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PERFORMANCE AND HEMATOLOGY INDICES OF BROILER CHICKENS FED MONO-CULTURE FUNGAL FERMENTED MANGO (*MANGIFERA INDICA*) KERNEL CAKE

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A 4-week feeding trial was conducted to investigate the effects of replacing maize with different mono-culture fungal fermented Mango kernel Cake at 20% inclusion level on performance and hematology indices in starter broiler chickens. Three hundred and sixty 1day-old broiler chicks were randomly allocated to six treatment groups of 60 chicks each in a completely randomized design. The treatments were the Control, Unfermented mango kernel cake (UMKC), *Rhizopus oligosporus* fermented mango kernel cake (Ro), *Aspergillus niger* fermented mango kernel cake (An), *Rhizopus stolonifer* fermented Mango kernel cake (Rs) and *Penicillium chrysogenum* fermented mango kernel cake (Pc). The highest total body weight gain of 494.45g was observed with birds fed control diet which was not significantly (p> 0.05) different from birds fed diets Ro (469.66g), An (483.92g) and Pc (492.52g) but differ (p< 0.05) from UMKC (408.04g). Birds fed Pc diet had higher feed intake that was significantly (p< 0.05) different from other treatments. Birds fed UMKC had a significantly lower protein, fibre and ash retention and lower feed efficiency than other treatments. However, fat retention does not differ (p> 0.05) among treatments. The hematological parameters of the birds fed different diets were within normal range except the values of PCV (16.00%) obtained for the birds fed UMKC which was significantly lower (p< 0.05) than other treatments. Fungi fermented mango kernel cake could be used to replace maize in diet of broiler starter without deleterious effects on performance, nutrient retention and hematological indices.

Key words: Mango Kernel Cake, Performance, Hematological indices, Broiler chicken

The global decline in food and feed reserves created impetus for the exploitation of seeds from tropical trees and plant residues that are presently wasted and are pollution hazards. Some of these tropical seeds are rich sources of plant protein, fat and carbohydrates. The use of carbohydrate-rich plant residues as substrates for improving the nutrient of foods or feeds by solid state fermentation using mono-culture of microorganisms has been given attention since the last decade. This stalwart attention partly results from the general awareness of the menace of malnutrition in the developing countries and the need to find a lasting solution to it. In the same vein, waste problems resulting from the production and processing of food intensifies. Thus, the synthesis of microbial protein from these by-products and waste could be a beneficial way of combating the problems of malnutrition and waste disposal (Hutagalung, 2006). In Nigeria and many developing countries around the world, the needs for protein sources are on the increase. Consumption of protein has been reported to

be 45g/day/person of which animal protein accounted for 17 g (USDA, 2012). As a result, efforts have been intensified to increase animal protein sources especially poultry meat in these countries. However, much success has not been made due high cost of feed for raising livestock. This necessitates the use of various agro-industrial wastes and seeds of some tropical trees as alternative source of feed for livestock (Martens et al., 2012). Mango (*Mangifera indica*) is one of the seeds common in Nigeria. Although the seeds of these tropical trees occur generally in the rural areas and are found in the cities when the whole fruits are transported to the markets for sales in Nigeria, the potential of the seeds for animal feed has not been fully exploited. In addition, these seeds contain anti-nutritional factors that may limit their utilization as livestock feed. It is quite possible to reduce or inhibit the anti-nutritional factors of these tropical seeds to improve the nutritive value of the seeds, thus improving livestock productivity. The objective of this study was to determine the effect of replacing maize with mono-culture fungi fermented mango kernel cake on performance and hematological indices in starter broiler chicken.

MATERIALS AND METHODS

Source and processing of mango fruits into mango kernel cake

One tonne of matured whole mango *(Mangifera indica)* fruits locally called "Oori" but commonly referred to as "Ogbomoso mango" was purchased from some mango plantations in Ogbomoso town, Oyo State, Nigeria. The peels and pulp of the mangoes were removed by washing in distilled water. Seeds were separated and cracked manually to remove the shells and hulls. The kernels were dried in the oven at $60\pm1\ ^0C$ for 24 h and ground using a local grinder to obtain mango kernel meal (MKM). The oil was extracted with n–hexane, using a giant soxhlet extractor containing one kilogram of the kernel meal at a time for 3 h to obtain mango kernel cake (MKC).

Mono-culture fermentation of mango kernel cake

Four fungi namely *Aspergillus niger, Rhizopus oligosporus, Penicillium chrysogenum* and *Rhizopus stolonifer* were obtained from naturally decomposed mango kernel as described by Kayode and Sani (2008). The suspensions of actively growing mid-log phase cultures of the fungi were separately prepared according to the methods described by Sani *et al*. (1992). One-kilogramme of autoclaved (sterile) mango kernel cake was mixed with one litre of sterile distilled water in a fermenter and stirred properly to obtain a uniform mash. Twenty mililitres from each of the mono-culture suspension ($5x10^4$spore/ml) was used as fermentation starter to inoculate each of the cakes in the fermenter before fermentation at ambient temperature ($27\pm5\ ^0C$) for 168 h (Kayode and Sani, 2008). Fermentation process of the MKC was terminated by autoclaving at 121 ^0C for 30mins, followed by drying in a Gallenkamp oven at 60 ± 1^0C until the moisture of dried MKC was between 7.0 and 11.0%.

Experimental diets

Six dietary treatments (1) were formulated to meet the NRC (1984) nutrient requirement of broilers. The experimental diet were the control (without mango kernel cake), Unfermented mango kernel cake (UMKC), *Rhizopus oligosporus* fermented Mango kernel cake, *Aspergillus niger* fermented Mango kernel cake, *Rhizopus stolonifer* fermented mango kernel cake and *Penicillium chrysogenum* fermented mango kernel cake. The diets were iso-nitrogenous (23% crude protein) and iso-caloric (2700 kcal/kg) as recommended by Oluyemi and Robert (1979). The test ingredients were incorporated as described in Table 1.

Management of experimental birds

Three hundred and sixty 1day-old broiler chickens were used for the trial. The birds were randomly allocated to six dietary treatments in a completely randomized design. Each treatment was replicated six times with ten birds per replicate. The chickens were reared in an electrically heated metabolic cage with feed and water

Table 1: Ingredient Composition of experimental diet

Ingredient	Diet composition in g/100g					
	Control	Umkc	Ro	An	Rs	Pc
Maize	45.00	36.00	36.00	36.00	36.0	36.00
Mango kernel cake	-	9.00	9.00	9.00	9.00	9.00
Soya bean meal	15.00	15.00	15.00	15.00	15.00	15.00
Groundnut cake	20.00	20.00	20.00	20.00	20.00	20.00
Brewers dried grain	10.00	10.00	10.00	10.00	10.00	10.00
Wheat offal	4.95	4.95	4.95	4.95	4.95	4.95
Fish meal (72%)	1.50	1.50	1.50	1.50	1.50	1.50
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	0.30	0.30	0.30	0.30	0.30	0.30
Salt (NaCl)	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25

Key: UMKC= Unfermented mango kernel cake, Ro= *Rhizopus oligosporus* fermented mango kernel diet, An= *Aspergillus niger* fermented mango kernel cake, Rs= *Rhizopus stolonifer* fermented mango kernel cake, Pc= *Penicillium chrysogenum* fermented mango kernel seed cake.

Table 2: Proximate composition (g/100g DM) of experimental diet

Nutrient composition	Dietary treatments					
	Control	Umkc	Ro	An	Rs	Pc
Dry matter						
Crude protein	23.92	23.57	23.95	24.15	24.03	23.81
Crude fat	5.28	7.50	5.56	4.81	6.23	6.23
Crude fibre	4.37	2.69	3.50	3.97	2.50	4.00
Total ash	7.20	7.18	9.08	8.60	7.95	9.96
Nitrogen free extract	59.23	59.06	57.91	58.47	59.29	57.27
Metabolizable energy (Kcal/kg)*	3065.2	3045.7	3056.2	3059.5	3012.0	3056.8

Key: UMKC= Unfermented mango kernel cake, Ro= *Rhizopus oligosporus* fermented mango kernel diet, An= *Aspergillus niger* fermented mango kernel cake, Rs= *Rhizopus stolonifer* fermented mango kernel cake, Pc= *Penicillium chrysogenum* fermented mango kernel seed cake. *= Metabolizable energy was obtained by calculation (Janssen *et al*. 1979)

supplied *ad-libitum*. Prior to the arrival of the day old chickens, the metabolic cage, feeders and drinkers were properly cleaned and disinfected with Morigad. Flat feeder's trays and fountain drinkers were used. Electrical bulbs (100 watts) were provided as source of heat for brooding. Ventilation was adequate; the brooding temperature of 32–35 ^{0}C was attained according to Oluyemi and Robert (1979). Routine medication (Vaccine and drugs) were administered as at when due. **T**he broilers chicks were weighed and randomly allocated to the dietary treatments. Records of feed consumption and body weight were taken weekly. Body weight gain and feed to gain ratio was estimated from the data collected.

STATISTICAL ANALYSIS

The experiment followed a completely randomized design (CRD). Data collected were subjected to one-way analysis of variance using the SAS (1999) software and means were separated using Duncan

Table 3: Performance characteristics of starter broiler chicken fed Mango kernel cake

Parameters	Dietary treatments						
	control	Umkc	Ro	An	Rs	Pc	SEM
Initial weight g/bird	31.84	33.14	33.85	31.88	33.96	31.54	
Final weight g/bird	526.28^{c}	441.18^{a}	503.61^{bc}	515.80^{bc}	463.51^{b}	523.44^{c}	580.63
Total weight gain	494.45	408.04	469.66	483.92	470.88	492.52	38.32
Weight gain (g/bird/week)	123.61^{b}	109.51^{a}	117.42^{a}	120.98^{ab}	117.72^{ab}	123.13^{b}	14.21
Feed intake (g/bird/week)	302.85^{a}	290.53^{a}	297.07^{a}	304.87^{a}	294.30^{a}	330.00^{b}	95.28
Feed conversion ratio	2.45	2.85	2.53	2.52	2.50	2.68	0.32
Mortality	-	4.7	-	-	-	-	-

a, b, c means having different superscript along the same row are significantly different ($p< 0.05$)

Key: UMKC= Unfermented mango kernel cake, Ro= *Rhizopus oligosporus* fermented mango kernel diet, An= *Aspergillus niger* fermented mango kernel cake, Rs= *Rhizopus stolonifer* fermented mango kernel cake, Pc= *Penicillium chrysogenum* fermented mango kernel seed cake.

multiple range test at significant level of 0.05.

RESULTS

Performance characteristics

Performance data of broilers fed diets containing Mango kernel cake (MKC) at 20% replacement value for maize at the starter phase is presented in Table 3. The highest final body weight of 526.28g/bird was observed with birds fed the control diet. This result was not significantly ($p> 0.05$) different from *Rhizopus oligosporus* fermented MKC (503.61), *Aspergillus niger* fermented MKC (515.80), and *Penicillium chrysogenum* fermented MKC (523.44g/bird). All the fermented MKC based diets were significantly ($p< 0.05$) higher than the diet containing unfermented mango kernel cake (UMKC) which gave the lowest final body weight of 441.18g/bird. There was also significant ($p< 0.05$) difference in the weight gain of the birds among the dietary treatments. The values

Table 4: Nutrient retention in starter broiler chicken fed Mango kernel cake

parameter	Dietary treatments						
	Control	Umkc	Ro	An	Rs	Pc	SEM
Dry matter	77.01^{c}	57.61^{a}	68.95^{bc}	68.37^{bc}	65.06^{b}	63.77^{b}	8.97
Crude protein	84.71^{d}	70.82^{a}	83.29^{c}	80.65^{b}	79.83^{b}	80.59^{b}	7.54
Crude fat	87.47^{b}	77.51^{a}	87.42^{b}	85.90^{b}	86.22^{b}	87.85^{b}	7.34
Crude fibre	61.27^{c}	50.75^{a}	57.62^{b}	56.30^{b}	58.24^{b}	55.51^{ab}	8.47
Total ash	75.91^{abc}	68.61^{a}	77.32^{c}	73.53^{ab}	73.36^{ab}	74.22^{b}	10.74

a, b, c means having different superscript along the same row are significantly different ($p< 0.05$)

Key: UMKC= Unfermented mango kernel cake, Ro= *Rhizopus oligosporus* fermented mango kernel diet, An= *Aspergillus niger* fermented mango kernel cake, Rs= *Rhizopus stolonifer* fermented mango kernel cake, Pc= *Penicillium chrysogenum* fermented mango kernel seed cake.

obtained for diets *Aspergillus niger* fermented MKC (120.98g/bird/week), *Rhizopus oligosporus* fermented MKC (117.72g/bird/week) and *Penicillium chrysogenum* fermented MKC (123.13g/bird/week) were significantly ($p< 0.05$) higher than the unfermented treatment which had the lowest body weight gain (109.51g/bird/week). There was significant ($p< 0.05$) difference in the weekly feed intake of *Penicillium chrysogenum* fermented MKC (330.00g/bird/week) compared with other treatment which were not significantly ($p> 0.05$) different from the control. The least feed intake was observed with the birds fed UMKC (290.53g/bird/week). The feed conversion ratio differs significantly ($p< 0.05$) with birds fed UMKC having the highest value. There was no significant difference among the fermented MKC and the control birds

Nutrient retention

The nutrient retention of broiler chicks fed diets containing the mono-culture fermented mango kernel cake at 20% replacement value for maize is shown in Table 4. There was significant difference in the dry matter intake of the fermented MKC diets compared with the unfermented MKC diet. The values obtained ranged between 63.77 to 68.95% for birds fed the fermented cake. While, the birds fed the control diet gave the highest value (77.01%) and those fed the unfermented cake (UMKC) gave the least value (57.61%). The crude protein retention was significantly ($p< 0.05$) higher in control (84.71%) and *Rhizopus oligosporus* fermented MKC (83.29%) than other treatments. Birds fed UMKC gave the least crude protein retention (70.82%). Significant ($p< 0.05$) increase was shown in the crude fibre retention, which range from 55.51 to 58.24% and ash retention, which range between 73.36 to 77.32 % in the fermented MKC diets in comparison to the unfermented MKC diet. There was no significant difference in the crude fat retention of birds fed various fermented mango kernel cake at the same replacement value for maize. Values obtained range between 85.90 to 87.85% for all the fermented diets and the control and were significantly higher than the value (77.51%) obtained for the diet containing the unfermented mango kernel cake. The only mortality that occurred was 4.7% among the birds fed unfermented MKC (Table 3). Nutrient retention of broilers fed monoculture fungal fermented MKC based diets were higher ($p< 0.05$) than those fed unfermented MKC. Protein retention of broilers fed the mono-culture fungal fermented diets (77.07–83.29%) and control (84.71%) were higher ($p< 0.05$) than those fed unfermented MKC based diet (70.82%).

Hematological parameters

Table 5 shows the hematology parameters and the immune status of broilers fed at 20% replacement for maize at the end of 4weeks. The range of white blood cells (12.05-13.07x10^{12}/l), packed cell volume (16.00-28.00%), red blood cell (1.59-2.27x10^{12}/l) and Hemoglobin (5.12-8.62g/dl) and the absolute erythrocytic indices MCH (28.13-39.62pg), MCV (100.62-125.79fl) and MCHC (25.60-33.44g/dl) obtained for all the diets were within the normal literature range for experimental birds, except the values of PCV (16.00%) obtained for the birds fed unfermented mango kernel cake. This value was significantly lower ($p< 0.05$) than other treatment diets including the control. There was significant ($p< 0.05$) difference in the value of lymphocytes count (87.00%) of broiler chickens fed *Penicillium chrysogenum* fermented MKC when compared with other treatment diets including control. The results showed significant differences ($p< 0.05$) among the mean values obtained for the various hematological parameters and immune status of the birds.

DISCUSSION

All the broiler chickens fed monoculture fungal fermented MKC at 20% replacement value for maize gained appreciable weight (469.66 to 492.52g/bird) in comparison with the weight gain (408.04g/bird) of the birds fed diet containing unfermented MKC at 20% replacement for maize during the trial (Table 3). The fermented MKC diets showed significantly ($p< 0.05$) higher values of feed intake and nutrient retention. In addition, the subsequent higher values of weight gain and lower feed conversion ratio of the birds

Table 5: Hematology indices of starter broiler chicken fed Mango kernel cake

Parameter	Dietary treatments						
	Control	Umkc	Ro	An	Rs	Pc	SEM
PCV (%)	22.00^{bc}	16.00^{a}	20.00^{b}	20.00^{b}	23.00^{c}	28.00^{d}	6.12
WBC ($x10^9$/l)	12.33^{ab}	12.14^{ab}	12.47^{ab}	13.07^{b}	12.34^{ab}	12.05^{a}	0.17
RBC ($x10^{12}$/l)	1.82^{ab}	1.59^{ab}	1.82^{a}	1.59^{ab}	1.86^{ab}	2.27^{b}	0.08
Hb (g/dl)	7.10^{c}	5.35^{ab}	5.12^{a}	6.30^{b}	6.70^{c}	8.62^{d}	0.29
MCV (fl)	120.88^{c}	100.62^{b}	109.89^{a}	125.79^{c}	123.66^{c}	123.35^{c}	41.82
MCH (pg)	39.01^{b}	33.65^{ab}	28.13^{a}	39.62^{b}	36.02^{ab}	37.97^{b}	12.68
MCHC (g/dl)	32.27^{b}	33.44^{b}	25.60^{a}	31.50^{b}	29.13^{a}	30.79^{b}	12.25
Neutrophil (%)	21.00^{b}	25.00^{c}	29.00^{d}	22.00^{b}	23.00^{bc}	13.00^{a}	15.44
Lymphocytes (%)	79.00^{bc}	75.00^{ab}	71.00^{a}	78.00^{abc}	77.00^{b}	87.00^{c}	15.44

a, b, c means having different superscript along the same row are significantly different ($p< 0.05$)
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indicated that the fermented MKC diets were adequate and well tolerated by the broilers than the unfermented MKC diet. The significant differences in feed intake and the weight gain of the birds among the fermented dietary treatments may be due to the reduction of anti-nutrients in the monoculture fungal fermented MKC and the synergistic interactions of the metabolic products of fermentation in the diets. The result of hematology parameters which differed significantly ($p< 0.05$) as shown in Table 5 still fall within the literature range as established by Mitruka and Rawnsley (1977). The values obtained for PCV (16.00%), which resulted in a significantly ($p< 0.05$) lower MCV (100.62fl) obtained for birds fed unfermented MKC may be attributed to muscular dystrophy or stifle development of the muscles of the chickens (Chauhan and Agarwal, 2000). While, the significantly low level of MCHC obtained for birds fed *Rhizopus oligosporus* fermented MKC may be attributed to microcytic anaemia (Adewuyi, 1995; Ochei and Kolhatkar, 2008). The significantly ($p< 0.05$) higher values of lymphocytes counts (87.00%) recorded for *Penicillium chrysogenum* fermented MKC may be attributed to conditions of hyperthyroidism or perhaps there was an insufficient adrenocortical production by the broiler chickens fed the diet (Chauhan and Agarwal, 2000). However, it was observed that most of the hematological parameters were not adversely affected in the broiler chickens fed on the mono-culture fungal fermented mango kernel cake. The weight gain of control (123.61g/bird/week) which was significantly ($p< 0.05$) higher than the values of UMKC (109.51g/bird/week) and *Rhizopus oligosporus* fermented MKC (117.42g/bird/week) could be due to the ability of the birds to retain more nutrients from the diets, which aid muscle development and subsequent increase in the total weight gain (Table 3).

CONCLUSION

Fermented mango kernel cake was capable of replacing maize at 20% in the diet of starter broiler chicks. The growth performance, nutrient retention and hematology indices obtained in birds fed mono-culture fungal fermented MKC was comparable to those fed maize based diet. Solid state fermentation of mango kernel cake using mono-culture of natural mycoflora of mango kernel improved the nutritional characteristics of mango kernel cake and reduced the inherent anti nutrients in MKC to a tolerable level by broiler chickens.

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