have pedal oedema caused by the effect of antinutrient in test ingredient on the kidney [27]. The high amount of AST in the blood can directly be related to the extent of liver and muscle tissue damage [28] which Voss et al. [29] attributted to toxins or infection. The high values recorded for treatment is similar to work of Belewu et al. [3]. Alkaline phosphatase is an enzyme found throughout the body. Like all enzymes, it is needed in small amounts to trigger specific chemical reactions. When it is present in large amounts, it may signify bone or liver disease or a tumor [30]. According to Mitruka and Rawnsley, [15], the normal range of serum cholesterol in chicken is 52.0-148mmol/L. High serum cholesterol level is a pointer to heart related disease such as arteriosclerosis and high blood pressure but low cholesterol is a good sign of good health. The ACP values were all outside the range (23.0-41.6U/L) described by Mitruka and Rawnsley, [15]. Despite the detoxification achieved with processing methods, the toxins residue particularly in fermented and boiled might have posed serious health challenge to the birds with under prolong exposure.

The lactobacilli count was more than the *E. coli* across the dietary treatments in the gut of the broiler chicks. The result of this study showed that the anti-microbial properties in the latex of *Jatropha curcas* are against both gram positive (*lactobacillus*) and gram negative (*E. coli*) bacteria. This result agreed with the findings of [31]. The action of these plants on microorganisms have been found to be due to the presence of certain substances such as alkaloids, glycosides, volatile oils, gums, tannins, steroids, saponins, phlobatannins, flavonoids and a host of other chemical compounds referred to as secondary metabolites that are present in them [32-34]. Since it has been observed that various treatment methods could reduce antinutrients [3,5,9,23,35-36] in plant origin feedstuffs, definitely this reduction will reduce the efficacy of the treated samples to reduce gram negative and or positive microbes in the birds fed such fed sample as it is evident in birds fed TDFM and LDFM as against the unfermented JCKM fed to broilers [13].

Table 2. Haematological parameters of broiler chicks fed processed and fermented *Jatropha curcas* kernel meals

Parameters	Control	RDFM	TDFM	CDFM	LDFM	ZDFM	SEM
PCV(%)	27.17	29.33	27.33	27.83	28.67	27.67	±0.14
Hb(g/dL)	9.05	9.78	9.11	9.28	9.28	9.22	±0.15
RBC($\times10^3$/µL)	2.66^{ab}	2.22^{b}	2.27^{ab}	3.09^{a}	2.74^{ab}	2.68^{ab}	±0.10
T WBC($\times10^6$/µL)	15.67	12.43	13.82	12.43	15.40	16.08	±0.52
Heterophil($\times10^6$/µL)	26.67	19.67	25.00	27.83	25.67	23.33	±1.31
Lymphocyte($\times10^6$/µL)	66.50	74.00	68.83	64.83	66.67	69.00	±1.27
Monocyte($\times10^6$/µL)	4.50	3.67	4.00	4.33	3.50	3.17	±1.19
Eosinophil($\times10^6$/µL)	3.50^{ab}	3.00^{ab}	2.00^{b}	3.00^{ab}	4.00^{a}	4.50^{a}	±0.26
Basophil($\times10^6$/µL)	0.50^{a}	0.00^{b}	0.17^{ab}	0.00^{b}	0.17^{ab}	0.17^{ab}	±0.06
MCV(FL)	104.26^{b}	138.44^{a}	107.67^{b}	98.11^{b}	107.41^{b}	104.95^{b}	±4.33
MCH(Pg)	34.73^{b}	46.14^{a}	35.88^{b}	32.69^{b}	34.87^{b}	34.97^{b}	±1.43
MCHC(g/dL)	33.31^{a}	33.32^{a}	33.32^{a}	33.32^{a}	32.53^{b}	33.23^{a}	±0.09

$^{a, b, c, d}$ = means within the same row bearing different superscripts differ significantly

PCV- Packed cell volume, Hb-haemoglobin, RBC-Red blood cell, T WBC- Total white blood cell, MCV-mean copuscular value, MCH-mean corpuscular haemoglobin, MCHC-mean corpuscular haemoglobin concentration. RDFM= Raw defatted fermented meal, TDFM= Toasted defatted fermented meal, CDFM= Cooked defatted fermented meal, Lye defatted fermented meal, ZDFM= Sand-roasted defatted fermented meal

Table 3. Serum chemistry of broiler chicks fed processed and fermented *Jatropha curcas* Kernel meals

Parameters	Control	RDFM	TDFM	CDFM	LDFM	ZDFM	SEM
Total protein (g/dl)	2.66	2.43	2.69	2.16	2.57	2.46	0.10
Albumin (g/dl)	1.52	1.52	1.42	1.48	1.59	1.73	0.70
Globulin (g/dl)	1.13	0.90	1.27	0.68	0.98	0.72	0.10
Creatinine (mg/d)	0.49	0.55	0.58	0.74	0.55	0.50	0.04
AST(U.l/l)	93.50	111.72	107.90	111.22	104.40	100.03	0.29
ALT(U.l/l)	4.73^{a}	4.10^{ab}	3.63^{ab}	1.89^{ab}	2.47^{ab}	4.35^{a}	0.33
ALP(U.l/l)	20.22^{b}	24.64^{a}	18.52^{bc}	10.63^{d}	15.50^{c}	14.95^{c}	0.88
Cholesterol (mg/d)	100.53^{bc}	77.03^{d}	92.20^{cd}	104.93^{bc}	125.53^{a}	114.60^{ab}	3.34
Tryglyceride (U.l/l)	69.25^{a}	35.50^{c}	47.39^{bc}	48.67^{bc}	54.30^{ab}	49.18^{bc}	2.27
ACP (U.l/l)	39.11^{a}	32.59^{b}	28.14^{b}	30.27^{b}	30.74^{b}	40.55^{a}	0.98

$^{a, b, c, d}$ = means within the same row bearing different superscripts differ significantly
AST: Aspartate amino transaminase, ALT: Alanine amino transaminase, ALP: Alkaline phosphatase
ACP: Acid phosphatase, RDFM= Raw defatted fermented meal, TDFM= Toasted defatted fermented meal, CDFM= Cooked defatted fermented meal, Lye defatted fermented meal, ZDFM= Sand-roasted defatted fermented

Table 4. Effect of processed-fermented JCKM diets on *Lactobacillus* and *Escherichia coli* population in the gut of broiler chicks

Treatment	*Lactobacillus*x10^3	*E.coli*x10^3
Control	14.40	13.70
RDFM	7.00	4.00
TDFM	16.60	12.20
CDFM	4.40	3.10
LDFM	23.00	20.50
ZDFM	7.35	6.25

RDFM= Raw defatted fermented meal, TDFM= Toasted defatted fermented meal, CDFM= Cooked defatted fermented meal, Lye defatted fermented meal, ZDFM= Sand-roasted defatted fermented meal

4. CONCLUSION

The haematological and serum biochemical indices observed showed that broiler chicks fed processed-fermented JCKM were not anaemic, although prolong exposure might posed serious health challenge to the birds. It can also be concluded that *Lactobacillus* thrived in the gut of the broiler chicks than *E. coli*, although the populations of both were reduced.

ETHICAL APPROVAL

All authors hereby declear that ‘‘principles of laboratory animal care (nih publication no. 85-23. Revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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