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Full Length Research Article

IMPACT OF FERMENTED FRUITS SEEDS AS NUTRIENT ADDITIVES ON THE PERFORMANCE OF BROILER BIRDS

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ABSTRACT

The increase in cost of Broiler Birds' feed, feed ingredients, and food security have raised concerns in exploring crop residues that can be nutritional, affordable and sustainable in Broiler Birds' feed formulation. In this study the potential of fermented mango seeds kernel, watermelon seeds, and African locust beans seed flour using Lactobacillus Plantrum isolated from Ogi and Nono supplemented as additive in Broiler Birds' feeds were evaluated. Three (3) replicate of 72 day old broilers were as seeds in a Randomized Block Design (RBD) of finisher and starter feeds. The results showed an improved performance of the feeds supplemented with fermented fruits seeds as compared to the control. Furthermore, the Anti-nutritional contents were markedly reduced with significant variation ($P \leq 0.05$) across the broiler diets T_2 and T_4 as compared to T_1. This implies that the fermented fruits additives could possibly provide the additional nutritional requirements for growth enhancement in broiler birds. Hence, these fermented fruits can be recommended as alternative additives in the formulation of Broiler Birds' feeds.

Keywords: Fermented Fruit Seeds, Nutrients Additives, Broiler Birds.

INTRODUCTION

Broilers Birds production is the most rewarding business adopted by most Nigeria. (Subhasheree*,* 2018). Unfortunately, one of the major obstacle in Broilers Birds production is high cost of feed and feed additives (Obasa *et al.,* 2017). The cost of broiler birds feeds including the additives is of major concern in broiler bird production since the cost of feed account for 70% - 85% of the total cost of production. (Maidala *et al.,* 2018).

In view of its scarcity and escalating cost, it is imperative to direct more effort toward exploring alternative feed additive that are available, suitable, affordable and sustainable with a good nutritional benefits and can enhance the conversion of the feeds to flesh for broiler feed production. (Moji *et al.,* 2014, Wafja et al., 2017; Mankind *et al.,* 2017).

Previous studies have shown that fermentation play a crucial role in enhancing organoleptic properties, preservation and reduction in Anti-Nutritional Factors (ANF) in feeds additives. (Demir et al; 2016).

However, this fruits seed are limited by several issues such as high and low fibre, protein content, mineral contents and the presence of anti-nutritional factors (ANF)in the alternative feed additives that can decrease feed digestibility and enhances palatability (Sugiharto and Ranjitkar 2019).

Fermentation of many plant materials is often associated with high number of lactic acid bacteria (Sugiharto et al, 2019) which alone or combination exhibit beneficial influence on gut ecosystem, immune functions and growth performances when incorporated into the feeds.

The mango, watermelon and African locust beans are common forest trees found both in wild and residential area of sub-Saharan African the plants are used for their fruit, pulp and seeds. The Mango Seed Kernel (MSK) is a good source of carbohydrate, also containing a number of Anti nutritional factors such as tannins, phytates, cyanide, Antitrypsin, oxalate and saponins which limit its utilization (Diarra, 2014). It also contain high amount of iron potassium, calcium, magnesium, sodium and phosphorus. (Kaur and Baur (2017). Mango seeds kernel can be used as alternative source of Antioxidant, vitamins, in livestock feeds which can be conveniently substituted with maize. If the Anti-nutritional factors are considered carefully. (Fowomola (2010)

Watermelon (Citrulus Lanatus) is a member of the family cucurbitaceae, containing a good sources of vitamin C (Manike et al; (2015). It seeds also contain a significant amount of minerals. It nutritional and minerals composition makes it's a good sources of protein and fibre in livestock diet. (Tabiri et al; (2016).

African Locust beans which is used for its pulps and seeds is said to have a great nutritional benefits African Locus beans which is used for its pulps and seeds is said to have a great nutritional benefits (Ifesan et al; 2017) due to its appreciable content of crude protein, fats, moisture, fibre, and dry matter (Sotean et al: 2014).

MATERIALS AND METHODS

Collection of sample

Samples of water melon, mango seeds, African locust beans seeds, fermented nono and ogi were obtained central market Kaduna, Kaduna state.

ISOLATION OF LACTIC ACID BACTERIA FROM FERMENTED CORN GRUEL (OGI) AND FERMENTED MILK (NONO)

Isolation of Lactic Acid Bacteria from Fermented Corn Gruel (Ogi) and Fermented Milk (Nono)

The media was prepared according to the manufacture's instruction and sterilized by autoclaving at 121 for 15 minutes, and was allow to cool, Twenty five (25) grams of ogi was weighed aseptically and homogenized in 225ml of sterile distilled water. Exactly 1ml aliquot of Fresh cow milk (Nono) and 1 ml homogenized Ogi samples were taken aseptically and serially diluted in separate test tubes up to 10-fold using 0.1% (w/v) bacteriological peptone. Exactly 1ml dilutions of both (Ogi and Nono) samples were each plated out separately in duplicate using spread plate method according to Cheesbrough (2009) on de Man Rosa Sharpe Agar (MRS) for 48 hours at 37^{o}C for the bacteria count. The colonies obtained after incubation were counted using the colony counter and were recorded as colony forming unit per milliliter (cfu/ml) according to the methods of (Oyeleke and Manga, 2008: Mohammed *et al.* 2017). Repeated subcultures of discrete colonies were made on fresh MRS agar to obtain pure cultures (Nwachukwu, *et al.,* 2016). Using the method of Oyeleke and Manga (2008), the pure isolates obtained were preserved in MRS agar slants and stored at 4°C for further identification and characterization.

Morphological Characterization of the Isolates
Colonial morphology such as the colonial shape which included both elevation and margin, arrangement, size, color, consistency as well as murkiness of the colonies (transparent, translucent, or opaque) was observed and recorded accordingly. Furthermore, colonies with shiny glistening surface are recorded as smooth, those that are dull, uneven, coarse, or have lusterless surface as rough, and slimy or sticky forms as mucoid (Bansal et al, 2013). Gram staining was carried out to ascertain the cell morphology and gram reaction.

Biochemical Characterization of LAB Isolated From Fermented Foods
Using standard methods as described by Cheesbrough (2009), biochemical tests which include: Oxidase, catalase, motility, Methyl Red and Voges Proskaur (MRVP), indole, Carbohydrate Fermentation and Citrate Utilization Tests (Oyeleke and Manga, 2008; Mohammed, 2011; Beatrice and Tega 2015) were conducted.

Molecular Identification of isolates by 16 sRNA PCR
Molecular confirmation of isolates was determining according to the 16 SDNA Region. The PCR was amplified and checked using 16s ribosomal DNA primers (27f (8f) forward 5-AGTTTGATCMTGGCTLAG3 and (926r)5-CCGTCAATTMTTRAGTTT-3ˡ).
The entire PCR reaction was loaded onto a 1% agarose gel and correct band size approximately 1000bp was excised, the DNA was recovered from the gel slices, the PCR product was run on of 1% gel e electrophoresis at 90v for 55 minutes and analysis allowed for proper separation of the PCR bands, there by confirmed the amplified Gene products. The PCR product was analysed by sanger sequencing at Inquba biotechnology, laboratory south Africa. The nucleotide sequence of the specific microorganisms isolated was subjected to automated PCR-cycle sequencing analysis was done at applied bio system and the sequence in the forward and reverse was analysed using bio system Gene amp 9700PCR system. The similarly search was conducted using nucleotide basic local alignment search tool (BLAST) at the national center for biotechnology institutes (NCB1) sever.

Procedure for seed processing

Mango Seed Processing
Mango seed kernel was obtained by cutting the mango seeds using a knife, the fresh mango kernel was chopped to reduce particle size, boiled in tap water at 100^0 C for 30 minutes, sundried for 72 hours, oven dried at 60^0 C for 30 Minutes, then milled into mango seeds kernel flour using an electric blender then shelved into an Air tight container till when ready for use as additive in feed formulation (Ibrahim et al; 2017).

Water Melon Seed Processing
Water melon seed was processed after obtained the seeds. The seed were sundried for 24 hours, then oven dried for 30 minutes at 60^0 C, It was then milled using an electric blender, and packed into air tight container till ready to be used as additive in feed formulation.

Parkia seed processing
Parkia pulp seed was extracted by opening the pulp to remove the seeds, then the seeds (300g) was soaked in 400ml of the distilled water for 1 hour. The soaked seeds were dried for 48 hours, then oven dried at 85^0 C for 30 minutes, then milled using an electric blender, then oven dried for 30 minutes, then packed into air tight container till ready to use as additive in feed formulation.

Fermentation Process
A bacterial suspension of 10^8 cells/ml *lactobacillus plantarum* was prepared and standardized using 0.5 Mc Falland's standard. It was then inoculated into each of the plastic container containing 12 kilogram of the fruits seed flours mixed with 800ml distilled water to form the starter culture. It was then covered and allowed to ferment for 72 hours while monitoring the temperature and pH after each 24 hours.
With little modification twelve kilogram of each fruit seed powder was moistened with 800ml of distilled water, then autoclaved at 121^0C for 15 minutes then allowed to cool down at room temperature. The prepared fruits seeds were inoculated with the starter culture and mixed then allow to ferment for 5 days after which the mixture was oven dried at 60^0C for 45 minutes as specified by Lawal (2005).

Procedure for Proximate Analysis

Two grams of each fruits seed flour was subjected to moisture, fibre, fats, protein and carbohydrate analysis

Anti-nutritional Analysis

Tannins
Tannin content of the two seeds samples were determined, (0.6 g) were extracted for 60s at room temperature ($28^oC \pm 2^oC$) with 3 ml) of methanol. Extract was reacted with 3 ml of 0.1M FeCl in 0.1N HCl and 33ml of 0.008M K_3 F_e (CN) $_6$. Absorbance of the colour developed was read at 720 nm. Catechin was used as standard. (AOAC 2006).

Phytates
Exactly 4g of seeds sample was soaked in 100 ml of 2% hydrochloric acid for 3 h and then filtered. 5 ml of 0.3% ammonium thiocyanate solution was added to 25 ml of the filtrate. Exactly 53.5 ml of distilled water was also added to the mixture. This was then titrated against a standard iron (III) chloride solution until a brownish yellow colour persisted for 5 min. The phytate content was calculated from the iron determinations, using a 4:1 iron-to-phytate molecular ratio (AOAC 2006).

Mineral Analysis
Sodium and potassium were determined using Gallenkamp Flame analyzer, while calcium, magnesium, iron, zinc and copper were determined using Buch Model 2005 Atomic absorption spectrophotometer Phosphorus level was determined using the phosphovanadomolybdate colorimetric techniques on JENWAY 6100 Ultraviolent Spectrophotometer Pearson (1976).

Feed formulation

Standard seed concentrated was used in bleeding of fermented fruits seed based on manufactures standards. The ingredients used for broiler feed formulation includes; Fermented fruits seeds (mango seeds kernel, watermelon seed, African locust beans seeds as additives, maize, soya beans meals, groundnut cake, wheat bran, bone meal, lysine, methionine and salt. As shown in Table 1 and 2)

T1 = Control (feed without fermented fruit seeds)
$T2_1$ = (Feed + fermented Mango seeds kernel)
$T3_1$ = (Feed + fermented water melon seeds)
$T4_1$ = (Feed + fermented African locust beans seeds)

Composition of experimental diets for broiler starter

Inclusion for starter diet

Ingredients	Parkia	Mango	Watermelon
Fermentated fruits seed flour	5.5%	5.5%	5.5%
Maize	49.50%	49.50%	49.50%
Soyabeans meal	26.45%	26.45%	26.45%
Groundnut cake	11.00%	11.00%	11.00%
Wheat bran	3:00%	3:00%	3:00%
Bone meal	3:50%	3:50%	3:50%
Lysine	0:25%	0:25%	0:25%
Methionine	0:20%	0:20%	0:20%
Salt	0:30%	0:30%	0:30%

Sources: Aliyu 2015

Composition of experimental diets for broiler finishers

Inclusion for broiler diets

Ingredients	Parkia	Mango	Watermelon
Fermentated fruits seed flour	5.5%	5.5%	5.5%
Maize	54.20%	54.20%	54.20%
Soyabeans meal	26.45%	26.45%	26.45%
Groundnut cake	11.00%	11.00%	11.00%
Wheat bran	3.00%	3.00%	3.00%
Bone meal	3.50%	3.50%	3.50%
Lysine	0:25%	0.25%	0.25%
Methionine	0.20%	0.20%	0.20%
Salt	0.30%	0:30%	0.30%

Source: Aliyu 2015

Experimental Design and Management of Experimental Animal

A total of 70 day-old broiler chicks of mixed sex purchased from a reputable hatchery used for this study on arrival of the chicks were weighed and assigned to 4 group of each was assigned eight replicates each for the experimental diets i.e. fermented products from fermented parkia, mango, watermelon, flour. A control conventional feed formulated without the fermented fruits seed flours. All the necessary routine management practices were obseved and the chicks were also vaccinated against new castle diseases and gumboro throughout the period of the experiment.

Data Collection

The birds were weighed at the beginning of the experiment and weekly thereafter for six weeks. Data were collected based on initial weight, final body weight, average weekly weight gain, feed and water intake.

Analysis of Data

Data obtained were analysed using the ANOVA with Duncan multiple range test used to find significant difference in data collected across the 4 four group (control feed, fermented water melon seeds, fermented mango seeds and fermented African locust beans seeds).

RESULTS AND DISCUSSION

The fermented product ogi and nono analyzed show the presence of lactic acid bacteria. Similarly, the occurrences of Lactic Acid Bacteria (LAB) in locally fermented foods was also documented by Mohammed and Ijah (2013) who reported that isolates isolated and characterized from fermented nono, cheese, (wara) and yoghurt were mostly LAB. Nono had the highest LAB counts of 5.8×10^6 (cfu/mg). While ogi had lowest LAB count of 4.3×10^6(cfu/mg). The LAB was identified as Lactobacillus *plantarum* with (40%), *Lactobacillus Acidophillus* with (20%), *Lactobacillus fermentum* (20%), *Lactococcus Lactis* (10%) and *Lactobacillus casei* with (10%). The fermentation of fruits seeds flour indicate that PH, temperature, microbial load, nti-nutritional factors, nutritional and mineral composition play a crucial role in the shelf – life, palatability, and digestibility of the fermented product. Fermentation changes palatability, beside augmenting the nutritional values and reducing ant nutritional factors of ferments products (Mua'azu et al., 2017). In this current study, fermentation increases the PH of most fermented fruits seed flour. The PH of the fermented mango seed kernel flour was (6.5), while that of watermelon was (6.0) and that of African locust beans decrease (5.0) fermentation.

This is similar with the report documented by Mohammed et al., (2017) who reported decrease in PH of products, turning fermentation and melting of certain cereal wearing food enriched with African locust beans. Thus, increase in PH is attributed to the fermentation condition and ability of *Lactobacillus plantarum* to with stand high PH, and also to dominate the fermentation process. Lower temperature also flavours increased PH values of the fermented substrates (Modupe et al., 2018). Nevertheless, increase in PH was also reported by previous research (Ifesan et al., 2014) reported increased PH to attribute to proteolysis activities of fermenting organism, which was similar to the PH result obtained in this current study. However, temperature is also a vital parameter used to monitor fermentation; it effects plays a crucial role in determining survival of the fermenting microbes and the nutritional benefit of fermented product.

(Ojewumi et al., 2018) reported that elevation of temperature (40^{o}c) in fermenting African locust beans seeds, result to decrease in the protein content of the substrates. Also, increased temperature attributes to donating of the microbes DNA, which affect the survival of the fermenting substrates. The stable temperature recorded in this study disagrees with the study of Ojewumi et al., (2018).

The nutritional benefit derived from fermentation is important, crude protein, fibre, ash and moisture of fermented product are mostly augmented Ibrahim et al., (2017).

In this study crude protein contents of all the fermented fruits seeds increased from (2.33% to 9.95%) for fermented mango seeds kernel, (2.63% to 43.69%) for watermelon seeds flour (21.44% to 41.23%) for African locust beans seeds flour. This could be attributed to extracellular protease activity exhibited by the fermenting microbes during fermentation, Apenal et al., (2015). This finding is similar to previous reports of Ojewumi et al., (2018) and Ibrahim et al., (2017) who reported the increased in crude protein content of fermented African locust beans seeds (52.71%) flour, and fermented mango seeds kernel (7.06% to 0.2171%) an increase in percentage crude fibre was noticed in only two of the fermented fruits seeds flour, (mango seeds kernel flour (2.90% to 8.15%) watermelon seeds flour (6.80% to 39.97%) and decrease in African locust beans seeds flour (19.02% to 10.77%). this could be due to a decrease in fermentation temperature and degradation of fibre by fermenting microbes result to decrease in fibre content of African locust beans. Employed in this study (Ifesan et al., 2017). This is accordance with previous reports of Ojewumi et al., (2018) and Ifesan et al., (2017) (2.16% to 14.30%) Ibrahim et al., (2017) (0.67% to 0.836%).

Ash content was noted to increase in all the fermented fruits seeds flour; this could be as a result of an increase in availability of minerals, from reduction of phytates. Employed in this study, which is in consistent with the reports or described by Ifesan et al., (2017) (0.61% to 3.56%) and (1.33% to 3.21%) Ibrahim et al., (2017).

Crude fats contents were observed to increase in only fermented mango seeds kernel, this could be due to increase in Lipolytic enzymes task with Hydrolyzing fats to glycerol and fat acid (Nduke et al., 2017).

Trace mineral are vital nutrients required is broilers bird's diets and are necessary for broiler bird's growth. (Ayoola 2018) they are prominent for digestive, physiological and biosynthetic process in broiler bird's development (LU et al; 2017).

During digestion numerous variety of minerals are absorbed and are formed, which can either enhance or reduce the functions of the dietary minerals ingested (Ayoola 2018).

Calcium (Ca^{2+}) is necessary for skeletal tissue and muscle contraction. (Ayoola 2018). Thus deficiency of dietary Calcium (Ca^{2+}) in broiler birds has a detrimental effect on serum plasma and muscle contraction. (Ayoola 2018). However, excess dietary calcium affect calcium hemostasis there by resulting to low absorption of phosphorous. (Ayoola 2018). In this study Calcium (Ca^{2+}) was increased in T_2 (Feed + fermented mango seeds kernel flours) and T_3 (feed + fermented water melon seeds flour) and reduced in T_4 (feed + fermented African locust beans seed flour). This could be attributed boiling and dehulling of the African locust beans seeds during processing. This result is in concern with (Ifeson et al; 2017). Who documented a decrease in the content of fermented African locust beans seeds and increase of calcium could be as result of protelytic activities of fermented Microbes. (Ifesan et al; 2017). Manganese is vital in broiler birds' diets manganese and carbohydrate, metabolism, bone development, fertility and maintenance of broilers performance (Ayoola 2018) dietary deficiency of manganese can result to decrease in enzymatic actions which could include Perosis and increase abdominal fat deposition. (Ayoola 2018). While excess dietary manganese result to increase in feed intake and impaired Hemoglobin formation (Ayoola 2018). In this current study (Mg^{2+}) manganese were observed to be reduced in only T_4 (Feed + fermented African locust beans flour) and increase in T_2 (Feed + fermented mango seeds kernel flour) this is in consistent with the finding of (Adeyomi et al; 2016 and Ibrahim et al; 2017) who observed that different seeds flour reacts differently with different microorganism.

Iron (F_2^{2+}) in broilers plays a crucial roles in egg yolk formation and serum formation (Lu et al; 2017).

Dietary deficiency of ($Fe2^{+}$) iron, could result to Anemia and increase in cupper absorption (Ayoola 2018). In this study, Iron was reduced in T_2 (feed + fermented mango seeds kernel). Which cloud have result to Anemia, in broiler birds excess Iron (Fe^{2+}) would result to maintaining broiler performance. Zinc (Zn^{4}) plays a prominent role in broiler birds' growth, (Ayoola 2018). Zn^{2+} Act as catalyst and cofactors to numerous classes of enzymes, also helps to augment cell proliferation, and gene regulation. Dietary zinc is also essential for modulating intestinal epithelium in broiler birds, dietary deficiency of zinc result to reduction in antibody responses and reduction in keratin and collagen synthesis the by causing bono deformities, abnormality poor feathering, and low tissue strength (Ayoola 2018) high Zn^{2+} could result to shallow respiration, Anorexia, reduced appetitive decrease body weight, weakness diarrhea and hemolytic Anemia kidney dysfunction, and Also causing systemically disruption of protein functions, enzymes and DNA. In this present study. Zn^{2+} was reduced in T_3 (feed + fermented watermelon seeds flour), resulting to low feed intake of broiler compared to others).

$No\overline{3}$ is necessary for broilers birds, in this study No3 increased in all the T_2 (feed +fermented mango seed kernel) T_3 (feed + fermented watermelon seeds) and T_4 (feed + fermented African locust beans seeds) variation in mineral content could be attributed to variety of soil composition.

(Lu et al; 2017, Ibrahim et al; 2017 and Eawomola 2010). Broiler diet can be potentially be formulated with lower dietary inclusion of minerals in feed additives without compromising broilers performances (Lu et al; 2017).

However, fermentation improves palatability, upgrade nutritional benefits, reduces, anti-nutritional factors contents to an acceptable level in all fermented product (Mua'azu et al: 2017). In this current study anti-nutrient factors contents, which is phytates in all the fermented fruits seeds flour reduced after fermentation.

This is attributed to seed processing such as boiling, dehulling drying and activities of fermenting microbes. (Ndukwe et al; 2017). The significant reduction in antinutritional content after fermentation has been documented by various researches using various substrates. (Ibrahim et al; 2017).

In Broilers Birds, palatability, digestibility and performance are directly partitioned (Orayaga et al: 2018). It' the feed is palatable the birds will consume more of the feeds, In this study significant differences was observed at weeks 4 and 5 when the finisher diet was introduced, T_2 (Feed + fermented mango seeds thermal was flour and T_4 (Feed + African locust beans seed flour) was more consumed.

At week 6. The consumption was statistically the same across diet this is attribute to the palatability of the feeds T_2, and T_4. Hence, the birds consumed more of T_2 in week 3. 4. 5. and 6. And T_4 in week 4. 5, and 6 mostly.

Nevertheless, significant differences (P > 0.05) was observed in the consumption most at week 4 and week 5 they no significant differences (P >0.05) cap as observed in a! diet on the feed intake of' the birds at week 1. 2 and 3. But at week 4. Significant difference was observed in the consumption.

Across birds fed diet T_2, T_3, T_4. However, high consumption of T_2 and T_3 are attributed to its digestibility and palatability of the fermented seeds flour used as additive in the Broilers Birds diet formulation. This is in agreement with the finding of Ibrahim et al: (2017) and Laval et al: (2018). But validates the report of Yahaya (2018) who blamed poor consumption in birds containing mango and African Locust beans diet. The increase in feed consumption of diets by Broilers Birds mostly result to increase in water intake in the current study. Broiler birds fed diets.

T_2 have the highest consumption of water intake across all the weeks.

Nevertheless, significant differences (P>0.05) was observed from week 2 to week 6 in water intake, due to increase in high water consumed by the birds fed diets 'I'2 mostly. Followed by these led by T_4 diet and T_3 diets. As more of consumed so did the birds consumed more water. This is consistent with the reports documented by Kaur and Brar (2017) who observed increased in water consumption with carbohydrate rich diets. However, there was no significant differences in water intake by birds fed all diet at week I which can he attributed to stress on arrival.

The increase in feed intake and water intake is an indicator of weight gain in Broilers birds. In week 1, 2 and 3 no significant differences in weight gain of birds fed different diet was documented in this current study. Hence significant difference was observed in weight gain when the finisher diets was introduced with birds fed T_4 diet having the highest weight gain at week 4. 5 and 6 followed by those birds fed diet T_2, while birds fed T_1 had the least final weight this reports is similar to the finding of Mua'azu et al; (2017) and Hishan (2014) who report increased weight gain of birds with finisher diet using African Locust beans seeds diet and watermelon seed diets.

Conclusion

This implies that fermented fruits seeds as additive used in Broilers Birds feeds formulation enhances palatability, digestibility and has no adverse negative effect on the performance of Broilers Birds.

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