POTENTIALS OF ETHANOLIC EXTRACTS OF (*Jatropha curcas*) ON THE GUT MORPHOLOGY OF BROILERS

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ABSTRACT

In a study conducted to evaluate the effect of *Jatropha curcas* leaf extract on the gut morphology of broiler chickens, one hundred and fifty day old Arbor Acre broiler chicks were randomly allocated to five treatments, each replicated five times, with six birds per replicate over a period of forty two days. The birds were allocated to the following treatments (T): T1 (Negative control, no antibiotics), T2 (positive control, with antibiotics), T3 (0.25g/100kg *Jatropha curcas* leaf extract), T4 (0.50g/100kg *J. curcas* leaf extract) and T5 (0.75g/100kg *J. curcas* leaf extract). Samples were collected from the ileum and duodenum for gut morphology assay, with the results of gut morphology revealing that *J. curcas* supplementation improved villi height in duodenum ($p<0.05$), while significantly decreasing values for villi height in the ileum ($p<0.05$). Crypt depth values were also significantly influenced by inclusion of antibiotics and 0.25% *J. curcas* extract.

Keywords: Performance, Jatropha curcas leaf extract, Gut morphology

INTRODUCTION

Over the past few years there has been increased concern about antibiotic-resistant bacteria and the inclusion of antibiotics in animal diets for growth promotion. This led to the ban of a number of antibiotic growth promoters (Dibner and Richards, 2005). Since the EU ban on using antibiotics as growth promoters in 2006 (Huff *et al.*, 2006), there has been an increase in the incidence of endemic diseases in poultry (Chee, 2008).

The practice of feeding livestock with antibiotics has been in use for over fifty years. Antibiotics affect microflora by altering the metabolism of microorganisms, and suppressing microbial growth in the gut (Gadd, 1997). Usage of antibiotics has negative effects on animal's health and production such as residual in tissues, long withdrawal period, and development of resistance in microorganisms, allergies, genotoxicity (Markovic, 2005) and harmful effects on human health by development of microbial resistance to specific products (Botsoglu and Fletouris, 2001; Williams and Losa, 2001; McCarteney, 2002). Consequently there is considerable research interest in the possible use of natural products, such as essential oils and extracts of edible and medicinal plants, herbs and spices, for the development of new additives in animal feeding. Plant extracts or phytogeni phytogenic feed additives have shown some capacity to replace or could be considered as potential alternatives to antibiotic growth promoters (AGP).

Phytogenic feed additives are plant extracts derived from herbs or spices, which have beneficial effect on animal production and health. A large variety of the plants have properties which could potentially improve feed intake, digestion, feed conversion and body weight gain (Lev'kova *et al.*, 2001, Williams and Losa 2001, Ertas *et al*, 2005). Plant extracts and spices as single compounds or as mixed preparations can play a role in supporting both performance and health status of the animal (Janssen, 1989; Horton *et al.*, 1991; Bakhiet and Adam, 1995; Skrabka Blotnicka *et al.*, 1997; Gill, 2000; Manzanilla *et al.*, 2001). Beneficial effects of herbal extracts or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and antihelminthic actions. Isoprene derivatives, flavonoids, glucosinolates and other plant metabolites may affect the physiological andchemical function of the digestive tract.

In particular, gut health has been affected, and without a healthy intestinal tract a broiler cannot reach its full performance potential. Due to this, there has been a drive in the market for feed supplements that will improve health and production of poultry, but remain safe for humans. There are thousands of species of medicinal plants used globally for the cure of different infections. Some of these plants include; *Anacardium occidentale* (cashew), *Pilostigma recticulatum, Anogeissus leiocarpa, Enantia chlorantha, Senna occidentalis, Jatropha curcas* "Lapalapa", *Azadirachta indica* "Dongoyaro". A great number of antibacterial agents exist for various purposes; some of these are usually in the form of plants. The action of these plants on microorganisms have been found to be due to the presence of certain substances such as alkaloids, glycosides, volatile oils, gums, tannins, steroids, saponins, phlobatannins, flavonoids and a host of other chemical compounds referred to as secondary metabolites that are present in them (Kochlar, 1986; Sofowora, 1993; Oyagade *et al.*, 1999).

Medicinal plants like *J. curcas* have played a major role in the treatment of various diseases including bacterial and fungal infections. The extracts of many *Jatropha* species including *J. curcas* displayed potent cytotoxic, anti-tumor and anti-microbial activities in different assays. The latex of *J. curcas* also showed antibacterial activity against

Staphylococcus aureus (Thomas, 1989), however the antimicrobial activity of the other parts have not been fully investigated.

Jatropha curcas Linn. (Family Euphorbiaceae) Physic nut, is a drought resistant shrub which is widely grown in Central and South America, South-east Asia, India and Africa. *J. curcas* plant has been initially considered a traditional herb in many parts of the world (Gubitz *et al.*, 1999). It has gained importance in Malaysia, but as a source of seed oil for biofuel production.

Traditionally, different parts of *J. curcas* have been used in treatment of different forms of infection. The leaves decoction is used as an antiseptic substance during birth, the root decoction is used to treat sexually transmitted diseases and the seed is used to treat skin diseases (Gubitz *et al.*, 1999; Joubert *et al.*, 1984). However, its application as a remedy for many of these ailments has not been fully substantiated by the actual bioactive compounds responsible for the various effects. The objective of this study was however to evaluate the effect of extracts of *Jatropha curcas* on the gut morphology of broilers

MATERIALS AND METHODS

The study was carried out at the poultry unit of the Teaching and Research farm, University of Ibadan, Nigeria (latitude7 73" N and longitude 3 5' to 3 36 E). Ibadan is located 228 m above sea level and has a mean annual rainfall of 1289.2 mm based on 27 year of records (Alabi and Ibiyemi, 2000). The experimental pens were thoroughly cleaned, washed and disinfected. The condition of housing and management of birds were the same in all groups.

Processing of Leaf Extract

Fresh leaves of *Jatropha curcas* were collected within Ibadan, South-Western Nigeria. The leaves collected per time was rinsed with clean water to remove any foreign matter, chopped and air-dried. The dried leaves were milled using a roller mill. One litre of an 80 % ethanol extraction fluid was mixed with 200 g of powdered plant material. The mixture was kept for 2-5 days in tightly sealed vessel at room temperature protected from sunlight, and stirred several times daily with a sterile glass rod. This mixture was filtered through muslin cloth and the resultant residue was reconstituted with 80% ethanol and the extraction process repeated 3-5 times until a clear colorless supernatant liquid could be obtained from the residue indicating that no more extraction from the plant material was possible. The extracted solvent was subjected to rota-evaporator (Model # R) to remove the ethanol. Rota evaporation was used to concentrate the smaller quantity of extract. A 250 ml aliquot of extracted liquid was subjected to rota evaporatoration for 3-4 hours. The resultant extract was then weighed and stored in desiccators until use. The dried extracts of *Jatropha curcas* was mixed in different concentrations with the feed to be presented to the birds.

Experimental Diets and Bird Management

A total number of 150 day old Arbor-acre broiler chicks were purchased from a commercial hatchery in Ibadan. The birds were randomly allocated to five dietary treatments (T1 - negative control, no antibiotics, T2 - positive control, with antibiotics, T3 - 0.25g/100kg *Jatropha curcas* leaf extract, T4 - 0.50g/100kg *J. curcas* leaf extract and T5 - 0.75g/100kg *J. curcas* leaf extract) in a completely randomized design. Each treatment was replicated 5 times, with each replicate having 6 birds each. Water and feed were supplied *ad libitum* while a standard medication and vaccination program was adopted as recommended by the parent breeding farm. Weekly feed intake and individual bird weights were recorded for six weeks. Weekly weight gains and feed conversion ratio were calculated from the data collected. The gross composition of experimental diet is shown in Table 1

Gut morphological Assay

After slaughtering, the small intestine was removed and washed with sterile phosphate buffered saline (PBS), then middle sections (3-4 cm) of duodenum and illume of one bird from each replicate were cut and prepared for histological indices assay. The histological indices were measured according to Iji *et al.* (2001). Intestinal tissue samples were fixed in buffered formalin and dehydrated, cleared and impregnated with paraffin. The processed tissue was then embedded in paraffin wax. Tissue sections, 6µm thick (3 cross-sections from each sample), were cut from the waxed tissue by a microtome, cleared of wrinkles by floating on warm water (55- 60oC) and were fixed on slides. A routine staining procedure was carried out using a combination of the periodic acid-Schiff method (PAS staining) with the basophilic dyes alcian blue (AB staining). Histological indices were determined by use of a light microscope (Olympus Corporation, Tokyo, Japan). Calculation was made for villous height: crypt depth ratio (VH: CD). Villus height was measured from the tip of the villus to the top of the lamina propria, and the crypt depth was measured from the base up to the region of transition between the crypt and villus.

Chemical analysis

Feeds were subjected to proximate analysis according to AOAC (1990) method.

Statistical analysis

Data generated were subjected to one-way analysis of variance (ANOVA) using the SAS (1999) package and the means separated using Duncan multiple range test of the same software at 5% level of significance.

RESULTS AND DISCUSSION

The villi height, crypt depth and villi height: crypt depth ratio (VH: CD) of broiler duodenum and ileum at 42 days of age are presented in table 2. Data shows that supplemented groups had improved villi height in duodenum in T3, T4 and T5 ($p<0.05$), while

decreased values were observed in Ileum for treatments T3, T4 and T5 as against T2 (positive control) ($p<0.05$). However, there was no significant difference between the duodenal villi height. The villi height of T2 (positive control) had the values for ileum ($P<0.05$) while T1, T3, T4 and T5 had lower values with T5 being the lowest. The crypt depth values for T2 and T3 were significantly different ($P<0.05$) while others treatments are not significantly different in duodenum. There were no significant difference among treatments for ileum ($P>0.05$).

The villi height: crypt depth values for the duodenum showed that the supplemented treatments (T3, T4, and T5) were significantly higher than both negative and positive control (T1 and T2) ($p<0.05$). There was significant difference observed among the supplemented diets, mainly between T3 and T5. The villi height: crypt values for Ileum were not significant different ($P>0.05$). T5 values of villi height: crypt had the least values when compared to other treatments.

The result from Table 3 shows that there was no significant difference among the treatment for duodenum, this agrees with the findings of Fukayama *et al.* (2005) who reported no significant difference between the villi height in duodenum of 21 days old broiler fed oregano extract. There were significant differences in the villi height in ileum but the value did not however translate into a difference in the birds' performance especially in terms of feed conversion ratio. The best FCR values were recorded in treatment 4 to treatment when compared with treatments 1 and 2 which are the treatment controls. Higher villi height indicates higher surface area for absorption and might be responsible for lower FCR values observed in treatment 4.

Crypt depth values for duodenum showed there were significant differences between treatments especially between treatment 2 and 3. Treatment 3 had the highest duodenal crypt value which does not translate into higher duodenal villi height. Treatment 4 showed a good correlation between crypt depth and villi height for duodenum as its higher crypt value translated into a higher villi height. This is because the crypt is known to be important in the formation of villi. Also the crypt depth determines the degree of exigency of cells (enterocytes) of the intestinal mucosa which leads to the formation and elongation of the villi. (Tiago *et al.*, 2012)

Yason *et al.* (1987) also referred to the crypt as the villus factory as a larger crypt indicates fast tissue turnover and a high demand for new tissue. On this finding, it can be deduced that higher gut morphological values like villi height to crypt depth ratio can help in improving nutrient absorption, gut function and overall performance of the birds. The result of this study showed that the villi height: crypt ratio between the treatment differ significantly in duodenum while no significant difference ($p>0.05$) was observed in the ileum. This shows that the intestinal health of the birds can be guaranteed when fed jatropha curcas leaf extract as Tiago *et al.* (2012) confirmed that the villus height: crypt depth ratio is a good indicator of intestinal health.

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Table 1: Gross Composition of Experimental Diets

Ingredients	Starter	Finisher
Maize	56.00	59.00
Soyabean meal	36.50	28.00
Fish meal 72%	2.20	1.30
Wheat offal	1.60	7.50
Dcp	1.50	1.50
Methionine	0.10	0.10
Salt	0.25	0.25
Premix	0.25	0.25
Total	100.00	100.00
Calculated Values		
Crude Protein (%)	23.02	20.00
Metabolizable Energy(kcal/kg)	2995.10	2950.05
Crude Fibre (%)	3.51	3.79
Calcium (%)	1.03	1.00
Available Phosphorus (%)	0.63	0.61
Lysine (%)	1.14	1.09
Methionine (%)	0.57	0.55
Chemical composition		
Dry Matter	90.20	90.50
Crude Protein	23.05	20.90
Crude Fibre	3.7	5.10
Ether Extract	2.7	2.50
Ash	6.5	6

Table 2: Gut Morphology of Broilers fed *Jatropha curcas* Extract

	DIETS					
Parameters	**1**	**2**	**3**	**4**	**5**	**SEM**
Villi Height						
Duodenum	196.38	151.30	176.28	212.40	207.20	19.5
Ileum	127.36	157.22[a]	128.76	138.70	93.13[b]	18.53
Crypt Depth						
Duodenum	28.52	21.58[b]	34.08[a]	30.38	27.78	3.45
Ileum	23.50	24.60	22.80	22.68	21.08	1.32
Villi height:Crypt depth ratio						
Duodenum	6.87	7.14	5.28[b]	7.34	7.62[a]	0.65
Ileum	5.53	6.55	5.60	6.26	4.40	0.84