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ORIGINAL RESEARCH ARTICLE

Performance, microbial load and gut morphology of weaned pigs fed diets supplemented with turmeric, ginger and garlic extracts

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ABSTRACT

Plant extracts have been proposed as effective additives in swine production for potential beneficial effects on pig performance and health. Changes in intestinal morphology, can lead to better nutrient absorption, decreased secretion in the gut and better overall performance. Hence, the experiment was conducted to assess the effect of turmeric, ginger and garlic extracts on performance, microbial loads and gut morphology of weaned pigs. A total number of 20 weaned pigs were randomly allotted into 4 treatments with 5 replicates each with a pig per replicate and arranged in a completely randomized design. T1 (control), T2 (2g turmeric/kg feed), T3 (2g ginger /kg), T4 (2g garlic /kg). Pigs in treatment 2 had a better feed utilization than treatment 1(control diet) and those in the other treatments. Enterobacter count was significantly higher in pigs fed treatments 1(6.38 cfu), 2 (6.29 cfu) and 3 (6.27 cfu), compared to their counterpart on treatment 4 (5.12). The total bacteria count was lower in treatment 4 (6.63 cfu). The result revealed a significant difference for ileum in the values recorded for villus height of pigs fed the experimental diets. Pigs fed diets in treatment 4 were significantly higher (1419.75µm) when compared to others with values of 709.25 µm, 1156.50 µm and 1068.00 µm in treatments 1, 2 and 3 respectively. Values observed on the villus width of the pigs fed experimental diet did not show any significant difference across the treatments. Garlic extract resulted in improved intestinal morphology characteristics of pigs.

Keywords: Garlic, Ginger, Gut morphology, Microbial load, Tumeric

INTRODUCTION

Achieving optimum performance and cost benefits from increasingly complex mixes of raw materials is an issue facing pig production in the tropics. Modern pigs have a great performance potential. Achieving this potential however, can be challenging in high throughput farms where production stressors influence performance. Good gut health contributes to an optimal performance. In livestock farming, infectious agents reduce the yield of farmed food animals. To control this, the administration of sub-therapeutic antibiotics and antimicrobial agents had been shown to be effective (Al-Dobaiba and Mousa, 2009). Optimal intestinal health is a positive co-operation between the microbiota present inside the gut and the gut barrier. For strong and consistent support through nutrition, it is important to take both into account. Production of pigs without using antibiotic growth promoters represents a challenge. Producing swine without in-feed antibiotics requires a combination of different strategies. These strategies can be divided into three categories: management strategies, nutritional strategies, and alternative dietary supplements. These can be accomplished through an improved immunological response to pathogens or via mechanisms that prevent the pathogens from adhering to intestinal tissue, and thus, reduce the damaging effects of the pathogens. Different approaches have been proposed within each strategy, but the similarity among the approaches is that they all aim at improving the pigs' ability to prevent pathogenic bacteria from colonizing in the intestinal system. Some of the approaches that are available to improve the pigs' ability to reduce the impact of intestinal pathogens are phytogenic, commonly defined as plant-derived compounds incorporated into diets to improve the productivity of livestock through amelioration of feed properties, promotion of the animals' production performance, and improving the quality of food derived from animals however, their mode of action and aspects of their application are still areas of interest. Although this definition is driven by the purpose of use, other terms are commonly used to classify the vast variety of phytogenic compounds, mainly with respect to origin and processing, such as herbs (flowering, non woody, and non persistent plants), spices (herbs with an intensive smell or taste commonly added to human

food), essential oils (volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation), or oleoresins (extracts derived by non aqueous solvents). Liu *et al*. (2013) earlier reported that capsicum oleoresin, garlic botanical, and turmeric oleoresin reduced diarrhea and decreased the adverse effects of *Escherichia coli* infection by reducing serum inflammatory mediators in pigs. Plant extracts have been proposed as effective additives in swine production for potential beneficial effects on pig performance and health (Manzanilla *et al*., 2004; Allan and Bilkei, 2005). Hence, this study was therefore conducted to assess the effect of turmeric, ginger and garlic extracts on performance, microbial load and gut morphology of weaned pigs.

MATERIALS AND METHODS

The experiment was carried out at the Pig Unit of the Teaching and Research Farm of the University of Ibadan, Ibadan, Nigeria. Twenty weaned pigs with an average weight of 8kg ±2 were obtained from a reputable commercial farm in Nigeria. The pigs were randomly allotted into 4 treatments with 5 replicates each with a pig per replicate and arranged in a completely randomized design, and were fed the experimental diets for 7 weeks. T1 served as the control, T2 contained 2g/kg turmeric, T3 contained 2g/kg ginger, while T4 had 2g/kg garlic. The animals were kept and monitored for one week for acclimatization before the commencement of the experiment. All the animals were of good health. The peeled turmeric, ginger and garlic were washed and air dried. They were ground to fine powder. Samples (10g) of which were collected along with feed and analyzed to determine their proximate composition.

Extraction of selected spices powder

One liter of an 80 % ethanol extraction fluid was mixed with 200 g of powdered plant material. The mixtures were kept for 2-5days in tightly sealed vessels at room temperature of 22^0C, protected from sunlight, and agitated several times daily with a sterile glass rod. This mixture is filtered through muslin cloth and the residue, adjusted to the required concentration (500 ml of 80% ethanol for the residue of 200 g of powdered plant material) with the extraction fluid for further extraction. Further extraction of the residue was repeated 3-5 times until a clear colorless supernatant extraction liquid was obtained indicating that no more extraction from the plant material was possible. The extracted liquid was subjected to rota-evaporation (Brinkmann rotavapor, Model # R) to remove the ethanol. The semisolid extract produced was then air dried to completely remove ethanol from the extract.

The experimental diet is as shown in Table 1.

Table 1: Gross composition of experimental basal diet

Ingredient	Percentage
Maize	38.00
Soybean meal	8.00
Wheat offal	20.00
Groundnut Cake	15.00
Palm Kernel Cake	10.00
Palm oil	5.00
Lysine	0.30
Dicalciphosphate	1.50
Common Salt (Nacl)	1.50
Premix (Grower)	0.40
Methionine	0.30
Total	100.00
Calculated Nutrient	
Metabolizable Energy(kcal/kg)	2662.46
Crude Protein%	19.11

The experimental diets were as follows
T1 is control, no turmeric, garlic or ginger in the diet
T2 is turmeric in the feed at (2g/kg) of diet,
T3 is ginger mixture in the feed at (2g/kg) of diet
T4 is garlic mixture in the feed at (2g/kg) of diet.

Data Collection

The feed intake was obtained by subtracting the leftover feed from the quantity served as follows: Feed intake= quantity of feed served-quantity of feed left. Body weight gains were determined by subtracting the initial body weight from the final body weight.

Feed conversion ratio (FCR) was calculated as the ratio of feed consumed to the body weight change and is expressed as:

$$FCR = \frac{\text{Average Feed Intake (g)}}{Average\ Weight\ Gain\ (g)}$$

Table 2: Proximate composition of experimental diet

Parameters (%)	Values
Crude protein	19.95
Ether extract	6.00
Ash	8.00
Crude Fibre	5.00
Dry Matter	91.12
Moisture	8.88

Viable bacteria are counted by diluting samples, plating the dilutions on solid medium, and counting the colonies that arise. Results are expressed as colony-forming units (CFU), since some bacteria may not produce colonies on the medium selected. Only plates

(or replicate plates from the same dilution) with 30-300 colonies are counted. Plates with fewer than 30 colonies give statistically unreliable results, while plates with more than 300 colonies are too crowded to allow all the bacteria to form distinct colonies. Usually, more than one dilution in a series is plated, just to be sure that results in a countable range will be obtained.

The concentration of bacteria in the original sample was calculated as: CFU ml^{-1} (or g^{-1}) = (colonies on plate)/(final plate dilution). Frequently, volumes other than one ml were used to inoculate the plate, for example, 0.1 ml is often used when surface-plating, as larger volumes may not be absorbed by the agar. Plating 0.1 ml of a given dilution is mathematically identical to plating a 1 ml of a further 1:10 dilution. For this reason, the size of the inoculum was usually incorporated with the dilution factor to give the "final plate dilution" (d x i). When 1.0 ml of a 10^{-4} dilution was plated, the final plate dilution is 10^{-4}. When 0.1 ml of the same dilution was plated, the final plate dilution was 10^{-4} X 10^{-1} = 10^{-5}.

The abdominal cavity was opened along the midline immediately after the pigs were euthanized. A 5-cm sample of the duodenum was collected; approximately 10 to15 cm distal to the stomach, and a 5-cm sample of the ileum was collected approximately 20 cm proximal to the ileocecocolic junction. A 5-cm sample was collected at the midpoint of the remaining small intestine, which was regarded as the jejunum. Each sample was ligated at both ends and a mixture of 30 mL per L glutaraldehyde and 40 mL per L paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) was injected. Each sample was then removed from the abdominal cavity and stored in the same fixative for 2 hours until preparation for light and scanning electron microscopy.

A 5 × 8-mm section from each 5-cm intestinal sample was fixed with Bouin's fixative solution (saturated solution of picric acid, 375 mL; formaldehyde buffered with CaCO3, 125mL; and acetic acid, 25 mL) for 1 week at room temperature, embedded in Paraplast Plus (Sigma-Aldrich Co, St Louis, Missouri), and cut into 5-µm cross sections using a microtome. Every 10^{th} section was collected and stained with hematoxylin-eosin Villus height, excluding the intestinal crypt, was measured in two villi in each section. Villi that included the lamina propria were selected at 40× magnification. Sixteen values of villus height were obtained from eight sections per pig, and the average of these values was expressed as the mean villus height for each pig. To measure villus area, the width of the villus was measured at the base and apex.

Villus area was calculated from villus height, basal width, and apical width. Two villi that included lamina propria were selected at 40× magnification for each section per pig. Ten values of villus area were obtained from eight sections per pig, and the mean villus area per pig was the average of these values. To measure one cell area on the 5-µm cross section, the area of the epithelial cell layer was measured in the middle of a villus that included lamina propria, and the number of cell nuclei within this layer was counted. The area of the epithelial cell layer was then divided by this number. This measurement was employed in one to two fields per section. Sixteen values of cell area were obtained from eight sections per pig, and the mean cell area per pig was the average of these values. To measure the cell mitosis number per crypt, five crypts with the approximate size of one microscopic field (400× magnification) were selected from four different sections for each pig and these four values were used to calculate the mean for that pig.

Statistical Analysis

The average weekly weight gain, final weight, total weight gain, total feed consumed, and feed conversion ratio (FCR) were compared by one factor analysis of variance programme of SAS (2010). The significant difference between the treatments means were determined using Duncan's Multiple Range Test of same package.

RESULTS

The proximate composition of the experimental diets (Table 2), performance characteristics (Table 4), microbial load (Table 5) and gut morphology (Table 6) of weaned pigs fed turmeric, garlic and ginger extracts are presented below. No significance differences were observed for the initial weights, final weights and FCR of the experimental animals.

Table 3: Proximate composition of garlic, ginger and turmeric extracts used as feed additives to the experimental feed for the weaned pigs

Extracts	%Crude Protein	%Ether Extract	%Ash	%Crude Fibre	%Dry Matter	%Moisture Content
Garlic extract	25.20	2.00	6.00	2.00	90.94	9.06
Ginger extract	8.05	3.00	7.00	3.00	93.34	6.66
Turmeric extract	14.70	3.00	8.00	3.00	91.96	8.04

Table 4: Performance characteristics of pigs fed dietary with extracts of turmeric, ginger and garlic

Parameters	T1 (control)	T2 (Turmeric)	T3 (Ginger)	T4 (Garlic)	SEM
Initial weight (kg/pig)	10.67	10.00	8.33	8.67	1.33
Final weight (kg/pig)	22.67	20.33	15.67	18.33	2.25
Weight gain (kg/pig)	12.00	10.33	7.33	9.67	1.33
Feed intake (kg/pig)	41.16^{a}	34.63^{ab}	24.83^{b}	36.59^{ab}	2.36
FCR	3.92	3.59	3.86	3.85	0.42

^{abc}: Means along the row with the same superscript are not significantly different (P>0.05);
T1:Control diet; T2: Turmeric extract (2g extract /kg feed); T3: Ginger extract (2g extract /kg feed); T4: Garlic extract (2g extract /kg feed)

The observed values for the feed intake on the experimental pigs ranged from 24.83kg/pig to 41.16kg/pig. The highest value was obtained from the treatment 1 (41.16kg) and the lowest value from treatment 3 (24.83kg).

The result from Table 5 revealed no significant differences in the total bacteria count. Pigs in treatment 4 had a mean of 5.12 for *Enterobacter* count which was significantly (P<0.05) lower compared to their counterparts on other treatments with values ranging from 6.27 (Treatment 3) to 6.38 (Treatment 1). Lactobacillus and *E. coli* counts did not show any significant differences across the treatments. The result as shown in Table (6) indicated a significant (P<0.05) difference in the values recorded for villus height in the ileum of pigs fed the experimental diets. Pigs fed Treatment 4 (garlic extracts) had significantly higher values (1419.75µm) when compared to others with values of 709.25 µm, 1156.50 µm and 1068.00 µm for Treatment 1, Treatment 2 and Treatment 3 respectively. Values observed on the villus width of the pigs fed experimental diets did not show any significant (P<0.05) difference across the treatments. The trend observed for the crypt depth of the ileum did not show any significant difference in pigs fed the experimental diets. Values observed for the jejunum showed significant (P<0.05) differences in both villus height and crypt depth. Villus height in pigs fed Treatment 3 (823.25 µm) and 4(804.75 µm) were similar but significantly different from the control (533.50) which had the least value, however, significantly (P<0.05) higher value was observed in treatment 2 (1087.25 µm). Result recorded for the crypt depth in the jejunum also showed significant difference with the highest mean value in pigs fed Treatment 3 (102.75µm) and least mean value in Treatment 1 (75.00 µm) which was the control diet.

DISCUSSION

The observed values for the experimental pigs in this research work suggest that the extracts of turmeric, ginger and garlic can only be used to improve the animal physiological system but not as growth promoter for animals. The result of the present study is in agreement with the report of Ilsley *et al*. (2005) who concluded that dietary curcumin had no influence on performance, FCR or immune status and serum immunoglobulin (IgA) of post weaning pigs. Less research work used ginger, garlic and turmeric for pigs, but most of the studies showed that the garlic or turmeric did not increase live weight gain and feed intake, for example Horton *et al.* (1991) fed male broiler chickens with diets containing dried garlic and noted that inclusion of garlic in the diet did not improve performance of broiler chickens. According to Bamidele and Adejumo (2012), garlic and ginger mixtures had no significant effect on growth performance of the experimental pullets used. It was concluded that garlic and ginger mixtures do not enhance growth performance and as such cannot be used as growth promoters for pullet birds.

Observed values for feed intake in the control diets, Treatments 1 and 4 were higher in ratio than in Treatment 2, while the lowest value was observed in Treatment 3. This however reveal a better feed utilization for pigs in fed Treatment 2 (Table 4).

Table 5: Bacteria count of pigs fed dietary with extracts of turmeric, ginger and garlic (cfu)

Parameters	T1 (control)	T2 (Turmeric)	T3 (Ginger)	T4 (Garlic)	SEM
Lactobacillus	5.78	6.31	6.32	5.75	0.14
Escherichia coli	6.76	6.28	6.40	5.77	0.22
Enterobacter	6.38[a]	6.29[a]	6.27[a]	5.12[b]	0.04
Total Count	6.78	7.65	7.77	6.63	0.34

[abc]: Means along the row with the same superscript are not significantly different (P>0.05); T1:Control diet; T2: Turmeric extract (2g extract /kg feed); T3: Ginger extract (2g extract /kg feed); T4: Garlic extract (2g extract /kg feed)

Table 6: Histological Parameters of Pigs Fed Dietary with Extracts of Turmeric, Ginger and Garlic

Parameters	T1 (control)	T2 (Turmeric)	T3 (Ginger)	T4 (Garlic)	SEM
ILEUM (µm)					
Villus height	709.25[c]	1156.50[b]	1068.00[b]	1419.75[a]	24.71
Villus Width	110.00	124.10	128.50	125.75	3.18
Crypt Depth	99.50	105.75	107.25	93.00	3.28
JEJUNUM (µm)					
Villus height	533.50[c]	1087.25[a]	823.25[b]	804.75[b]	23.19
Villus Width	102.65	126.00	109.50	112.75	4.47
Crypt Depth	75.00[b]	99.50[a]	102.75[a]	84.50[b]	2.54

[abc]: Means along the row with the same superscript are not significantly different (P>0.05); T1:
T1:Control diet; T2: Turmeric extract (2g extract /kg feed); T3: Ginger extract (2g extract /kg feed); T4: Garlic extract (2g extract /kg feed)

This observation is similar to the findings of previous researchers (Elagib *et al*., 2013). Fadlalla *et al*. (2010) and Raeesi *et al*. (2010) reported better FCR when supplementing garlic to broiler diets, although Onibi *et al*., (2009) found no significant effect when garlic was incorporated into the diet of broiler birds while Jamroz *et al*. (2005) reported improved FCR due to the addition of a plant extract, containing cinnamaldehyde, carvacrol and capsaicin. A similar result was found by Wang *et al*. (2010) on weaned pigs. However, Corrigan *et al*. (2001) reported that the supplementation of garlic in the diet increased feed intake of nursery pigs. The result showed that *E. coli* number observed increased from Treatment 1(6.76 cfu) through treatment 4 (5.77 cfu) irrespective of the extracts used. The result showed that no significant differences were observed across the treatments. Treatment 1 encouraged higher proliferations of this bacterium compared to other treatments but lower value was observed in the Treatment 4. This is in supported with the result of Rees *et al*. (1993) who reported that *Esherichia coli* strains and other intestinal bacteria are more easily inhibited by garlic than the normal intestinal flora. The lactobacillus contents were high in Treatment 2 and 3 compared to Treatment 4 (garlic extract). This high number of lactobacilli may depress the growth performance of broiler chickens leading to competition in nutrient uptake or impaired fat absorption due to bile acid deconjugation (Miles *et al*., 2006). Lactobacillus *spp.* is very small in treatment 4, Reuter *et al*. (1996) earlier reported that garlic inhibits Lactobacillus, also lactobacillus plantarum was found to be stimulated most frequently by spices, and the effect appears to be associated with the natural species rather than with their oleoresins.

The lower value of *Enterobacter* observed in treatment 4 can be related to earlier work in *in-vitro* studies that have shown that garlic extract have strong antibacterial properties against *Escherichia coli*, *Salmonella typhimurium* (Johnson and Vaughn, 1969), *Enterobacter aerogenes* (Arora and Kaur, 1999). Numerous studies reported that garlic can be used effectively to inhibit the growth of enteropathogenic bacteria, including 20 different serogroups of *Escherichia coli*, 8 serotypes of *Salmonella*, and *Aeromonas hydrophila* (Johnson and Vaughn, 1969). Most of the digestive and absorption processes of ingested feeds take place in the intestine. It has been reported that intestinal villus and epithelial cell morphology is associated with intestine function and growth rate (Ruttanavut *et al*., 2009). The increase in the villus height of the pigs fed garlic extract diet in treatment 4 showed that garlic has the ability of increasing the villus height and thus the intestinal surface area for absorption, this is in agreement with the observed

effect of garlic meal in present thinner intestinal epitheliums which enhance the absorption and reduce the metabolic demands of the gastrointestinal system (Visek, 1978); because Caspary (1992) reported that increase in the villus height suggests an increased surface area capable of greater absorption of available nutrients also large sized villi have been associated with activated cell proliferation (Lauronen *et al*., 1998). Krinke and Jamroz (1996) reported a reduced cell proliferation and a thinner epithelial in chicks fed on diet containing antibiotic.

The crypt depth in this experimental study showed corresponding increase as the villus increases because long villi indicate a faster multiplication of the base of the crypt which migrated faster to the tip of villi (Nordstorm and Dahlqvist, 1973). A shortening of the villi decreases the surface area for nutrient absorption. The crypt can be regarded as the villus factory, and a large crypt indicates a fast tissue turnover and a high demand for new tissue (Yason *et al*., 1987). A decrease in either villus height or crypt may lead to a reduction in nutrient absorption. Though the increase in villus height and crypt depth on pigs fed garlic extract diet in treatment 4 showed no corresponding increase in the growth rate and this showed that though there was increase in villus height, its functions were not well activated because greater villus height and numerous epithelia cells are indicators that the function of the intestinal villi is activated (Langhout *et al*., 1999; Yasar and Forbes, 1999) and this is because the muscularis which contain the epithelia cells when measured in this study became shorter as the villus height increases. This agrees with the findings of Nordstrom and Dahlqvist (1973) who reported that long villi indicated a faster multiplication of the base of the crypt which migrated faster to tip of villi and the turnover of the epithelial cells would therefore be shorter. Numerous epithelial cells will be observed in pigs with increased body weight (Yamauchi *et al*., 2006). The function of the epithelial layers is fundamental to the digestion and absorption of nutrients from the intestinal lumen. The epithelium is covered by a layer of mucous which acts as a layer of protection, lubrication and transport between luminal contents and epithelial cells (Uni *et al*., 1998). Changes in the properties of this barrier could affect the absorption of both dietary and endogenous macromolecules and ions such as in nutrition, the same trend was observed in the ileum and jejunum in this study.

CONCLUSION

It is therefore concluded that garlic extracts improved intestinal morphology, reduced bacterial load especially the *E-coli* and total microbial count compared to other extracts although the feed conversion ratio was no significantly affected. Changes in intestinal morphology, can lead to better nutrient absorption, decreased secretion in the gut and better overall performance.

CONFLICT OF INTEREST

Authors declare that no conflict of interest exist concerning this manuscript.

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