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Apparent nutrients digestibility, villi morphometry and intestinal microbiota of broiler chickens fed graded levels of chestnut (*Castenea sativa*) as eubiotics

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***Target Audience:** Poultry farmers, Poultry Researchers, Feed millers*

Abstract

A total of 300-day-old cobb 500 broiler chicks were allotted in a completely randomized design to five dietary treatments each replicated thrice, with 20 chicks per replicate. Chestnut phytobiotic was included at 0g, 100g, 125g and 150g/100 Kg diet while Oxytetracycline was included at 111g/100kg diet. Data was collected on nutrient digestibility, villi morphometry and intestinal microbial activity. All data collected were subjected to analysis of variance and significant differences among treatment means were compared using the Duncan's Multiple Range Test. Birds fed diets containing 100g phytobiotics was significantly (P<0.05) higher in values for red blood cell, monocytes, eosinophils and basophils. There were significant (P<0.05) differences for values of Lactobacillus spp and Bacillus spp, whereas non-significant (P>0.05) differences were observed for Escherichia coli, Clostridium spp and Salmonella spp. Intestinal bacteria count revealed that Lactobacillus spp, a beneficial bacteria was significantly higher and best in diet containing 100g phytobiotics (15.33x10^{3}cfu/g) when compared to that of the control (5.00x10^{3}cfu/g) but similar to that fed oxytetracycline (10.67x10^{3}cfu/g). Supplementation with phytobiotics also increased the concentration of Bacillus spp. In conclusion, supplementation of chestnut phytobiotics at 100g/100Kg significantly improved Lactobacillus spp and Bacillus spp which are beneficial bacteria.

***Key words:** Chestnut, digestibility, villi morphometry, intestinal microbiota, broiler, eubiotics*

Description of Problem

Using antibiotics as growth promoters causes weight increase, better feed conversion and low cost of therapy. Besides positive effects, it is possible for certain negative effects such as production of resistant strains of enterobacteria to occur. However, concerns of consumers over antibiotic-resistant bacteria and drug residues in poultry meat in recent years have generated controversial views concerning use of antibiotics. The European Union has banned the use of human antibiotics as growth promotants in animal feed since 2006.

Now the poultry industry is looking for alternative products, such as prebiotics, probiotics, essential oils, organic acids, enzymes, minerals (Zn and Cu compounds), herbs and spices etc. with claim to affect the composition or activity of intestinal microbiota. The above-mentioned alternative substances are referred to as natural growth promoters (1) as most of them are of natural origin. In recent years, some of those products have been described by the general term 'eubiotics', which is related to the Greek term 'eubiosis', referring to an optimal balance of microflora in the gastrointestinal tract. The main purpose of using such eubiotics is to maintain the intestinal eubiosis, which will result in an improved health status and performance in farm animals. The combination of eubiotics in one product has shown to confer benefits beyond those of either on its own (2).

Among the mentioned alternatives, phytobiotics have drawn a lot of attention because of being natural, nontoxic and residue free. Phytogenic feed additives (phytobiotics or botanicals) are commonly defined as plant-derived compounds incorporated in to diets to improve the livestock productivity through amelioration of feed properties, improvement of nutrient digestibility, absorption and elimination of pathogens in the gut (3,4). However, the use of phytobiotics feed additives like plant extracts, hydrolysable tannin from Chestnut (*Castenea sativa)* have potentials which can be harnessed in animal production especially for gut health in broiler chickens. The protection of the gut environment is now known to play an important role in reducing disease in animals (5).

In this scenario, raw plant extracts and derived tannins are showing promising results for food animal production (6). Miadiasan® feed additive is a blend of two products comprising phytobiotics (plant extract, hydrolysable tannin) from Chestnut (*Castenea sativa*) which may help to maintain chickens intestine, health and improve immunity. The specific objective was the evaluation of Chestnut (*Castenea sativa*) phytobiotics in broiler diets and the effect on digestibility, villi morphometry and intestinal microbiota.

Materials and Methods

Experimental site

The experiment was conducted at the Livestock Section, Division of Agricultural Colleges, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Zaria is located in the Northern Guinea Savannah Ecological Zone on longitude 11° 09' 01.78'N and latitude 7°39' 14.79' E, 671m above sea level. The climate is characterized by a well-defined dry and wet seasons with annual rainfall ranging from 700-1400mm.The maximum temperature varies from 26-320C depending on the season while the mean relative humidity during the dry and wet season are 21 and 72%, respectively (7).

Experimental design and management of birds

Three hundred day old broiler chicks was allocated to five dietary treatments with 3 replicates of 20 birds each in a Completely Randomized Design (CRD). The birds were housed in deep litter pens and managed with all necessary routine management practices. Feed and water was provided *ad libitum* for the period of the experiment.

Preparation of Test ingredients: The test ingredient (Chestnut feed additive) is a finished product from manufacturer and need no further processing. Miadiasan® feed additive is a blend of two products comprising phytobiotics (plant extract, hydrolysable tannin) from Chestnut (*Castenea sativa*).

Experimental diets

Treatment diets were formulated for starter and finisher chickens, respectively. The phytobiotics additives was added as non-inclusive part of the diets as follows:

Diet 1: (Control diet) – without phytobiotics
Diet 2: 100g of phytobiotics/100 Kg diet
Diet 3: 125g of phytobiotics/100 Kg diet
Diet 4: 150g of phytobiotics/100 Kg diet
Diet 5: Oxytetracycline (Sub-therapeutic dose) at 111g/100Kg diet (as recommended by manufacturer).

Digestibility Study

Apparent Nutrient Digestibility trial was carried out at the end of the experiment. Three birds were randomly selected from each treatment and kept in individual cages for total faecal collection. The birds were allowed a period of 3 days to adjust to the cage environment and thereafter offered a known amount of experimental diet. Total faecal droppings were collected for five consecutive days, weighed and oven-dried at 65 °C. The dried faecal samples were assayed for their nutrient contents using the methods described by (8) at the Biochemical Laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria. Nutrient digestibility was determined for crude protein, ether extract, crude fibre and nitrogen-free extract using the formula:

% Apparent Nutrient Digestibility

$$= \frac{\text{Nutrient in feed - Nutrient in feaces}}{\text{Nutrient in feed}} \times 100$$

Villi morphometry

Intestinal segment samples (approximately 2 cm in length) from ileum was taken from birds after slaughter to ascertain villi morphometry. Intestinal Tissues were harvested and\fixed in 10% formolsaline. They were histologically processed according to the method of (9). They were dehydrated through ascending grades of alcohol (70%, 90% and 100%) for 2 hours each. The tissues were cleared in xylene for 2 hours. They were impregnated and embedded in paraffin wax, thereafter sectioned at 5 micron thickness using Rotary microtome machine (Leica RT 25 made in England). Sectioned tissues were mounted on slides, dried and stained using Hematoxylin and Eosin (H and E) stain. Stained sides of the tissues were photomicrographed using Amscope Digital Camera for microscope verision 2.0, made in Japan. Histomorphometric analysis for the villi was carried out using Digimizer image analysis software version 4.5 made in USA (10). The gut histomorphometry indices that was evaluated include villus height, from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, and the villus height to crypt depth ratio. This was carried out at the Histology Laboratory of the Department of Anatomy, Ahmadu Bello University Zaria.

Intestinal bacterial count

At the end of the finisher phase, bacterial cell numbers in the ileum was

determined for *Escherichia coli, Lactobacilli spp, Clostridium spp, Salmonella spp, Bacillus spp* using different selective media for isolation of bacteria groups and characterization based on sugars fermentation using Microbact 12E kit and conventional biochemical methods (11). This was carried out at the clinical pathology laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Statistical analysis

All data obtained from the feeding trial was statistically analysed using the General Linear Model Procedure of Statistical Analysis Systems (12). Significant difference between treatments means was separated using Duncan's Multiple Range Test (12).

Results and Discussion

Table 1 shows the total tract apparent nutrient digestibility of broiler chicken fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotics feed additives. The results show non-significant (P>0.05) differences in ether extract, crude protein, crude fibre and nitrogen free extract. Significant (P<0.05) difference was observed in crude protein. Birds fed diets containing 100g phytobiotics were significantly (P<0.05) better in crude protein (76.15%) compared to that of control (66.54%) but were however, statistically similar to that fed 125g (73.71%), phytobiotics, 150g (72.77%), and oxytetracycline (75.43%) supplementation. This present work is in agreement with the report of (13) who reported that supplementation of either antibiotic growth promoters or phytogenic feed additives (phytobiotics) to the basal diet significantly increased the apparent total tract digestibility of CP when compared to the control group.

Table 1: Apparent total tract percent nutrient digestibility of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa)* phytobiotics feed additives

	Levels of phytobiotics					
Parameter	**0g**	**100g**	**125g**	**150g**	**Oxytet**	**SEM**
Crude Protein (%)	66.54^{b}	76.15^{a}	73.71^{ab}	72.77^{ab}	75.43^{ab}	4.12
Ether Extract (%)	67.56	76.09	71.18	70.71	73.15	4.45
Crude Fibre (%)	40.50	56.81	41.00	44.38	50.37	8.91
Nitrogen Free Extract (%)	67.29	73.40	68.52	68.03	69.06	4.65

a,b: Means with different superscripts on the same row are significantly different (P<0.05): Oxytet; Oxytetracycline; SEM: Standard error of mean.

Table 2 shows the gut histomorphometry of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotic feed additive. There were significant (P<0.05) differences in all the parameters measured except for crypt depth. Villi area, perimeter, height, width and villi height/crypt depth ratio were higher for birds fed 125g of phytobiotics than other treatment groups except for control and oxytetracycline, which were similar in villi height/crypt depth ratio. Villi area, perimeter, height, width, crypt depth and villi height/crypt depth ratio values were similar for birds fed control and oxytetracycline. The morphology of intestinal villi and crypts has been associated with intestinal function and growth in chickens. Higher intestinal villi are associated with increased absorptive surface area of the intestine and thus, an increased absorptive capacity with resultant higher body weight gain (14). A lower villus/crypt ratio has been associated with the presence of toxins, poor nutritive absorption and increased secretion in the gastrointestinal tract, diarrhea, reduced disease resistance and lower overall performance. A large crypt indicates a fast tissue turnover and a high demand for new tissue (15).

Table 2: Gut Histomorphometry of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa)* phytobiotics feed additives

	Levels of phytobiotics					
Parameter	**0g**	**100g**	**125g**	**150g**	**Oxytet**	**SEM**
Area (µm)^{2}	26231^{b}	19447^{b}	45696^{a}	16585^{b}	25346^{b}	6678.52
Perimeter (µm)	939.22^{ab}	696.85^{b}	1071.28^{a}	693.68^{b}	861.71^{ab}	106.80
Villi height (µm)	411.85^{ab}	287.36^{b}	458.83^{a}	277.99^{b}	368.21^{ab}	56.16
Villi width (µm)	138.08^{ab}	127.44^{ab}	160.05^{a}	123.86^{b}	124.60^{ab}	18.05
Crypt depth (µm)	68.57	75.58	71.08	71.85	69.04	12.20
Villi height/crypt (µm)	5.94^{a}	3.85^{b}	6.40^{a}	3.90^{b}	5.93^{a}	0.64

a,b; Means with different superscripts on the same row are significantly different (P<0.05): Oxytet; Oxytetracycline; SEM: Standard error of mean.

Table 3 shows the intestinal bacteria count of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotics feed additives. There were significant (P<0.05) differences for values of *Lactobacillus spp* and *Bacillus spp*, whereas non-significant (P>0.05) differences were observed for *Escherichia coli*, *Clostridium spp* and *Salmonella spp*. The results show that there was no presence of *Clostridium spp* among the treatment groups. *Lactobacillus* spp was significantly higher and best in diet containing 100g phytobiotics (15.33×10^{3}cfu/g) when compared to that of the control (5.00×10^{3}cfu/g) but similar to that fed oxytetracycline (10.67×10^{3}cfu/g). However, the results showed a decrease in the *Lactobacilli spp* count (15.33 x10^3cfu/g, 8.00 x10^3cfu/g, 4.00 x10^3cfu/g) as the levels of phytobiotics increases (100g, 125g, 150g). The results revealed that inclusion of phytobiotics at 100g improved the beneficial bacteria (*Lactobacillus spp*). Supplementation with phytobiotics also increased the concentration of *Bacillus spp*, which is also a beneficial bacteria. Significant (P<0.05) differences were observed for birds fed 125g phytobiotics (9.67 x10^3cfu/g) when compared to the control (4.00 x10^3cfu/g) but similar to that fed 100g phytobiotics (6.33 x10^3cfu/g) and oxytetracycline (6.33 x10^3cfu/g). *Lactobacillus spp* and *Bacilllus spp* are beneficial harmless microbes in the microbiota. Phytobiotics have shown positive effects by increasing the beneficial bacteria and displacing the pathogenic bacteria. The colonization of the GIT by the beneficial bacteria (*Lactobacillus spp* and *Bacillus spp*) suppresses the activity and habitation of potentially pathogenic species (16). The results revealed that chestnut phytobiotics do not act in bactericidal manner but in a bacteriostatic manner, which means it helps to colonize the GIT with beneficial bacteria and reduces the virulence of pathogenic bacteria. This work is in agreement with the report of (13) who reported that phytogenic feed additive (phytobiotics) significantly reduced the cecal population of coliforms and fortified the gut microbiota with beneficial bacteria, such as *Lactobacillus spp.* Once the *Lactobacillus spp.* are established, they might selectively exclude the pathogens from adhering due to their fast colonization, proliferation, and acidification properties in the GIT (17). However, (18) reported that diets containing tannin caused positive effects on the immune system, and exerted potent bactericidal and coccidiostatic properties in broiler chickens.

Table 3: Intestinal bacteria count of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa)* phytobiotics feed additives

	Levels of phytobiotics					
Parameter (10^3cfu/g)	**0g**	**100g**	**125g**	**150g**	**Oxytet**	**SEM**
Escherichia coli	12.66	13.67	13.67	11.00	10.33	4.45
Lactobacilli spp	5.00^{bc}	15.33^{a}	8.00^{b}	4.00^{c}	10.67^{ab}	3.06
Clostridium spp	0.00	0.00	0.00	0.00	0.00	0.00
Salmonella spp	1.00	2.00	1.00	2.00	2.67	1.33
Bacillus spp	4.00^{b}	6.33^{ab}	9.67^{a}	3.00^{b}	6.33^{ab}	2.80

a,b,c ; Means with different superscripts on the same row are significantly different (P<0.05): Oxytet; Oxytetracycline; SEM: Standard error of mean.

Conclusion and Applications

It can be concluded that, Chestnut (*Castenea sativa*) phytobiotic used as natural growth promoter:

1. Improved the integrity of villi, nutrient digestibility and intestinal microbial balance. It also improved the activities of beneficial bacteria in the gastro intestinal tract of broiler chickens thereby making more nutrients available from the feed consumed.
2. Can be used by poultry farmers at 100g/100 Kg feed as replacement for antibiotics in broiler production which significantly improved *Lactobacillus spp and Bacillus spp* that are the beneficial bacteria and in turn resulted in improved gut health of broiler chickens.

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