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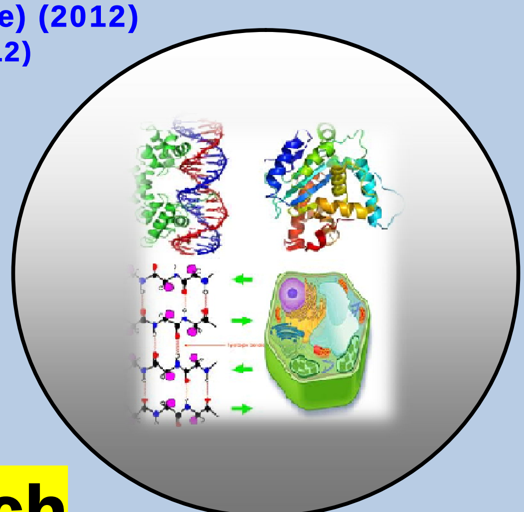

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RESEARCH PAPER

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Assessment of Prebiotic Roles of *Musa paradisiaca* (Plantain) Peels for Controlling Spread of *Salmonella* and other Infectious Disease Agents by Broilers

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ABSTRACT

Plantain fruit is widely consumed in Nigeria, Africa and some other parts of the world. The peel (epicarp) which has been reported to constitute about 40% of the whole fruit weight is considered useless and often discarded. In this study, we evaluated the effects of these peels on the intestinal microbiota of broilers by monitoring the intestinal beneficial bacteria flora and infectious pathogens commonly shed by poultry. The dried peels-powder were mixed with poultry feed in a ratio of 10% ($^{w}/_{w}$). Thirty broiler-chicks were divided into two groups. Group A (test) was fed with the feed-plantain peels mixture, while Group B (control) was fed with conventional feed. Faecal specimens of the broilers were collected and analysed for bacterial and parasite loads. The growth performance of the broilers was also evaluated. There was significant (P<0.05) increase in the numbers of Lactobacillus and Enterococcus spp, with concomitant decrease in numbers of Salmonella, Escherichia coli and Cryptosporidium in the test group. The growth performance of the test group was significantly (P<0.05) better than control. The findings of this study show that plantain peels can play important roles in controlling spread of infectious disease agents and improve broilers' health.
Key words: Prebiotic, Infectious disease, Musa paradisiaca, Microbiota and Broilers.

INTRODUCTION

Prebiotics are non-digestible food substances that when consumed provide a beneficial physiological effect on the host, by selectively stimulating the growth or activity of a limited number of indigenous beneficial bacteria (Cummings *et al*., 2001). These prebiotics resist digestion by host enzymes not only in the stomach, also intestine and reach the colon where they are metabolized by the indigenous bacteria, thereby directly providing the host with energy and metabolic substrates (Cummings *et al*., 2001; Wang and Gibson, 1993). An ideal prebiotic usually have the ability to alter the faecal microflora composition towards a more beneficial community structure (Chakraborti, 2011). Thus, prebiotics exert their beneficial effects on the host indirectly, by stimulating the beneficial functions of the intestinal microflora. The microflora of the intestine plays important protective roles in humans and animals health (Cummings *et al*., 2001). Among several beneficial roles of these beneficial commensal microorganisms include competing for available nutrients in gut microenvironment consuming all resources and out compete pathogens. The ability of the intestinal microflora to synthesize vitamins, exert trophic effects on intestinal epithelial cells, and salvage energy from unabsorbed food by producing short-chain fatty acids. Furthermore, these beneficial bacteria inhibit the growth of pathogens, sustain intestinal barrier integrity and maintain mucosal immune homeostasis (Canny and McCormick, 2008; Ewaschuk and Dieleman, 2006). Prebiotics have recently come into use as an alternative to probiotics. While probiotics are meant to be beneficial microorganisms in the gut, prebiotics selectively stimulate the beneficial microorganisms that already live there. Thus, prebiotics have two clear advantages relative to probiotics: firstly, there are no critical problems of inability of the ingested probiotic to survive the acid conditions of the stomach and secondly, there is no introduction of foreign microbial species into the gut (Falcao-e-Cunha *et al*., 2007; Macfarlane *et al.,* 2006). In addition to these, prebiotics also have the advantage of relative ease of manufacture because they can be either directly extracted from natural sources or be produced by partial acid or enzymatic hydrolysis of polysaccharides or by trans-glycosylation reactions (Falcao-e-Cunha *et al*., 2007; Macfarlane *et al.,* 2006). The role of prebiotics in modulation of bowel function has been widely studied and reported, but more recent studies have focused on their protective role against infections and diseases (Chakraborti, 2011; Gibson and Roberfroid, 1995; Hamilton-Miller, 2004; Licht *et al.,* 2011; Lomax and Calder, 2009). The most widely studied prebiotics are inulin and non-digestible oligosaccharides such as oligofructose (Guarner, 2007; Leenen and Dieleman, 2007; Lomax and Calder, 2009; Watzl *et al.,* 2005). However, studies show that there may be other candidate-prebiotics such as saponin, sorbitol, mannitol and lactulose (Chakraborti, 2011; Ukwah and Ezeonu, 2008).

M. paradisiaca (plantain) peel is a popular African fruit. It is consumed as an energy yielding food and desert. It is an important contributor to the diets of many low and middle class people in many African settings including Nigeria (Ighodaro, 2012). The peels are most commonly discarded by consumers with few pig farmers giving it to their animals. These plantain peels have been reported to contain tannins, flavonoids, terpenoids, alkaloids and glycosides (carbohydrates) (Ighodaro, 2012; Zhang *et al.,* 2011).

These constituents have been shown to have different biological effects including lipid lowering, anti-nutritional and antimicrobial effects (Ighodaro, 2012; Oluwatofarati, 2010). In this study, we evaluated the prebiotic effects of plantain peels on intestinal microbiota of broilers including shedding of pathogenic organisms.

MATERIAL AND METHODS

Preparation of Plantain Peels as feed additive

Healthy ripe plantain fruits were obtained from Kpirikpiri and Meat markets in Abakaliki, Ebonyi, Nigeria and the botanical authentication was done at the department of Applied Biology (Botany unit), Ebonyi State University, Abakaliki, Nigeria. The ripped peels were removed the aid of hand knife and cut into smaller pieces for easy drying. The dried peels were ground into homogenous powder using a milling machine. The plantain peel powdery samples were weighed and 10 g of the powder were mixed with 90 g of the conventional poultry feed, making 10% ($^{w}/_{w}$).

Study Birds and Feed Administration

Thirty day old chicks brought from Obasanjo Farm were used for the study. The sexes of the birds were determined by J.O. Uguru (animal scientist) after arrival from Obasanjo farm and only the males chicks were selected for the study. The birds were divided into groups A and B. Each group was made up of 15 birds, divided into three replicates of 5 birds per replicate. The 10% ($^{w}/_{w}$) peel powder-feed was administered to group A and group B was administered conventional feed (Control). The control group was administered with antibiotics and anti-coccidiosis as conventionally raised. However, no drug was administered to the test except the peel additive. The birds were monitored for 8 weeks. The birds were handled in accordance to the ethical guideline for animal use in experiments.

Faecal Sample Collection and Analysis

Fresh faecal samples were collected weekly throughout the entire study periods using sterile containers. Six birds were sampled weekly from each group, two from each replicate.

Bacteriological Analysis of Feacal Samples

All the stool samples were processed within 3 h of collection. A small quantity of faecal sample (0.5 g) was homogenized in 5 ml of phosphate buffered saline (NaCl – 0.8% w/v; KH_2PO_4 – 0.2% w/v; Na_2HPO_4 - 0.115% w/v at pH 7.4), which was autoclaved at 121^0C for 15 minutes, 15 pounds pressure. The emulsified faecal samples were serially diluted in 10-folds in sterile phosphate buffered saline. Thereafter, 0.1 ml of the 10^{-5} dilution was inoculated onto two sets of replicate agar media [Eosin Methylene Blue (EMB) agar (Fluka) and De Man, Rogosa and Sharpe agar (Fluka)]. The media were prepared according to the manufacturers' instructions. One set of cultures was incubated aerobically and the other set anaerobically at 37^oC for 48 h.

Identification and Enumeration of Intestinal Bacteria

The isolated organisms were identified and enumerated by conventional microbiological methods including cultural morphology, Gram staining reaction, catalase and biochemical reactions.

Evaluation of Growth Performance

The growth performance of broilers was evaluated by measuring their body weight weekly on scale for eight weeks. The weight gained was determined by subtracting the current body weight from the previous weight.

Identification of Intestinal *Cryptosporidium*

Faecal Sample analysis

The samples collected were analysed by homogenising a pea-sized (0.5 g) portion of faecal specimen in 3 ml of 10% formol-saline in a test-tube. The faecal suspensions were filtered through a 2 mm pore size sieve. Four millilitres (4 ml) of ethyl-acetate was added to the faecal suspension, shaken vigorously for 1 min and then centrifuged at 3000 rpm for 10 min. The faecal debris at the interface was loosened with an applicator stick and the supernatant decanted. The tube was tapped gently to loosen and re-suspend the faecal deposit at the bottom. The deposit was put on clean a grease-free glass slide, covered with a cover slip and examined using x10 and x40 objective lens of the microscope. The deposit was allowed to dry and then stained by modified Ziehl Neelsen staining technique (Cheesbrough, 2005).

Modified Ziehl Neelsen staining technique

The faecal smear was fixed in methanol for 2 min. The fixed smear was drained and stained with cold carbol fuschin for 15 min and washed off with water. The stained smear was decolorized with 1% acid-ethanol for 15 seconds and washed with water. The decolorized film was counter-stained with 0.25% malachite green for 30 s and washed with water. Then, the stained slides were kept in a draining rack to dry, before examination under the microscope using x100 objective lens (Cheesbrough, 2005).

Statistical Analysis

The data generated were analyzed using GenStat 11th Edition. Comparison of the growth performance of the broilers, stimulation of beneficial bacteria and pathogen shedding in the control and test groups were made using analysis of variance (ANOVA) and significance was test at 95% confidence. The significant difference between the mean weights gained for the two groups were determined by ANOVA at 95% confidence level. Also, relationship between weight gained by the broilers and population of intestinal beneficial bacteria isolated was tested using correlation at 95% confidence level.

RESULTS

Effect of Oral administration of plantain peel powder on the intestinal microbiota

Four bacterial organisms were consistently isolated from the faecal samples: *Enterococcus* spp., *Lactobacillus* spp., *Salmonella* spp. and *E. coli.* In the chicks fed with feed supplemented with plantain peel powder, there were significant ($P < 0.05$) increases in the number of *Enterococcus* and *Lactobacillus* spp. starting from the second week, while the numbers of *Salmonella* and *E. coli* decreased (Figure 1). The data also showed that the population of *Lactobacillus* significantly ($P<0.05$) supassed those of *Enterococcus* from week 6 till week 8 (end of monitoring period) in the test group as compared with the control (Figure 5). On the other hand, the population of enteropathogens (*E. coli* and *Salmonella* which were the most consistent pathogens isolated and monitored) was significantly ($p<0.05$) reduced in both control and test groups but decreased more in the test group after the 4th week till 8th week as compare to the control group which had a slight increase within the same period (Figures 1, 2 & 6). *Salmonella* species were not detected in 6th and 7th weeks but reappeared in very low number on 8th week in the test group as compared with the control (Figure 6).

Comparative evaluation of the effects of the peel on probiotics and *Salmonella* species indicated that the population of *Salmonella* species decreased most significantly ($p<0.05$) from the 3rd week when the number of *Lactobacillus* and *Enterococcus* became most significantly ($p<0.05$) increased (Figure 1).

Effect of Oral administration of plantain peel powder on shedding of *Cryptosporidium*

In chicks fed with peel powder-feed blend, *Cryptosporidium* oocysts were detected in the faecal samples up to the third week of study, after which the shedding of oocysts stopped. Degree of infection (reflected by the number of oocysts observed microscopically) also dropped from week 1 to week 3. In the control chicks, however, shedding of oocysts continued up to weeks 5 to 7, even though there was reduction in degree of infection (Table 1). The peel powder significantly ($p<0.05$) reduced the shedding of *Cryptosporidium* in the test group as compared with the control (Table 1).

Effect of Oral administration of plantain peel powder on growth performance

The growth performance of the test group was significantly ($p<0.05$) better than the control (Figure 3). The test group gained more weight from a week old till the 5th week of age as compared with the control. However, the weight gain of the test group dropped on the 6th week till 8th week as compared with the control which gained more weight (Figure 4).

The data also indicated that growth performance of the broilers appeared not to be correlated with the populations of the *Lactobacillus* and *Enterococcus* as the decrease in their numbers in the control did not significantly affect the growth of the broilers.

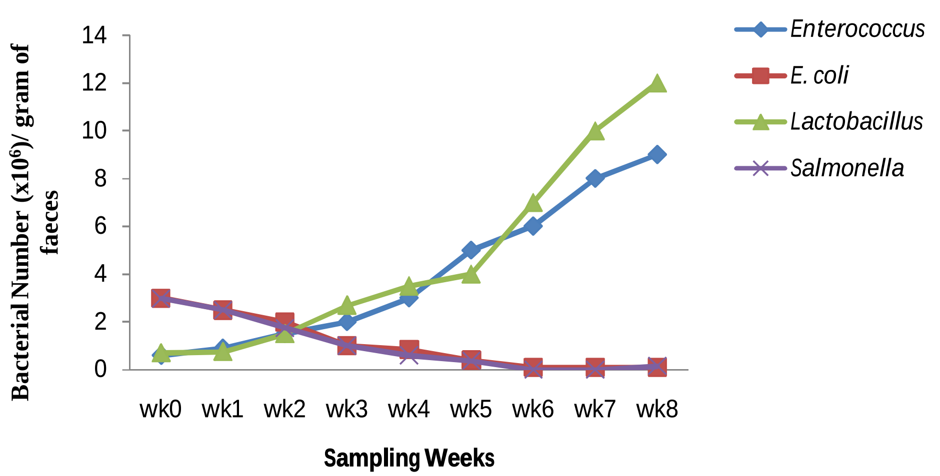

Figure 1. Population of Bacterial flora in Broilers Fed with *Musa paradisiaca* (Plantain) Peel

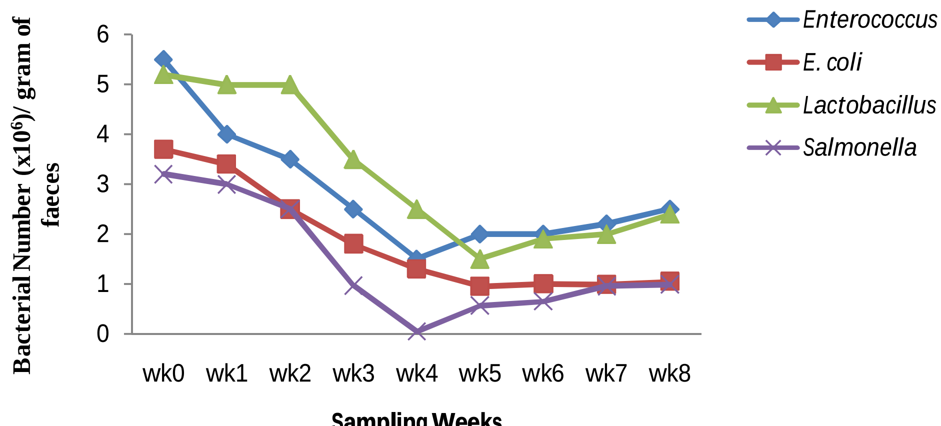

Figure 2. Population of Bacterial flora in Broilers Fed with Conventional Feed (Control)

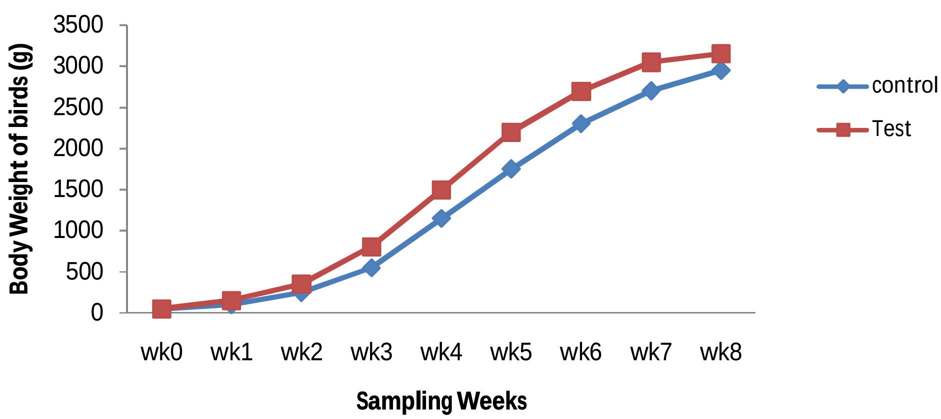

Fig 3. Effects of Plantain Peels Additive on Growth Performance of Broilers

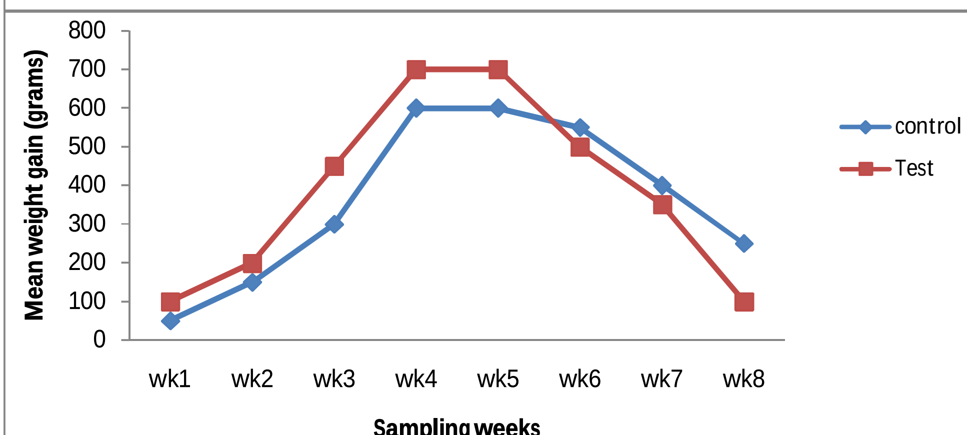

Figure 4. Mean weight gain of Broilers Fed with Plantain Peel and Control

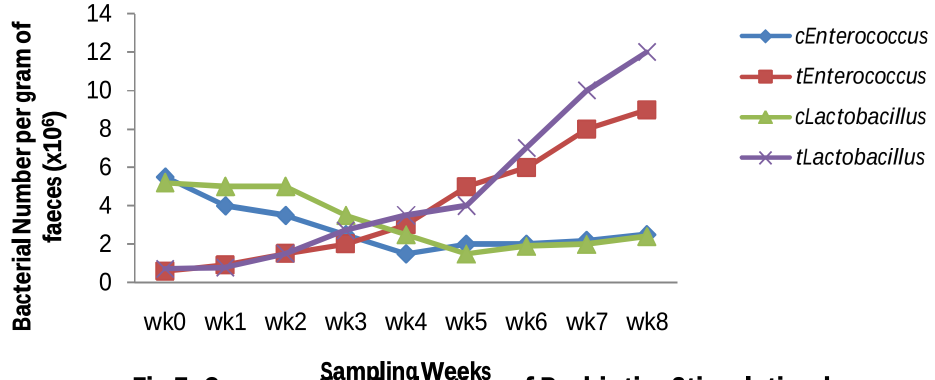

Fig 5. Comparative Evaluation of Probiotics Stimulation by Plantain Peels and Conventional Feed in the Guts of Broilers

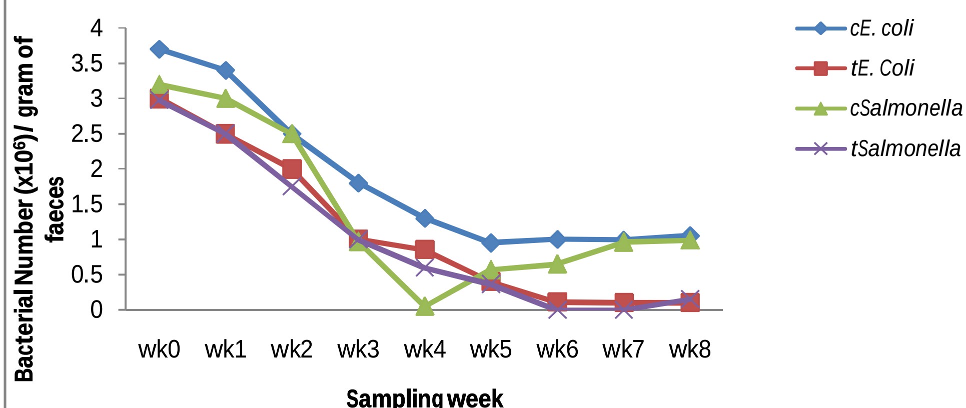

Fig 6. Comparative Evaluation of Intestinal Pathogens Shedding by Broilers Fed with Plantain Peels and Conventional Feed

Table 1. Effects of *Musa paradisiaca* Peel Powder on shedding of *Cryptosporidium* oocyst by Broilers.

	Test	**(10% w/w)**	**Control**	**(No Additive)**
	Percentage of oocyst shedding	**Degree of Infection**	**Percentage of oocyst shedding**	**Degree of Infection**
Week 0	0	-	0	-
Week 1	6(40%)	++	9(60%)	++
Week 2	6(40%)	+	6(40%)	+
Week 3	3(20%)	+	3(20%)	+
Week 4	0	-	3(20%)	+
Week 5	0	-	1(6.7%)	+
Week 6	0	-	0	-
Week 7	0	-	1(6.7%)	+
Week 8	0	-	0	-

Keys: - = Not Detected; + = 0 – 10 oocysts per field; ++ = 11 – 50 oocysts per field.

DISCUSSION

Plantain peel has been reported to contain carbohydrates, glycosides and fibre (Ighodaro, 2012). Some well-studied prebiotics are mostly complex carbohydrates found in fruits and plants. In this study, results showed that *Enterococcus* and *Lactobacillus* were significantly ($p<0.05$) stimulated in broilers by the supplementation of poultry feed with plantain peel powder. *Enterococcus* and *Lactobacillus* are bacteria known as probiotics which have been studied extensively and found to impart positively on host health (Crittenden *et al*., 2003; Cummings *et al*., 2001). The stimulation of these two organisms indicates that the peel contains prebiotics. The stimulation of *Lactobacillus* was significantly ($p<0.05$) higher than that of *Enterococcus* from the 6th week to 8th week of the study (Figure 1). This finding is attributable to report that *Enterococcus* is a good producer of folate with *Lactobacillus* being a good consumer of the folate (Crittenden *et al*., 2003; Ezeonu and Ukwah, 2009).

There was also concomitant reduction in numbers of *Salmonella* and *E. coli* (less beneficial bacteria). This is also a hallmark of known prebiotics; the ability to enhance the growth of beneficial bacteria, while reducing the non-beneficial bacteria (Gibson and Roberfroid, 1995; Roberfroid, 2007). The week of disappearance of *Salmonella* and *E. coli* coincides with the time when the population of *Lactobacillus* and *Enterococcus* increased most significantly (Figure 1). The reduction and subsequent disappearance of these bacteria may be attributable to the antagonistic action of *Lactobacillus* and *Enterococcus* and/or antimicrobial activity of the peel (Ighodaro, 2012; Oluwatofarati, 2010). The reduction of some intestinal pathogens like *Salmonella* and *E. coli* is in agreement with the report of Ighodaro that the peel extract was found to be active against some pathogenic microbes (Ighodaro, 2012). These results are also in line with the reports of another study, in which administration of a plant extract to rabbits inhibited colonization by *Staphylococcus aureus* and *E. coli* (Ezeonu *et al.,* 2012). The reappearance of *Salmonella* on week 8 may be attributable to the ability of *Salmonella* to translocate intracellularly and appear later. Translocation is a mechanism employed by some organisms including *Salmonella* to evade effects of antibiotics and other hazardous substances in the intestine (Bovee-Oudenhoven *et al.,* 2003; Deiwick *et al.,* 2006). Salmonellosis, a gastrointestinal and zoonotic disease is highly prevalent in Nigeria like other countries in the world and poultry is one of the major sources of transmission (Saidu *et al.,* 1994). The reduction of *Salmonella* shedding as found in this study is indicative of the potential benefits of plantain peels additive in poultry feed in the control of infectious disease spread.

The evaluation of the effects of the plantain peel powder on the growth performance of the birds and their weight gain showed that the peel significantly ($p<0.05$) increased growth performance of the broilers as compared with the control (Figure 3). However, the test group gained more significant ($p<0.05$) weight from first week till 5th week as compared with the control but weight gained by the test group dropped significantly ($p<0.05$) and continually from 6th week till the last week of study (week 8) (Figure 4).

Coccidiosis is a common self-limiting parasitic disease associated with birds especially poultry and can cause huge loss to commercial poultry farmers (Fanatico, 2006). Poultry can be given anti-coccidiosis after one week of age to prevent the disease from occurring (prophylaxis) (Fanatico, 2006).

In this study, our finding showed that shedding of coccidian parasites was greatly reduced from two weeks of age and subsequently eliminated from the 4^{th} week in the test group as compared to the control which had a significantly ($p<0.05$) low reduction in number of coccidian shed (Table 1). The elimination of coccidian parasites after three weeks by the peel powder is attributable to the significant improvement of growth performance and health status of the birds. A good growth performance is associated with good health and improved immunity (Fanatico, 2006). Studies have shown that coccidiosis is best managed by improvement of host immunity (Fanatico, 2006). The inability of the coccidian parasites continual colonizing the intestine of the test group is possibly due to the immune boosting potentials of the peel powder and the antagonistic actions of the stimulated bacterial flora (*Lactobacillus* and *Enterococcus)*. Poultry faeces are organic compound rich in nitrogen and phosphate making it a good fertilizer for plants (Wikipedia, 2014). In Nigeria including Abakaliki where this study was carried out, farmers especially those in rural settings use poultry faeces as manure for growing crops with no treatment to get rid of zoonotic infectious agents like *Salmonella* and *Cryptosporidium*. The reduction and elimination of infectious pathogens in broilers as shown in this study is indicative of protective roles of plantain peels additive in the transmission of zoonotic diseases to farmers. It is interesting that while the addition of plantain peel powder could eliminate the shedding of *Salmonella,* although reappeared later, the same level of elimination was not offered against *E. coli.* These findings suggest that protection conferred by probiotic organisms in the gut could vary with the type of pathogen and probiotic organism involved. The findings of this study are also in contrast and partly agrees with other studies in which inclusion of fructo-oligosaccharide prebiotics in water and feed of animals protected against *E. coli* but not *S. typhimurium* infection (Chakraborti, 2011; Hamilton-Miller, 2004).

CONCLUSION

This study has just provided information on the prebiotic nature of *Musa paradisiaca* (plantain) peel powder and also provided clues that these peels can be used as an additive in raising poultry, preventing gastrointestinal diseases and control environmental spread of microbial pathogens from poultry. Hence can reduce/lower chances do infectious disease agents transmission from birds to farmers who use the poultry faeces as manure for growing crops without prior treatment. The findings of this study are also suggestive that the use of the peel powder can reduce the cost of poultry production by removing the cost of antibiotics and anti-coccidiosis that are always administered to birds.

We recommend that future work involving molecular techniques are employed to detect strict anaerobic beneficial bacteria that may play important roles in the prebiotic effects of this plantain peels and as well determine its exact cost effectiveness in poultry production as compare with the conventional method.

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