



Research Article

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# *Rhizopus Oryzae* May Reduce Toxic Effects of Raw Mango Seed Kernel Amendment in the Diet of Broilers



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## Abstract

Studies on the histological and haematological indices of broilers fed *Rhizopus oryzae* fermented mango (*Mangnifera indica*) seed kernel was conducted. Mango seed kernels were seeded with *Rhizopus oryzae* and allowed to ferment for ten days. Bird fed fermented mango seed kernels had a mean weight of 2.35kg before slaughtering and 2.2kg after slaughtering. The mean weight of the organs were 13.3g for lungs, 8.5g for heart, 4.1g for the intestine, 1.3g for spleen, 47.9g for liver, 5.4g for bursa and 11.2g for kidney. Histological observation revealed hemorrhages and necrosis in the lung and intestine of the birds and the bursa was inflamed. The mean haematological value was 25.5% for PCV,  $2.10 \times 10^6$  M/ $\mu$ l for RBC and  $21.65 \times 10^3$  M/ $\mu$ l for WBC. The full blood count revealed that the chicken had neutrophils value of 9%, lymphocytes 86%, monocytes 5% and eosinophils 1.0%. In conclusion, *Rhizopus oryzae* could be exploited to reduce the toxic effects of raw mango seed kernels and 50% of maize could be replaced with fermented mango seed kernel in the diet of broilers without adverse effect on growth, histological and hematological indices.

**Keywords:** Broilers; Bursa; Mangnifera indica; Rhizopus oryzae; Poultry feed

## Introduction

The production of most domestic farm animal species has increased over the years and this trend needs to be continued over the near future. The world population is rapidly growing, so poultry meat has to be well positioned to meet demand for increased supply. Poultry is the quickest source of meat and its production involves the least hazardous and arduous process in relation to other livestock enterprises. Although it is impossible to produce meat that are guaranteed to be free from pathogens, but it is relatively free from some pathological, ecological and economical constraints which effects the commercial production of other breeds and classes of livestock in Nigeria [1]. Poultry has seen the greatest increase in production and again, this trend will likely continue poultry nest is well positioned to meet demands for increased supply from our out growing population [2].

To some extent chicken is an affordable source of meat and it is first or second for per capita consumption. This competitive situation has occurred due to continued improvements in efficiency of production that often necessitate acceptance

of how new ideas and innovation by poultry producers. The broiler chicken industry has shown unparalleled growth over the last 30 years [2]. As livestock production is becoming more commercialized in response to increased consumer demand, a major consideration is the extent to which a particular country can satisfy increased demand for feedstuffs on its own account and the extent to which it will have to rely on imports. However an obvious trend occurring is that this population is quickly aging and also living in urban settings of ever increasing size. This increase in size is developing more in developing countries especially Africa and Asia.

In recent time the world is facing so many challenging of climate change some of which include adverse environmental conditions resulting to poor yield and eventual food shortage. Africa has always had difficulty feeding its' growing population, and with increased urbanization, this situation is expected to deteriorate further. The climate is rapidly changing; the production of maize cannot keep pace with its demand for food. Many plant species such as corn and soybean

are now routinely genetically modified and used as ingredients for poultry in many countries. However, this is not the case in Nigeria and maize which is short in supply and expensive is main energy source. It is therefore necessary to look for locally available safe, cheap and nutritionally adequate substitutes for maize in poultry feed formulation. Mango kernel could be useful in this regard as a substitute because it is a waste, and causes environmental pollution.

Mango (*Mangifera indica*) is a tree crop well adapted to all ecological zones in Nigeria and the trees are found all over the country [3]. Its protein is comparable to that of maize but it has higher fat than maize. Mango kernel, a by-product of mango pulp is reported to be a good source of starch [4]. In India, mango kernel is consumed by human beings in form of porridge [4,5] but in Nigeria, it is regarded as waste thus contributing to environmental pollution. Many cultivars of both indigenous and improved mango seed (containing the kernel) poses a serious environmental problem because it has no food, feed and industrial use in the area. There are few reports on the use of mango kernel in livestock feeding but the level of inclusion in poultry diet has been low because of presence of tannins which have been reported to reduce chick growth [3,6]. Recently replaced 20% of dietary maize in broiler chickens with raw mango kernel meal and observed no significant differences in performance between the control kernel meal diets because of its abundance availability and cheap cost, it would be interesting to investigate higher levels of mango kernel meal in poultry diets [3]. This research work is aimed at evaluating the impact of fermented mango kernel meal will either increase or decrease their weight as well as their internal organs such as heart, lungs, liver, kidney and bursa.

## Materials and Methods

### Experimental design

Completely randomized design was used where 21 day-old broiler chicks (Rhodes Island) were purchased and distributed into 3 groups.

### Experimental diets

Two types of diets were formulated, compounded and fed to the sampled birds for a period of eight (8) weeks.

- o Conventional (poultry) feed
- o Compounded feed with fermented mango seed kernels

### Haematological test

At the end of four weeks of feeding, two birds from each of the replicates was selected at random. Blood was collected by wing venipuncture from the right wing. At day 42, two birds were randomly selected from each replicate for haematological test. Five millilitres (5ml) of blood was gently drawn out with the aid of a 5 ml hypodermic syringe. Three millilitres (3ml) of

the blood was put into a labeled blood collection vial containing Ethylene Diamine Tetra-acetic Acid (EDTA) as anticoagulant and the rest of the blood (approximately 2ml) put into a vial that contained no anticoagulant. The vial with EDTA was gently shaken to facilitate the dissolution of the anticoagulant in order to prevent clotting of the blood. The blood samples which contained no anticoagulants were kept at room temperature for approximately 45 min in order to clot and the serum decanted into clean, labeled tubes. Haematological parameters such as Red Blood Cells (RBC), White Blood Cell (WBC), Packed Cell Volume (PCV) and absolute counts of neutrophils, basophils, eosinophils, monocytes and lymphocytes were recorded according to Jain [7].

### Organ histopathology

The birds were slaughtered per treatment at the end of the eight feed trial; live weight, slaughtered weight, dressed weight and weight of liver, heart, kidney, spleen, lung, and bursa were recorded. The selected internal organs were removed, weighed, fixed in 10% formalin solution and later processed for histopathological examination at the Veterinary Anatomy of Usmanu Danfodiyo University Sokoto, Sokoto as described by Drury and Wallington [8], Ewuola [9].

## Results

**Table 1:** Results of the weights of live and carcass of the broilers.

Feed	Live weight	Weight of carcass
A1	2.5kg	2.3kg
A2	2.2kg	2.1kg
B1	2.7kg	2.6kg
B2	2.3kg	2.1kg
C	3.8kg	3.6kg

The birds (broiler chicken) were weighed before and after slaughtering and the result presented in Table 1. From the result, Chicken fed conventional feed mixed with fermented mango kernel (1:1) had a mean weight of 2.35kg before and 2.2kg after slaughtering, while those fed with fermented mango kernel alone (B1 and B2) had a mean weight of 2.5kg before and 2.35kg after slaughtering, and finally, the control group designated as group C had a weight of 3.8kg and 3.6kg. The control weighed 3.8kg which had higher body weight compared to the rest of the birds.

**Table 2:** Weight of organs of bird after slaughtering (Grams).

Feed	Lungs	Heart	Intestine (section)	Spleen	Liver	Bursa	Kidney
A1	7	4.3	2.1	0.5	22.8	1.6	5.5
A2	6.3	4.2	2	0.8	25.1	3.8	5.7
B1	9.3	6.5	1.5	0.9	35.9	4	8.6
B2	5.5	5.7	1.4	0.9	31.6	2.5	7.6
C	8.6	5.7	1.9	1.6	32.1	Not inflamed	6.1

Internal organs of the individual slaughtered birds such as: lungs, liver, kidneys, heart, spleen and intestines were also weighed (Table 2). The mean weight of organs for birds (A1 & A2) are 13.3g for lungs, 8.5g for heart, 4.1g for intestine, 1.3g for spleen, 47.9g for liver, 5.4g for bursa and 11.2g for kidney. And for birds (B1 & B2) 14.8g for lungs, 12.2g for heart, 2.9g for intestine, 1.8g for spleen, 67.5g for liver, 6.5g for bursa and 16.2g for kidney.

**Table 3:** Result of Histological indices of broilers fed *Rhizopus oryzae* fermented mango seed kernels rations

Feed	Lungs	Heart	Intestine	Spleen	Liver	Bursa	Kidney
A1	Haemorrhage	N	Necrosis	N	N	INF	N
A2	Haemorrhage	N	Necrosis	N	N	INF	N
B1	N	N	Necrosis	N	N	INF	N
B2	N	N	Necrosis	N	N	INF	N
Control	N	N	N	N	N	N	N

**Key:** N: Normal, INF: Inflamed

The mean haematological values of broilers fed with *Rhizopus oryzae* fermented mango seed kernel had a lower PCV than the normal range of PCV found in chickens which is 31-36%. The (A1, A2) had a PCV of 25.5%, the (B1, B2) had a PCV of 26.5% and the control had a PCV of 30%. The normal range for RBC in chicken is 3 - 4M/ $\mu$ l but all the birds had a lower range compared to the normal parameters. Chickens (A1, A2) had an RBC of  $2.10 \times 10^6$ , chickens (B1, B2) had an RBC of  $2.12 \times 10^6$ , and the control (C) had an RBC of  $2.24 \times 10^6$ . For the WBC, chickens (A1, A2) had a low WBC than the normal range (31.56) which is  $21.65 \times 10^3$ , chickens (B1, B2) 1 had  $22.3 \times 10^3$ , and the control had  $32.3 \times 10^3$  (Table 4).

**Table 4:** Mean haematological values of broilers fed *Rhizopus oryzae* fermented mango seed kernels rations.

Haematology	Normal range	Feed A	Feed B	Control
PCV (%)	27 - 35	26.5	25.5	30
RBC ( $\times 10^6$ /M/ $\mu$ l)	4-Mar	2.1	2.12	2.24
WBC ( $\times 10^3$ /M/ $\mu$ l)	31.56	21.65	22.3	32.2

The full blood count of broilers fed with *Rhizopus oryzae* fermented mango seed kernel had a neutrophil of 9, those fed with *Rhizopus oryzae* fermented mango seed kernel and commercial feed had a neutrophil of 9.5 and the control had 16. Broilers fed with *Rhizopus oryzae* fermented mango seed kernel had a lymphocyte of 86, those fed with *Rhizopus oryzae* fermented mango seed kernel and commercial feed had a lymphocyte of 85.5, and the control had 77. Broilers fed with *Rhizopus oryzae* fermented mango seed kernel had a monocyte of 5, those fed with *Rhizopus oryzae* fermented mango seed kernel and commercial feed had a monocyte of 4.5 and the control had 6. Broiler fed with *Rhizopus oryzae* fermented mango seed kernel had an eosinophil of 1.0, those fed with *Rhizopus oryzae* fermented mango seed kernel and commercial feed had an eosinophil of 1.0, and the control had 1.0. And finally broilers fed with *Rhizopus oryzae* fermented mango seed kernel had a basophil of 0, those fed with *Rhizopus*

The gross lesions observed showed intestinal necrosis and haemorrhages (Table 3) in the lungs of birds fed with mixed conventional and fermented mango seed kernel (A1, A2) and intestinal necrosis in birds fed with Fermented mango seed kernel only (B1 & B2), and both groups with inflamed Bursa except the control group which was normal and the rest of the organs were normal in each of the birds such as the heart, spleen, liver, bursa and kidney.

*oryzae* fermented mango seed kernel and commercial feed had a basophil of 0, and the control had 0 (Table 1-5).

**Table 5:** Result of full blood count of broilers fed *Rhizopus oryzae* fermented mango seed kernels rations.

Parameter	Feed A	Feed B	Control	Normal range
Neutrophils (%)	9	9.5	16	-
Lymphocytes (%)	86	85.5	77	64 - 82
Monocytes (%)	5	4.5	6	6-4
Eosinophils (%)	1	1	1	4-2
Basophils (%)	0	0	0	2

## Discussion

Birds fed *Rhizopus oryzae* fermented mango seed kernel alone had higher weight gain compared to that fed *Rhizopus oryzae* fermented mango seed kernel and commercial feed (1:1). There was no much difference observed between the *Rhizopus oryzae* fermented feed and commercial feed in terms of growth performance. This implies that *Rhizopus oryzae* fermented feeds was able to support the broilers chicken energy and growth requirement. Diarra and Usman [10] reported that 20% of maize could be replaced with boiled mango kernel meal in the diet of broilers without adverse effect on growth and blood parameters. Joseph and Abolaji (1996a) observed no adverse effect on broilers which have been fed 10% of raw mango seed kernels and two-fold improvement on the 10% inclusion level of incorporation into broilers chicken rations [11]. This suggests that *Rhizopus oryzae* fermented mango seed kernel is safe for the broilers consumption particularly because it is a waste product.

Gross examination of the internal organs revealed that the organs were normal except for the bursa of fabricius which was inflamed in broilers fed *Rhizopus oryzae* fermented mango seed kernel and commercial feed (1:1) and *Rhizopus oryzae* fermented feed. However, the inflammation was more pronounced in birds fed *Rhizopus oryzae* fermented mango

seed kernel. This may be indication of gumboro disease (highly contagious viral disease). This may not be linked to the feed but may suggest that the two feeding trails may contain certain contaminants such as residual anti nutritional factors such as tannin and cyanide (Mohammad and Oloyede, 2009), microorganisms and/or their toxins, which might have suppress the immune system making them susceptible to viral infection since an important part of immune system.

Histological indices revealed hemorrhage in the lungs of broilers fed *Rhizopus oryzae* fermented mango seed kernel and commercial feed (1:1) while necrosis was observed in the intestine of broilers fed *Rhizopus oryzae* fermented mango seed kernel and commercial feed (1:1) and *Rhizopus oryzae* fermented feed. The hemorrhage may be due to heat stress not due to the feed. Aengwanich and Simarak [12] reported that hemorrhage can caused in the lungs broilers due to heat stress which leads to blood being largely transferred to the lung by increased heart rate making the lung saturated by this fluid, followed by the increasing backpressure from the pulmonary arteries and right ventricle chamber, and caused right ventricular hypertrophy, right atrium enlargement and other tissue hypoxia. The necrosis observed in the birds could be an indication of necrotic enteritis which is an intestinal disease caused by bacteria in dirt, soil, faeces and, to some extent, in the intestinal tracts of healthy chickens. This type of broiler disease is caused by *Clostridium perfringens* [13-15]. Hafiz [16] reported that it is difficult to determine the true cause of enteric disorders in poultry because is of infectious or non-infectious origin. This may suggest that the necrosis observed has no link to the feed.

The PCV of broilers fed *Rhizopus oryzae* fermented mango seed kernel (25.5%) was lower than that of the control and *Rhizopus oryzae* fermented mango seed kernel and commercial feed (1:1). The RBC of broilers fed *Rhizopus oryzae* fermented mango seed kernel ( $2.10 \times 10^6$ ), *Rhizopus oryzae* fermented mango seed kernel and commercial feed ( $2.12 \times 10^6$ ) and the control ( $2.24 \times 10^6$ ) were all lower than the normal range ( $3-4 \times 10^6$ ) and this could be as a result of anaemia. Anaemia is a reduction in the haemoglobin concentration of the blood [17]. There was decrease in neutrophil and monocyte, while lymphocyte had increase. The eosinophils values were similar to those reported by Adeyemo and Longe [18]. Reduction in the haemoglobin may be accompanied by a fall in the red cell count (RBC) and packed cell volume [17]. The major function of the red blood cells is to transport haemoglobin, which in turn carries oxygen from the lungs to the tissues [19]. The WBC of broilers fed *Rhizopus oryzae* fermented mango seed kernel and *Rhizopus oryzae* fermented mango seed kernel and commercial feed were lower than the control. The reductions in the WBC and lymphocytes would predispose the animals to reduced immunological responses to infections. This is because the main function of white blood cell is to combat and prevent infection. A high WBC usually means that the body is fighting

an infection [20]. This reduction in haematological parameter may be due to diseases which were confirmed in the histological indices of the broilers chicken and may not have link to the feed. Available information indicates that haematological values of avian species are significantly influenced by poultry diseases including fowl typhoid [21], mycoplasmosis [22], avian coccidiosis [23], infectious bursal disease [24,25], Newcastle disease [26] and toxoplasmosis [27]. Mohammad and Oloyede [28] reported significant reductions in the haematological contents of the blood of broiler chicks fed *A. niger*-fermented *T. catappa* seed meal-based diet [28-31].

## Conclusion

The result revealed that there was increase in weight of the birds and hemorrhage was found in the lungs of birds and necrosis found in the lungs and liver of chicken fed with *Rhizopus oryzae* fermented mango seed kernel and chicken fed with *Rhizopus oryzae* fermented mango seed kernel and commercial feed. The bursa of these chickens was inflamed. From this result it is concluded that 50% of maize can be replaced with fermented mango seed kernel meal in the diet of broilers without adverse effect on the growth, histological and haematological parameters.

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