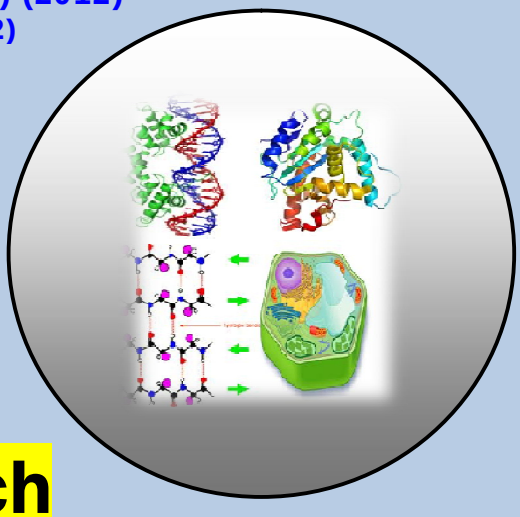
In vivo Studies on the Prebiotic Effects of *Vernonia amygdalina* Leaf Powder on Broilers' Intestinal Microflora and Pathogen Shedding

By

B.N. Ukwah and I.M. Ezeonu

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Index Copernicus International Value
IC Value of Journal 4.21 (Poland, Europe) (2012)
Global Impact factor of Journal: 0.587 (2012)



J. Biol. Chem. Research
Volume 31 (1) 2014 Pages No. 464-473

Journal of Biological and Chemical Research
(An International Journal of Life Sciences and Chemistry)

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 31, No. 1: 464-473 (2014)
(An International Journal of Life Sciences and Chemistry)

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

B. N. Ukwah
http:// www.jbcr.in
jbiolchemres@gmail.com
info@jbcr.in

RESEARCH PAPER

Received: 26/01/2014 Revised: 08/03/2014 Accepted: 15/03/2014

In vivo Studies on the Prebiotic Effects of *Vernonia amygdalina* Leaf Powder on Broilers' Intestinal Microflora and Pathogen Shedding

B.N. Ukwah* and I.M. Ezeonu

***Medical Microbiology and Parasitology Unit, Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Ebonyi State University, Abakaliki, Nigeria**
Department of Microbiology, Faculty Biological Science, University Nigeria, Nsukka

ABSTRACT

Vernonia amygdalina leaves, commonly called bitter leaf is widely consumed in Africa especially in Nigeria. The leaf extract has been reported to contain some antimicrobial compounds and prebiotic potentials. However, no studies have been conducted using the leaf powder. In this study, we evaluated the effects of the leaf powder on the intestinal microflora and growth performance of broiler chicks. A total of 45 day-old chicks, consisting of three groups and three replicates of five birds per group (A, B and C) were used for the study. Dried Vernonia amygdalina leaves were ground to a homogenous powder. The powder was mixed with conventional poultry feed as an additive to achieve 1%, 2% and 0% ($^{w}/_{w}$) concentrations. The different concentrations were administered to groups A, B, and C (control), respectively, for 8 weeks. Fresh faecal specimens of the broilers were collected weekly and analysed for bacterial and parasitic loads. Growth performance of the broilers was determined on each sampling week by measuring the body weight. The analysis of the faecal samples showed a significant (P<0.05) stimulation of Lactobacillus and Enterococcus species on the groups A and B as compared with the control but significantly (P<0.05) higher in group A than group B. Salmonella and E. coli which were initially abundant, continued to decrease in population till the 4th week when Salmonella was no more detected but reappeared in scanty numbers from the 7th to 8th week in all the groups. Coccidian parasites including Cryptosporidium were supressed in groups A and B as in the control (C). The growth performances of group A and control were significantly (P<0.05) higher than group B. The result of this study has shown that Vernonia amygdalina leaf powder contain prebiotic components and can play important roles in reduction of intestinal pathogens shedding in broilers.

Key words: Prebiotic, V. amygdalina Leaf Powder, Broilers, Intestinal microflora, and Pathogens.

INTRODUCTION

Prebiotics are non-digestible ingredients that when consumed provide a beneficial effect on the host, by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria in the colon (Roberfroid, 2007 and Mitchell, 2010). These prebiotic substances survive digestion in the stomach and reach the colon where they are metabolized by the bacteria, thereby providing the host with energy and metabolic substrates (Wang and Gibson, 1993; Cummings et al., 2001). Effective prebiotics usually have a specific fermentation in the colon and 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 has important protective and metabolic functions (Canny and McCormick, 2008). Among the several functions these commensal bacteria play is competition for available nutrients in ecological niches and subsequent consumption of all resources. The host also benefits from the ability of the intestinal microflora to synthesize vitamins like folate which is important in regeneration of dead cells including intestinal epithelial cells. Some produce short-chain fatty acids like butyrate and propionate that help to salvage energy from unabsorbed food (Crittenden *et al*, 2003; Scheppach, 1994; Wong *et al.,* 2006). These beneficial bacteria therefore have the ability to inhibit the growth of pathogens, sustain intestinal barrier integrity and maintain mucosal immunity (Binder, 2010; Ewaschuk and Dieleman, 2006; Canny and McCormick, 2008). Prebiotics have recently become an alternative to probiotics. 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 (Macfarlane et al., 2006; Falcao-e-Cunha et al., 2007). 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 transglycosylation reactions (Macfarlane et al., 2006). The role of prebiotics in modulation of bowel function has been widely studied and reported (Gibson and Roberfroid, 1995; Hamilton-Miller, 2004; Lomax and Calder, 2009), but more recent studies have focused on their protective role against infections and diseases (Lomax and Calder, 2009; Chakraborti, 2011; Licht et al., 2011). The most widely studied prebiotics are inulin and non-digestible oligosaccharides such as oligofructose (Leenen and Dieleman, 2007; Lomax and Calder, 2009; Guarner, 2007). However, recent studies show that there may be other candidate-prebiotics such as xylitol, sorbitol, mannitol, saponnin and lactulose (Ukwah and Ezeonu, 2008; Chakraborti, 2011). *V. amygdalina* (bitter leaf) is a popular African vegetable. It is used in various food preparations and in ethnomedicine for the treatment of malaria and gastrointestinal infections. The aqueous leaf extract has also been shown to have blood sugar and lipid-lowering effects in experimental animals (Adaramoye et al., 2008). The prebiotic potentials of *V. amydgalina* leaf extract and its effect on bowel function in humans has previously been reported (Ezeonu and Ukwah, 2009). The ability of this extract to act as carbon and energy source for some intestinal bacteria, *in vitro* has also been reported (Ukwah and Ezeonu, 2008). This present study evaluated the prebiotic and protective roles of the leaf powder against intestinal pathogens including bacteria, yeasts and coccidian parasites in broilers.

MATERIAL AND METHODS

Test Plant

The plant *Vernonia amygdalina* (bitter leaf) was used for this study. The plant was collected and authenticated by a botanist of the Herbarium section in the Department of Botany, Ebonyi State University Abakaliki. The leaves of the plant were used for the study.

Preparation of Leaf Powder and Feed Mixture

The leaves collected were washed in clean water and air dried on a clean surface at room temperature. The dried leaves were pulverized into a homogenous powder using grinder. The leaf powder and the conventional feed were mixed to achieve 1%w/w and 2%w/w.

Study Birds

Forty-five day old chicks brought from Obasanjo Farm were used for the study. The birds were divided into three groups A, B and C. Each group was made up of 15 birds, divided into three replicates of 5 birds per replicate.

Evaluation of the Effects of the Leaf Powder on Poultry Intestinal Microflora

The 1%w/w leaf powder feed was administered to group A, 2%w/w was administered to group B and group C was administered conventional feed (Control). The birds were monitored for 8 weeks.

Faecal Sample Collection and Analysis

Fresh faecal samples were collected weekly throughout the entire study periods using sterile containers.

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 microaerophically 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.

Identification of Intestinal *Cryptosporidium* and Yeasts

Faecal Sample analysis

The samples collected were analysed by homogenising a pea-sized portion of faecal specimen in 3 ml of 10% formol-saline in a test-tube. The faecal suspensions were filtered through a 2 mm sieve. Four millilitres of ethyl-acetate was added to the faecal suspension, shaken vigorously for 1 min and then centrifuged at 3000 rpm for 10 min. The ether-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 (Chessbrough, 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 (that is 1 volume of concentrated HCl into 99 volumes of 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 (Chessbrough, 2005).

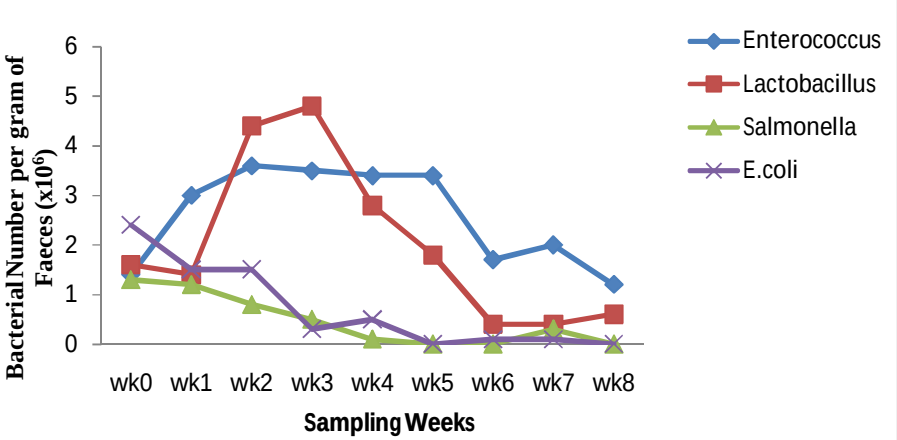


Figure 1. Effects of Oral Administration of 1% *V. amygdalina* Leaf Powder on Intestinal Microflora of Broiler

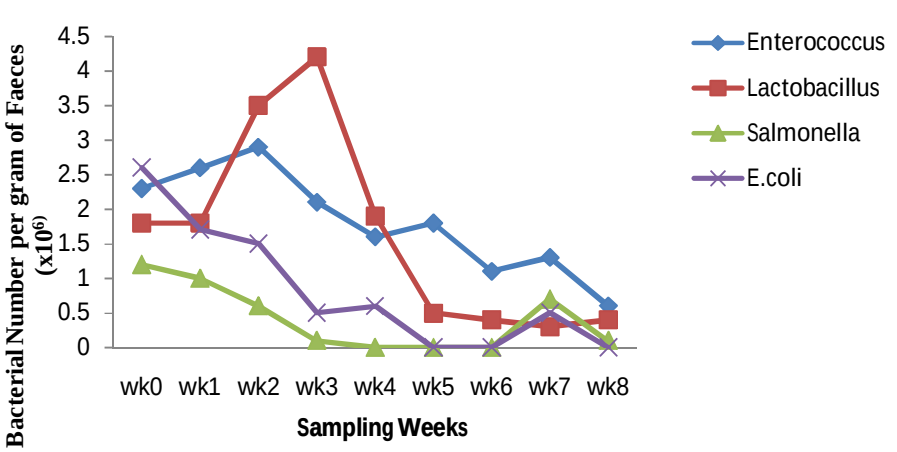


Figure 2. Effects of Oral Administration of 2% *V. amygdalina* Leaf Powder on Intestinal Microflora of Broiler

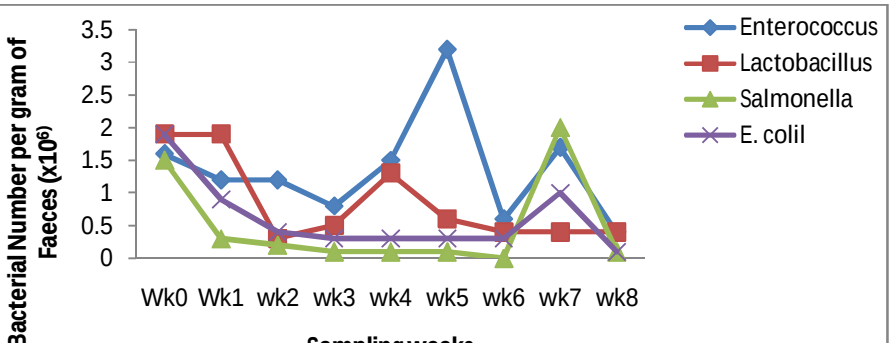


Figure 3. Effects of Oral Administration of Conventional Feed on Intestinal Microflora of Broiler (Control).

Table 1. Effects of *V. amygdalina* Leaf Powder on shedding of *Cryptosporidium* by Broilers

	Group A(1	%w/w)	Group B(2	%w/w)	Control	(No Additive)
	Percentage of oocysts Shedding	Degree of Infection	Percentage of oocysts Shedding	Degree of Infection	Percentage of oocysts Shedding	Degree of Infection
Week 0	0	-	0	-	0	-
Week 1	6(40%)	++	6(40%)	+	9(60%)	++
Week 2	6(40%)	+	6(40%)	+	6(40%)	+
Week 3	1(6.7%)	+	1(6.7%)	+	3(20%)	+
Week 4	0	-	1(6.7%)	+	1(6.7%)	+
Week 5	0	-	0	-	1(6.7%)	+
Week 6	0	-	0	-	3(20%)	+
Week 7	1(6.7%)	+	0	-	3(20%)	+
Week 8	0	-	0	-	0	-

Keys: - = Not Detected; + = 0 – 20 oocysts per field; ++ = 21 – 50 oocysts per field

Table 2. Effects of *V. amygdalina* Leaf Powder on shedding of Yeasts by Broilers

	Group A(1	%w/w)	Group B(2	%w/w)	Control	(No Additive)
	Percentage of Yeasts Shedding	Degree of Infection	Percentage of Yeasts Shedding	Degree of Infection	Percentage of Yeasts Shedding	Degree of Infection
Week 0	0	-	0	-	0	-
Week 1	3(20%)	+	0	-	0	-
Week 2	0	-	0	-	6(40%)	+
Week 3	0	-	3(20%)	+	0	-
Week 4	0	-	0	-	3(20%)	+
Week 5	0	-	0	-	0	-
Week 6	0	-	0	-	0	-
Week 7	0	-	0	-	0	-
Week 8	0	-	0	-	0	-

Keys: - = Not Detected; + = 0 – 20 oocysts per field; ++ = 21 – 50 oocysts per field

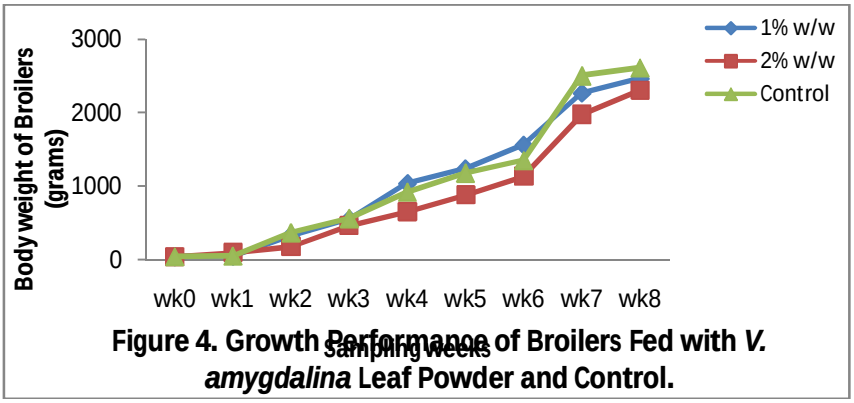


Figure 4. Growth Performance of Broilers Fed with *V. amygdalina* Leaf Powder and Control.

RESULTS

The growth of *Enterococcus* in group A and B was significantly ($p<0.05$) stimulated more than *Lactobacillus* after the first week of feeding with *V. amygdalina* leaf powder. The population peaked on week 2 and remained relatively stationary till week 5 (Figure 1 & 2). The population of *Lactobacillus* increased significantly ($p<0.05$) higher than *Enterococcus* from week 2 to week 3 and dropped below the *Enterococcus* on week 4 (Figure 1). The number of *Salmonella* and *E. coli* significantly ($p<0.05$) decreased from week 1. *Salmonella* species were not been detected in week 5 and 6 but reappeared in week 7 and disappear in week 8 (Figure 1). Similar results were obtained in group B fed with 2%w/w *V. amygdalina* but *Salmonella* disappeared from week 4 and reappeared in week 7 in low numbers (Figure 2). The population of the intestinal microflora were inconsistent in the group fed with the conventional feed (control). The numbers of *Lactobacillus* decreased from week 1 till week 2 and increased from week 3 till week 4. The number remained relatively low and stationary from week 5 till the end of the study. That of *Enterococcus* decreased as in *Lactobacillus* but increased more than *Lactobacillus* from week 4 till week 5, decreased in week 6 and increased again in week 7 (Figure 3). *E.coli* and *Salmonella* were detected throughout the study in the control, although in lower number than their initial populations (Figure 3). Group A birds weighed significantly ($P<0.05$) more than control birds between the 4^{th} and 6^{th} weeks but their weights dropped significantly ($P<0.05$) lower than control from 7^{th} week to 8th week. The growth performance of groups A and C were significantly ($p<0.05$) better than group B (fed with 2%w/w *V. amygdalina*) (Figure 4). Shedding of *Cryptosporidium* was significantly ($p<0.05$) reduced in both groups A and B but ceased on week 4 in group B in which the oocyst was detected only in one of the birds as compared with the control (group C). *Cryptosporidium* oocyst was later detected in group A in low numbers only in one of the 15 birds on week 7 (Table 1). The shedding of yeasts was highly minimal in the groups A and B as compared with the control (Table 2).

DISCUSSION

The stimulation of *Enterococcus* and *Lactobacillus* as well as the inhibition of pathogens indicates that *V. amygdalina* leaf powder had prebiotic effects as previously reported for its extract (Figures 1 & 2). The initial significant ($p<0.05$) higher increase in the numbers of *Enterococcus* in the first week as compared with *Lactobacillus* as observed in this study is

Consistent with the finding of our earlier study on human subjects fed with the leaf extract (Ezeonu and Ukwah, 2009). The growth of *Lactobacillus* was observed to surpass that of *Enterococcus* from week 2 and dropped below *Enterococcus* on week 4 and week 5 (Figures 1 & 2). This higher increase in the population of *Lactobacillus* on week 2 as compared with *Enterococcus* is attributable to the report that *Enterococcus* produces folate and *Lactobacillus* utilizes preferentially (Crittenden, 2003). The population of the *Enterococcus* persisted more than that of *Lactobacillus* in both groups fed with 1%w/w and 2%w/w of *V. amygdalina* leaf powder respectively (Figures 1 & 2). The selective stimulation of *Lactobacillus* and *Enterococcus* in preference of other organisms is consistent with the reports of Cumming *et al.,* (2001) that prebiotics selectively stimulate the growth of a limited number of bacteria in the colon. The persistence of *Enterococcus* is indicative of its important role in protecting the host against infectious agents as seen with the disappearance of *Salmonella* and *E. coli* within the same period (Figure 1 & 2). Modulation of intestinal microflora is characteristic of effective prebiotics. The finding of this study is in agreement with this report that prebiotics modulate the population of intestinal microbial community.

The initial presence and inconsistent nature of the microbial community (including *Lactobacillus, Enterococcus E. coli* and *Salmonella*) in the control group is indicative of the transovurian and natural colonization and succession of the microrganisms that take place in the birds (Figure 3).

Some pathogenic organisms including *Salmonella* species are constitutive in poultry (i.e it can be transmitted trans-ovarian to the chicks). The species of this *Salmonella* commonly associated with severe diseases in both poultry and human are *Salmonella enterica* and *Salmonella enterica* subspecies *enterica* (OIE, 2012; CFSP, 2009). The finding of this study showed that *Salmonella* was totally eliminated from weeks 5 and 4 in the birds fed with 1% and 2% *V. amygdalina* leaf powder respectively as compared with the control (Figures 1, 2 & 3). The total elimination of *Salmonella* in groups A and B respectively is indicative of the usefulness of *V. amygdalina* leaf powder in poultry breeding and showed it cannot only protect birds from pathogens but can also reduce pathogens' shedding and spread to the environment. The ability of the leaf powder to reduce and eliminate pathogen shedding in these birds is attributable to its antimicrobial properties and prebiotic potentials as reported by Ezeonu and Ukwah (2009) and Enyi-Idoh, *et al.,* (2012).

Bacterial translocation is the ability of some organisms to enter into the cell when intestinal environment becomes unfavourable (Deiwick *et al.*, 2006). This characteristic has been reported in *Salmonella* species (Bovee-Oudenhoven *et al.,* 2003; Deiwick *et al.*, 2006). The reappearance of *Salmonella* species on week 7 after initial elimination is suggestive of the fact that *Salmonella* can translocate intracellularly.

Cryptosporidiosis is a disease of both poultry and human caused by a protozoan parasite called *Cryptosporidium* (Ukwah and Ezeonu, 2013). This parasite is one of the coccidian parasites that is commonly associated with birds and can cause a massive death of poultry. Ensue of the huge economic loss it causes to commercial poultry farmers, farmers often treat their birds with anti-coccidiosis leading to increase in the cost of poultry production. In this study, the shedding of coccidian parasites was evaluated and the results showed that shedding of *Cryptosporidium* oocysts was significantly ($p<0.05$) reduced by the leaf powder at

Both 1%w/w and 2%w/w supplementation. Although, both percentages (1% and 2%) supplementation effectively inhibited the shedding of oocysts, the oocyst was detected in 1(6.7%) of the 15 birds fed with 1% w/w of the *V. amygdalina* leaf powder on week 7 but no mortality was recorded. However, oocyst shedding was last detected in week 4 in 1 bird (6.7%) of the 15 birds fed with 2%w/w of the *V. amygdalina* leaf powder with no further detection till the end of the study. The complete inhibition of *Cryptosporidium* oocysts shedding shows that 2% supplementation is more effective in protecting the birds against this pathogen as compared with the 1% (Table 1). This finding is suggestive of anti-cryptosporidial potential of the *V. amygdalina* leaf powder.

The evaluation of the growth performance of the broilers in this study showed that 1%w/w *V. amygdalina* leaf powder supplementation significantly ($p<0.05$) enhance growth more than the 2% supplementation but non-significant ($p>0.05$) as compared with the control. The non-significant drop ($p>0.05$) in weight of the group fed with 1% on week 7 and 8 as compared with the control is attributable to the lipid lowering effects of the plant powder as reported by Adaramoye et al., (2008) (Figure 4). The significant ($p<0.05$) drop in body weight of group B (2%) as compared with group A and C may be attributable to excessive reduction in cholesterol level of those broilers as the plant leaf extract has been reported to contain compounds like saponin, thanin and flavonoids which lower cholesterol (Ukwah and Ezeonu, 2008; Zhang *et al.,* 2011).

The shedding of yeasts was completely eliminated by the leaf powder at all concentration (Table 2). Although presence of yeasts in poultry is not always a challenge to poultry farmers except if there is outbreak of avian gastric yeast (AGY) called *Macrorhabdus sp* which can be very frustrating to farmers. This yeast infection can cause huge losses to poultry farmers due to mortality of birds (Wissman, 2006). The finding of this study indicates that the leaf powder may be protective against such yeast pathogen, even though the pathogen was not identified in this study.

It is interestingly shown in this study that the addition of *V. amygdalina* leaf powder could eliminate the shedding of *Salmonella, E. coli* and *Cryptosporidium*, although the *Salmonella* and *E. coli* reappeared later in both groups. The elimination of *Cryptosporidium* complete without reappearance in group B but this group had decreased growth performance making it not highly recommended as compared with 1%w/w supplementation. 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 agreement with other studies in which inclusion of fructo-oligosaccharide prebiotics in water and feed of animals protected against *E. coli* but not completely against *Salmonella* infection (Chakraborti, 2011; Licth et al., 2011). This study has provided supportive information on the prebiotic potentials of *V. amygdalina* leaves and also provided clues that these leaf powder at low concentration can be of use as an additive in feeds for raising poultry, preventing gastrointestinal diseases and contamination of environment with microbial pathogens from poultry. The study has also shown that the use of the *V. amygdalina* leaf powder can reduce the cost of poultry production by removing the cost of antibiotics, anticoccidiosis and vitamins that are always administered to birds.

ACKNOWLEDGEMENTS

We acknowledge the kind gestures of the Dean, Faculty of Biological Sciences, Ebonyi State University for allowing us use the animals house during this study.

REFERENCES

Adaramoye, O.A., Akintayo, O., Achem, J. and Fafunso, M. 2008. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. Vascular Health and Risk Management, 4: 235-241.

Binder, H.J. 2010. Role of Colonic Short-Chain Fatty Acid Transport in Diarrhea. *Annual Review of Physiology,* ***72:*** 297-313.

Bovee-Oudenhoven, I.M.J., Bruggencate, S.J.M., Lettink-Wissink, M.L.G. and Van der Meer, R. 2003. Dietary fructo-oligosaccharides and lactulose inhibit intestinal colonization but stimulate translocation of *Salmonella* in rats. *Gut*, ***52***: 1572-1578.

Canny, G.O. and McCormick, B.A. 2008. Minireview: Bacteria in the intestine, helpful residents or enemies from within? *Infection & Immunity*, ***76***: 3360-3373.

Centre for Food Security and Public Health, Iowa State University, Iowa, USA, 2009. Fowl Typhoid and Pullorum Disease. 1-5 [*www.cfsphiastate.edu*]

Chakraborti, C.K. 2011. The status of synbiotics in colorectal cancer. *Life Science Medical Research,* 2011: 1-13.

Cheesbrough, M. 2005. *District Laboratory Practice in Tropical Countries*. Tropical Health Technology and Butterworth – Heinemann Ltd Cambridge Low price ed. Vol. 2, pp 63-70.

Crittenden, R.G., Martinez, N.R. and Playe, M.J. 2003. Synthesis and utilisation of folate by youghurt starter cultures and probiotic bacteria. *International Journal of Food Microbiology*, ***80***: 217-222.

Cummings, J.H., Macfarlane, G.T. and Englyst, H.N. 2001. Prebiotics digestion and fermentation. *American Journal of Clinical Nutrition,* ***73***: 415-420.

Deiwick, J., Salcedo, S.P., Boucrot, E., Gilliland, S.M., Henry, T., Petermann, N., Waterman, S.R., Gorvel, J.P., Holden, D.W. and Méresse, S. 2006. The Translocated *Salmonella* Effector Proteins SseF and SseG Interact and Are Required To Establish an Intracellular Replication Niche. *Infection and Immunity*, ***74(12)***: 6965–6972.

Enyi-Idoh, K.H. Etim, S.E. Ofor, U.A. Mandor, B.I. and Ogar, M. 2012. Evaluation of the antibacterial potential of *Vernonia Amygdalina* on foodborne pathogens isolated from kunu sold in Calabar, Nigeria. *Journal of Microbiology and Biotechnology Research,* ***2(5)***; 778 – 782**.**

Ewaschuk, J.B. and Dieleman, L.A. 2006. Probiotics and prebiotics in chronic inflammatory bowel diseases. *World Journal of Gastroenterology,* ***12***: 5941-5950.

Ezeonu, I.M. and Ukwah, B.N. 2009. *In vivo* studies on the prebiotic effects of *Vernonia amygdalina* leaf extract. *Bioresearch,* ***7****:* 396-401.

Falcao-e-Cunha, L., Castro-Solla, L., Maertens, L., Marounek, M., Pinheiro, V., Freire, J. and Mourao, J.L. 2007. Alternatives to antibiotic growth promoters in rabbit feeding: a review. *World Rabbit Science,* ***15***: 127-140.

Fanatico, A. 2006. Parasite Management for Natural and Organic Poultry: Coccidiosis. *A Publication of ATTRA - National Sustainable Agriculture Information Service* • ***1-800***-346-9140 • *www.attra.ncat.org.*

Gibson, G.R. and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microflora: introducing the concept of prebiotics. *Journal of Nutrition*, ***125***: 1401-1412.

Guarner, F. 2007. Studies with inulin-type fructans on intestinal infections, permeability and inflammation. *Journal of Nutrition*, ***137***: 2568S-2571S.

Hamilton-Miller, J.M. 2004. Probiotics and prebiotics in the elderly. *Postgraduate Medical Journal*, ***80:*** 447-451.

Leenen, C.H. and Dieleman, L.A. 2007. Inulin and oligofructose in chronic inflammatory bowel disease. *Journal of Nutrition*, ***137***: 2572S-2575S.

Licht, T.R., Ebersbach, T. and Frokiaer, H. 2011. Prebiotics for prevention of gut infections. *Trends in Food Science Technology*, ***xx***: 1-13. [http://www.sciencedirect.com.]

Lomax, A.R. and Calder, P.C. 2009. Prebiotics, immune function and inflammation: a review of the evidence. *British Journal Nutrition*, ***101***: 633-658.

Macfarlane, S., Macfarlane, G.T. and Cummings, J.H. 2006. Prebiotics in the gastrointestinal tract. *Alimentary Pharmacological Therapy*, ***24***: 701-714.

Mitchell, D. 2010. Prebiotics may help prevent colon cancer. [http://www.examhealth.com/1275/100/33330/prebiotics-mayhelp-prevent-colon-cancer.html]

OIE 2012. Fowl Typhoid and Pullorum Disease, *OIE Terrestrial Manual, Chapter 2.3.11:* 1-13.

Roberfroid, M. 2007. Prebiotics: The concept revisited. *Journal of Clinical Nutrition*, ***137***: 830S-837S.

Scheppach, W. 1994. Effects of Short Chain Fatty Acids on Gut Morphology and Function. *Gut,* **35**: S35-S38. *http://gut.bmj.com/content/35/1_Suppl/S35*

Ukwah, B.N. and Ezeonu, I.M. 2008. *In vitro* stimulation of growth of intestinal bacteria by prebiotic components of *Vernonia amygdalina* leaf extracts. Journal of Applied Natural Science (JANS), ***3***: 2-7.

Ukwah, B.N and Ezeonu, I.M. 2013. Prevalence of *Cryptosporidium* and other intestinal parasites using different diagnostic techniques in Enugu Metropolis, South-Eastern Nigeria. *African Journal of Microbiology Research,* ***7***(15): 1366 – 1370.

Wang, X. and Gibson, G.R. 1993. Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *Journal of Applied Bacteriology*, ***75:*** 373-380.

Wissman, M.A. 2006. Avian Gastric Yeast**.** *www.exoticpetvet.net*

Wong, J.M., De Souza, R., Kendall, C.W., Emam, A. and Jenkins, D.J. 2006. Colonic health: fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology, 40(3)*:235-243.

Zhang, T., Gang, Li and Haizhen, M.O. and Chunxiang Zhi, 2011. Persimmon Tannin Composition and Function. International Conference on Agricultural and Biosystems Engineering. *Advances in Biomedical Engineering,* ***1-2.***

Corresponding author: Dr. B.N. Ukwah, Medical Microbiology and Parasitology Unit, Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Ebonyi State University, Abakaliki, Nigeria. **Email:** bnukwah@gmail.com; boniface.ukwah@ebsu-edu.net;