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Promoting bird performance using phytogetic as a healthy modulator of gut morphometry in broiler birds

**Dudu, Francess^{1a}, Ogidikpe, Keniebi Alfred¹, Deemua, Oyinmiesendei Marvel¹,
Youngman, Soseipiribo tubonimi¹, Ofongo, Ruth Tariebi S¹.**

¹DEPARTMENT OF ANIMAL SCIENCE, NIGER DELTA UNIVERSITY
WILBERFORCE ISLAND BAYELSA STATE, NIGERIA

Introduction

Poultry production is one of the fastest-growing sectors of meat-producing agriculture, driven by the increasing global demand for affordable sources of animal protein to meet the needs of the rapidly expanding human population (Bogale and Engida, 2022; El-Sabrou *et al.*, 2022). In order to sustain this growing demand for foods of animal origin, continuous efforts are being made to develop innovative strategies that improve efficiency and productivity in poultry production systems in order to sustain the growing demand for food of animal origin (El-Sabrou *et al.*, 2022). Over the past decades, antibiotic growth promoters (AGPs) have been widely used in poultry diets because of their beneficial effects on growth performance, feed efficiency, and overall health status of broiler chickens (Mirzaei *et al.*, 2022). However, the long-term and indiscriminate use of antibiotics as growth promoters has been associated with a reduction in beneficial gut microflora, the emergence of antibiotic-resistant pathogenic microorganisms, and the

accumulation of antibiotic residues in animal products and the environment, thereby posing a serious threat to public health (Kurniawati *et al.*, 2021; Mirzaei *et al.*, 2022).

Consequently, increasing concerns over antimicrobial resistance and food safety led to the ban on in-feed antibiotic growth promoters in many regions, particularly in the European Union in 2006. This development stimulated growing interest among poultry nutritionists and researchers in the search for safe, natural alternatives capable of maintaining productivity without compromising animal or consumer health (Upadhaya and Kim, 2017; El-Saadany *et al.*, 2022; El-Sabrout *et al.*, 2022). Among the numerous alternatives investigated, plant-derived products, collectively referred to as phytogetic feed additives (PFAs), have gained considerable attention and wide consumer acceptance (Saeed *et al.*, 2018). These medicinal herbs, spices, and aromatic plants have long been recognized for their roles in improving food flavor, aroma, and health benefits in both humans and animals (Elgammel and Alagawany, 2015; Abd-El-Hack *et al.*, 2019). Furthermore, their application in livestock production has increased due to their reported ability to enhance product quality, including carcass traits and meat characteristics (Abdelnour *et al.*, 2018; Velmurugan *et al.*, 2018).

Phytogetic feed additives are natural plant-derived products incorporated into animal diets to enhance productivity through improved digestibility, nutrient absorption, and suppression of pathogenic microorganisms in the gastrointestinal tract (Bölükbaşı *et al.*, 2009). They encompass a broad range of plant-derived substances obtained from various plant parts, including roots, bark, leaves, flowers, fruits, seeds, herbs, and spices, and are commonly incorporated into animal diets as dried plant materials, powders, extracts, oleoresins, or essential oils (Biswas *et al.*, 2024). Plant-based additives have been reported to be relatively safe, less toxic, residue-free, and environmentally friendly when compared with synthetic antibiotics and inorganic chemicals (Wang *et al.*, 2022), making them suitable feed stuffs for sustainable poultry nutrition.

Uziza (*Piper guineense*), also known as West African black pepper, is a perennial climbing vine belonging to the *Piperaceae* family. It is indigenous to West Africa where it is cultivated and utilized extensively, particularly in countries like Nigeria, Ghana, and Cameroon. Uziza is esteemed for its dual role as both a

culinary spice and a medicinal plant (Madueke *et al.*, 2021). It is rich in bioactive compounds, revealing the presence of several constituents such as alkaloids, glycosides, tannins, flavonoids, terpenes; sesquiterpenoids and monoterpenoids, saponins and secondary metabolites (Gbadamosi *et al.*, 2011; Ejele *et al.*, 2012; Udoh *et al.*, 2012; Oyemitan *et al.*, 2015). These metabolites confer medicinal properties such as antioxidant, anti-inflammatory, anti-tumor, anti-allergic and antiplatelet properties, anti-malarial, antihypertensive, antiarrhythmic and anti-cancer properties (Adesokan and Akanji 2010; Okoye and Ebeledike 2013).

Pawpaw (*Carica papaya*) is a tropical plant that has attracted considerable scientific interest. Native to tropical America, it is widely distributed throughout Africa and other tropical regions. Pawpaw has been identified as a rich natural source of papain, a proteolytic enzyme known for its digestive and therapeutic applications (Koul *et al.*, 2022). The plant contains several phytochemicals, including alkaloids, saponins, tannins, glycosides, and flavonoids, many of which possess pharmacological significance (Wang *et al.*, 2022). These compounds have been reported to exhibit antimicrobial activity, enhance physiological functions, and support overall health (Wang *et al.*, 2022). Pawpaw seeds specifically contain bioactive alkaloids such as flavonoids, phenolic compounds, and fatty acids such as palmitic, oleic, stearic, and linoleic acids, and have been traditionally used in the treatment of various ailments (Olorunsola *et al.*, 2023). Traditionally, different parts of the pawpaw plant have been used for medicinal purposes. Moreso, recent studies have highlighted the potential of pawpaw seed and leaf products as natural feed additives with antimicrobial, antioxidant, and growth-promoting properties in poultry productions (Koul *et al.*, 2022). Despite the increasing number of studies on phytogenic feed additives, there are limited information regarding the effects of black pepper and pawpaw supplementation on gut morphometry. Therefore, the present study was designed to bridge this knowledge gap by evaluating their potential as natural alternatives to antibiotic growth promoters in broiler chicken diets.

Materials and Methods

Experiment location

The experiment was carried out in Niger Delta University Teaching and Research farm, Wilberforce Island, Bayelsa State which is geographically located within latitude 4° 15'N, 5 ° 23' S and longitude 5° 22'W and 6° 45' E. The experimental area is located in the rainforest vegetation.

Preparation of experimental materials

Fresh pawpaw seed was obtained from Amassoma in waterside market and black pepper seed were gotten from Yenagoa in Swalli Market, the pawpaw seeds were washed thoroughly and sun dried for four days, while black pepper was sundried for a day. After sun drying, both seeds were taken to the mill and grounded to powder form and stored separately in a plastic airtight container.

Experimental design and management of experimental animals

Two weeks prior to the arrival of the broiler chicks, the pens were subjected to thorough washing with disinfectant (Izal solution), followed by scrubbing, cleaning, and removal of debris and cobwebs. A total of two hundred and forty (240) day old Arbor acre broiler chicks were purchased from Chi farm limited Ibadan, Oyo State. The birds were housed in a deep litter system where they all received uniform care and management. The birds were randomly allotted into six treatment groups designated as T1, T2, T3, T4, T5 and T6 respectively. Each treatment had four replicates with ten birds per replicate in a completely randomized design. T1 (control) contained 0% of any experimental diet, while T2 contained 1% antibiotic, T3 contained 1% black pepper seed powder, T4 contained 2% black pepper seed powder, T5 contained 1% pawpaw seed powder and T6 contained 2% pawpaw seed powder.

Sample collection

On day 42 of the experiment, two broiler chickens randomly selected from each replicate were slaughtered, allowed to bleed, and defeathered. After that, its gastrointestinal tract (GIT) was carefully removed for both gut morphometric and immune response analyses.

For gut morphometry, a small tissue was cut from the ileum segment of the small intestine for the morphological measurement. For the immune response evaluation and histopathology test, the spleen, proventriculus and a segment of the ileum were removed and collected. All the samples were immediately stored in a container containing 10% formaldehyde.

Statistical analysis

All data obtained from the study were tested using one-way analysis of variance (ANOVA) according to statistical analysis system (SAS, 2003). Where Significant differences were observed and means were separated by Duncan's Multiple Range Test of the same software. Results were considered statistically significant at ($P < 0.05$).

Results and Discussion

The results of gut morphometry in broiler birds administered antibiotics, *Carica papaya* and *Piper guineense* supplemented diets at 1% and 2% are presented in Table a. There was significant difference ($P < 0.05$) observed in the villi height among all the treatment. Supplementation of broiler diet with *Carica papaya* at 2% resulted in significantly higher villi height (2.92), followed by birds fed diet supplemented with 1% *Carica papaya* (2.85) then by control diet (2.79). Birds fed *Piper guineense* 1% and 2% (2.44 and 2.58) had comparatively lower values, while antibiotics treated group recorded the least villus height (2.24). Crypt width also showed significant difference ($P < 0.05$) among treatments. The highest value was also observed in birds fed diet supplemented with 2% *Carica papaya* (2.76), followed by *Piper guineense* 2% (2.65) and control (2.74). Moderate value was recorded in *Carica papaya* 1% (2.56). The least value was recorded in birds fed antibiotics (2.36) and *Piper guineense* 1% (2.25). However, no significant difference ($P > 0.05$) was observed in crypt depth, villus width and muscle thickness, although numerical variations were observed across groups.

Table a

Parameters	Control	Antibiotics	<i>Piper</i> <i>guineense</i> 1%	<i>Piper</i> <i>guineense</i> 2%	<i>Carica</i> <i>Papaya</i> 1%	<i>Carica</i> <i>Papaya</i> 2%	PV	SEM
Villi height	2.79 ^{ab}	2.24 ^d	2.44 ^{ab}	2.58 ^{bc}	2.85 ^{ab}	2.92 ^a	0.01	61.3
Villi width	2.71 ^a	2.24 ^a	2.36 ^a	2.54 ^a	2.71 ^a	2.80 ^a	0.14 ^{ns}	5.82
Cryptal depth	687.81 ^a	619.93 ^a	552.33 ^a	713.11 ^a	640.42 ^a	737.73 ^a	0.48 ^{ns}	25.68
Cryptal width	2.74 ^{ab}	2.36 ^{bc}	2.25 ^c	2.65 ^{ab}	2.56 ^{abc}	2.76 ^a	0.04	25.68
Muscle thickness	262.09 ^a	2.55.22 ^a	246,70 ^a	249.55 ^a	245.43 ^a	292.43 ^a	0.53 ^{ns}	7.69

DISCUSSION

The present study is in agreement with those of Shittu *et al* (2024) who reported that an inclusion of Negro pepper, garlic and ginger in broilers diet can improve the gut histomorphometry of broiler birds. Oloruntola *et al* (2016) reported an improved intestinal morphology in broiler fed phyto-genic feed additives. The result of the present study is also in accordance with Avishek *et al.*, (2022) who reported a significant increase ($P<0.01$) on the villi height, villi width and crypt depth. Similarly, Windish *et al* (2008) noted that plants derived feed additives enhance gut health through bioactive compounds that stimulate epithelial development.

However, these findings are contrary to those of Megaache *et al* 2018 who reported that phyto-genic has no effect on the different parts of the broilers gut. Cross *et al* (2007) and Hernandez *et al* (2004) observed no

significant effects of phytogenic additives on gut morphometry. Adegbeye *et al* (2020) also reported that birds fed control diet had longer villi height than those fed supplemented with phytogenic. Additionally, improved intestinal morphology in broiler chickens administered antibiotics which contrasts with the lower values recorded in this study.

Moreover, Miles *et al* (2006) reported the moderate values observed in *Piper guineense* may be attributed to the presence of piperine a bioactive compound found in *Piper guineense*, which enhances digestive enzymes and nutrient utilization (Platel and Srinivasan, 2004; Adosiaku *et al.*, 2012). In contrast, the significantly lower values recorded in the antibiotic group may be due to suppression of gut microflora, which are essential for stimulating normal intestinal development (Dibner and Richards, 2005). In contrary Shahan *et al.*, 2022 reported that the effect of antibiotics on intestinal microflora was time dependent.

Conclusions and Outlook

In conclusion, this study revealed that *Carica papaya* seed meal supplementation improved gut morphometric parameters and could be used as a suitable alternative to antibiotics.

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