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ORIGINAL RESEARCH ARTICLE

Effect of combination of turmeric, ginger and garlic extracts on performance, microbial load and gut morphology of weaned pigs

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ABSTRACT

This study was conducted to evaluate the effect of combinations of turmeric, ginger and garlic extracts on performance, microbial load and gut histomorphological parameters of weaned pigs. Fifteen mixed sex weaned pigs were randomly allotted into five treatments consisting a control (T0) with no extracts; T1 with combinations of extracts turmeric/ginger, T2 with combinations of extracts turmeric/garlic, T3 with combination of extracts ginger/garlic, T4 with combinations of extracts turmeric/ginger/garlic. A total number of 15 weaned pigs were randomly allotted into 5 treatments with 3 replicates each (1 animal per replicate) and arranged in a completely randomized design. Feed intake (FI), body weight gain (BWG) were measured weekly throughout the experiment while feed conversion ratio (FCR) was calculated, data was collected on microbial load and gut histomorphological parameters of ileum and jejunum of the experimental animals. No significant variation was observed for final weight with values ranging from 18.00kg to 22.67kg. The same trend was observed for feed intake and feed conversion ratio for all treatments with values ranging from 34.67kg to 41.81kg and 3.89 to 4.46 respectively. Total microbial count was however significantly improved in pigs fed T3 and T4 with values 7.70 and 7.65 cfu/kg respectively. No significant (P>0.05) variation was observed for Lactobacillus and E. coli. across the treatments with values ranging from 5.74 to 6.33cfu and 5.43 to 5.77 cfu/kg respectively. Enterobacteria were not found in pigs fed T4, however, no significant difference was observed in the pigs fed T0, T1, T2, and T3 diets. Furthermore, the addition of extracts improved significantly (P<0.05) the villus height, villus width and crypt depth in ileum in all treatments with values ranging from 709.25 to 1284.25 µm, 110 to 128.50 µm and 87.65 to 112.00 µm respectively. The same trend was observed for villus height, villus width, and crypt depth in jejunum in all treatment with values ranging from 533.50 to 1453.75 µm, 94.75 to 131.00 µm and 75.00 to 116.00 µm respectively. From this study, it can be concluded that combinations of turmeric and ginger can serve as a better growth promoter since the dietary supplementations resulted in an increase in the villus height, villus width and crypt depth of intestinal mucosa of pigs thus increasing the surface area for nutrient absorptions which will aids the digestion flow and absorption processes of digested food in the Gut Intestinal tracts of the animals.

Keyword: Turmeric, Ginger, Garlic, Performance, Microbial load, Gut histomorphology Weaned Pigs

INTRODUCTION

The livestock industry has become an important economic activity in many countries. Attempts to prevent and control diseases have led during recent decades to a substantial increase in the use of veterinary medicines. Antibiotics have been extensively used as animal production to prevent pathogens and diseases so as to improve growth. However, its excessive use as a growth promoter has resulted in resistance of bacteria to drugs as well as residue in meat which is deleterious to human who are the consumers (Sorum and Sunde, 2001). It is in view of this that attention is being focused on setting new regulations for more natural product that are friendly to animals, the consumer and the environment (Wenk, 2000) meanwhile during the past decade many studies investigated the use of new and promising alternative such as probiotics, prebiotics, enzymes, and phytobiotics (plant extract) in animal feeding (Sarica *et al*., 2005). Some phytogenic feed additives have been successfully incorporated into the diets of poultry birds without any deleterious effect or toxic residues (Oyekunle and Owonikoko, 2002). There are several naturally existing medicinal plants which could be used in preventing pathogens and disease which in turn improve growth. Prominent among these medicinal herb plants are turmeric (*Curcuma longa*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*) (Oyekunle *et al*. 2002).

Turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinale*) are rhizomatous herbaceous perennial herb, are members of the *Zingiberaceae* family. The rhizomes from *C. longa* and ginger (*Zingiber officinale*) are considered to have natural medicinal properties, including anti-inflammatory (Ammon *et al*., 1993), antioxidant , anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, antifungal, antiprotozoal (Antony *et al*., 1999), antiviral, antifibrotic, antivenom, antiulcer, hypotensive, and hypocholesterolemic (Al-Yahya *et al*., 1989) , antineoplastic, analgesic, anti-cancer, anti-emetic, anti-hypertensive (vasodilatator), antithrombotic, hypoglycaemic, hypolipidemic, and antipyretic properties because they contains a number of moniterpenoids, sesquiterpenoids, curcuminoids, sesquiterpene-Zingiberene, and pungent phenol compounds such as gingerols and shogaols (Soni *et al*., 1997). Different researchers have worked on the effect of ginger on growth performance in broilers, and controversial results were reported. Taylor (2001) showed that the use of ginger powders significantly increased body weight and improved feed conversion compared to birds fed with control diet. In contrast, Zhang *et al.*, (2009) examined the effect of processed ginger on growth performance and showed that the ginger additive had no significant effect on the feed efficiency, while body weight and daily weight gain of birds fed with ginger supplements were higher than control group.

Garlic (*Allium sativum*) is the most important specie of the onion genus, *Allium* belonging to the family *Alliaceae* (Eric, 2010). Intact garlic bulbs contain alliin (S-allyl cysteine sulfoxide), the precursor of allicin, which is hydrolyzed by enzyme allinase upon crushing to its active form, the allicin (S-allyl-2propenthiosulphinate) as the agent responsible for garlic's potent antibacterial properties. Garlic has been shown to increase feed palatability and thus feed intake (Horton, Blethen and Prasad, 1991). Allicin is the most potentially active component of garlic that is responsible for its characteristic odour, flavour as well as most of its biological properties (Chowdhury *et al*., 2002). Tollba and Hassan (2003) found that garlic as a natural feed additive, improved broilers growth, feed conversion ratio (FCR) and decreased mortality rate. The use of whole plant (bulb) and plant extract singly have been documented with positive effect. However, the effect of combining the plant extracts has not been well investigated to show possible synergy or antagonism. The aim of this study was to determine the effect of the combinations of turmeric, ginger and garlic extracts on growth performance and gut morphology of weaned pigs.

MATERIALS AND METHODS

The experiment was carried out at the Piggery Unit of Teaching and Research, Farm, University Of Ibadan, Ibadan, Oyo State, country?

Processing of test samples

Turmeric, ginger and garlic samples were purchased from Bodija market in Ibadan, Oyo State. The samples were sliced to increase surface area, air dried and milled to powder. Extraction was carried out using ethanol (Mohan, 2004). A liter of 80 % ethanol solution was mixed with 200 g of powdered turmeric, ginger and garlic respectively. The mixtures were kept for 5days in tightly sealed vessels at room temperature of 22˚ C and away from sunlight, and stirred several times daily with sterile glass rod. The mixture was filtered through muslin cloth and the residue, if necessary, adjusted to the required concentration (500 ml of 80% ethanol for the residue of 200 g of the turmeric, ginger and garlic powdered 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-evaporatoration (Brinkmann rotavapor, 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 h. The semisolid extract produced was kept in the deep freezer at -80˚ C overnight and then subjected to freeze drying for 24 hrs at -60˚ C at 200 millitorr vacuum. Extract from this method was then weighed and stored at 22˚C in desiccators until further use.

Animal management

Fifteen weaned pigs were used for the experiment, they were randomly allotted into 5 treatments with 3 replicates per treatment in a completely randomized design

Experimental diet

T-1 control (no extract)

T-2 turmeric and ginger extracts (2 g/kg of feed, 1g each)

T-3 turmeric and garlic extracts (2 g/kg of feed, 1g each)

T-4 ginger and garlic extracts (2 g/kg of feed, 1g each)

T-5 turmeric, ginger and garlic extracts (2.01 g/kg of feed, 0.67g each)

Data collection

Data was collected on the following parameters

Growth performance

Feed intake was obtained by subtracting the leftover feed from the quantity served. Feed conversion ratio was obtained by dividing the average daily feed intake by the average bodyweight gain. Weight gain of the pigs was determined by subtracting the initial live weight from the final live weight.

Microbial load

Total microbial count, *E. coli*, enterobacters and *lactobacillus* were cultured from fresh pig intestinal contents after 49 days of feeding. Serial dilutions were plated on nutrient agar, MacConkey and eosine methylene agar plates respectively with lactose and incubated 24hrs at 39 °C. Colony forming units (CFU) were enumerated for each animal.

Table 1: Gross composition of experimental basal diet g/100g

Ingredient	Percentage
Maize	38.00
Soybean meal	8.00
Wheat offal	20.00
GNC	15.00
PKC	10.00
Palm oil	5.00
Lysine	0.25
Dicalciphosphate	1.50
Salt	1.50
Premix	0.50
Methionine	0.25
Total	100.
Calculated Nutrient	
Metabolizable Energy(kcal/kg)	2662.46
Crude Protein%	19.11
Chemical Composition	
Crude protein	19.95
Ether extract	6.00
Ash	8.00
Crude Fibre	5.00
Dry Matter	91.12
Moisture	8.88

Gut histomorphological parameters

The tissue samples for histology were taken from the ileum and jejunum. The samples were fixed in 10% buffered formalin and stored in a bottle with tightly sealed lid until further processing. The processing consisted of serial dehydration, clearing, and impregnation with wax. Tissue sections, 5 µm thick (3 cross-sections from each sample), were cut by a microtome and were fixed on slides. A routine staining procedure was carried out using hematoxylin and eosin. The slides were examined on an Olympus AX70 microscope (Olympus Corporation, Tokyo, Japan). Twenty well-oriented villi and crypts from ileum and jejunum were measured along their length (height and depth, respectively) and width. The villus height (VH) was measured from the crypt-villus junction to the brush border at the tip. Villus width (VD) was measured parallel to the adjoining villus. The crypt depth (CD) was measured from them base near the lamina propria to the crypt-villus junction. All measurements were made to the nearest micrometer. The total of the intact well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section for each sample. The criterion for villus selection was based on the presence of intact lamina propria. Villus height was measured from the tip of the villus to the villus-crypt junction, whereas crypt depth was defined as the depth of the invagination between adjacent villi.

Chemical composition

Chemical composition of extracts and diet were carried out using the methods as outlined by Association of Official Analytical Chemist (AOAC 1990).

Statistical analysis

The data collected were subjected to a one way analysis of variance in a completely randomized design (CRD). The difference between the treatments means were separated by Duncan's Multiple Range Test of SAS (2010).

RESULTS

Performance characteristics of pigs fed mixture of turmeric, ginger and garlic extracts diet

The result in Table 2 reveals no significant (P>0.05) difference in final weight across the treatment. The result also reveals no significant (P>0.05) difference in weight gain across the treatment with the observed value range from 12.00kg to 9.22kg. Likewise no significant (P>0.05) difference was observed in feed intake with pigs in T3 having the highest value (41.81) for feed intake and pigs fed combination of T4 having the lowest value (34.67) for feed intake. The result also reveal no significant (P>0.05) difference in feed conversion ratio with pigs fed combination of T3 extracts having the lowest value (3.80) for FCR and pigs fed combination of turmeric and ginger having the highest value.

Microbial load of pigs fed combination of turmeric, ginger and garlic extracts diet

The result as shown in Table 3 indicated a significant (P<0.05) variation for total microbial count, ranging from 5.90 to 7.70cfu with T3 having the highest value and T2 having the least value.

Table 2: Performance of pigs fed combination turmeric, ginger, garlic diet

Parameter(kg)	T0	T1	T2	T3	T4	SEM
Initial weight(kg)	10.67	9.67	7.67	8.33	8.00	1.24
Final weight(kg)	22.67	19.00	18.00	19.33	18.00	2.56
Weight gain(kg)	12.00	9.33	10.33	11.00	10.00	1.55
Feed intake(kg)	41.16	35.93	39.85	41.81	34.67	3.26
FCR	3.92	4.46	4.02	3.80	3.92	0.42

SEM = Standard error of mean, FCR = Feed conversion ratio, T0 – Control, T1 – Turmeric + Ginger, T2 – Turmeric + Garlic, T3 – Ginger+ Garlic, T4 –Turmeric +Ginger + Garlic.

Table 3: Microbial load of pigs fed combination turmeric, ginger, garlic diet

Parameter(CFU)	T0	T1	T2	T3	T4	SEM
Total count	6.78^{b}	6.23^{bc}	5.90^{c}	7.70^{a}	7.65^{a}	0.12
Lactobacilus	5.78	5.74	6.33	6.33	6.21	0.12
E. coli	5.77	5.53	5.58	5.64	5.43	0.25
Enterobacteria	6.38^{a}	6.20^{a}	6.23^{a}	6.29^{a}	0.00^{b}	0.03

^{abc} :Mean along the row with same superscript are not significantly different (P>0.05)

SEM = Standard error of mean, E.coli = *Escherichia coli* ,T0 – Control, T1 – Turmeric + Ginger, T2 – Turmeric + Garlic, T3 – Ginger+ Garlic, T4 –Turmeric + Ginger + Garlic

The result for *lactobacillus* and *E. coli*shown on Table 5 indicated no significant (P>0.05) variation observed from the experiment conducted. However, the result shows significant (P<0.05) difference for enterobacteria between T4 and the other treatments with values ranging from 0.00 to 6.38.

Gut histomorphological parameters of pigs fed combination of turmeric, ginger and garlic extracts diet

Histomorphological data obtained for both ileum and jejunum revealed significant (P<0.05) difference in all parameters observed in the experimental research. The result as shown in Table 4 indicated significant (P<0.05) difference for ileum in the villus height of pigs across the treatment with pigs in T1, T2, and T3 having 1284.25, 1144.75 and1032.25µm respectively and pigs that received the T0 and T4 had the least values (709.25 and 926.75µm). The result for villus width showed significant (P<0.05) difference between treatments with pigs fed combination of extracts were significantly higher than the pigs fed with control diets. The highest value was recorded in pigs in T3 (128.50 µm) and the least was the T0 (110.00 µm). The villus width showed significant (P<0.05) difference between pigs fed the control diet (110.00) and pigs in T3 (128.50). The crypt depth also showed significant (P<0.05) difference between the treatments with pigs in T3 and T4 (112.00 and 102.00 µm) was numerically higher than the control(99.50 µm) and the least value was recorded with pigs fed diet containing combination of turmeric and ginger (87.65 µm).

Data obtained for jejunum reveals significant (P<0.05) difference in all parameters. The result obtained for villus height reveals significant (P<0.05) difference across the treatment with pigs in T2 (1453.75) having the highest numerical value compared with the control (533.50) which has the least value. The significant increases are seen as treatment effect. The result for the villus width also shows significant (P<0.05) difference increase between the treatments, highest numerical value was recorded in pigs fed ginger and garlic extracts (131.00) while those fed combination of turmeric, ginger and garlic extracts have the least observed value of (94.75). Significant difference (P<0.05) of crypt depth was observed between T3 and the other treatments.

DISCUSSION

There is limited literature on studies using combination turmeric, ginger and garlic in pigs on microbial load as well as gut morphology but on hematology and serum. The effects of dietary treatment on feed intake, weight gains, feed conversion ratio (FCR), are presented in Table 2. The results of the present study were consistent with the reports of Moorthy *et al*.,(2009), who found that feed intake in the control treatment was numerically higher than treatments containing ginger powder, turmeric powder, mixture of ginger powder and turmeric powder. However, Ademola, *et al.*, (2009) and Doley *et al*.,(2009), observed no differences was observed in feed consumption level of broilers fed with powdered ginger and pepper extracts for a period of 6 weeks the research work was conducted. The conclusive result from Ademola *et al* (2009) and Doley *et al* (2009) research work also agrees with the work of Bamidele and Adejumo 2012, which indicated that garlic and ginger mixtures had no significant effect on growth performance. Studies showed that the garlic or turmeric did not increase live weigh gain, feed intake of laying hens or egg weight (Maercks, 1979). Hence could not be used as growth promoters for growing pullets at the levels of inclusion; however Ademola *et al.* (2009) reported from his previous research that mixtures of garlic and ginger significantly improved the growth of the chicks than garlic and ginger as

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Table 4: Gut histomorphological parameters of pigs fed combination of turmeric, ginger and garlic extracts diet

Parameters	T0	T1	T2	T3	T4	SEM
Ileum(µm)						
Villus height	709.25^{e}	1284.25^{a}	1144.75^{b}	1032.25^{c}	926.75^{d}	17.08
Villus width	110.00^{b}	122.05^{ab}	119.25^{ab}	128.50^{a}	121.50^{ab}	3.13
Crypt depth	99.50^{abc}	87.65^{c}	90.75^{c}	112.00^{a}	102.00^{ab}	2.61
Jejumum(µm)						
Villus height	533.50^{e}	1305.25^{b}	1453.75^{a}	878.75^{d}	1157.25^{c}	9.71
Villus width	102.65^{bc}	115.55^{ab}	125.75^{a}	131.00^{a}	94.75^{c}	3.75
Crypt depth	75.00^{b}	77.50^{b}	78.75^{b}	92.25^{b}	116.00^{a}	3.86

$^{abc:}$ Means along the row with the same superscript are not significantly different (P >0.05)

SEM: Standard error of mean, T0- Control, T1 – Turmeric + Ginger, T2 – Turmeric + Garlic, T3 – Ginger+ Garlic, T4 –Turmeric +Ginger + Garlic

sole agent in broiler diets. However, this can also be relevant to pigs for research discussion since pigs is a referred monogastric animal such as broiler, layers and rabbit.

It can then be inferred that the effect of garlic and ginger on the growth performance of poultry birds (broilers/layers) is dependent on its dose and preparation. Taylor (2001) showed that the use of ginger and garlic powders significantly increased body weight and improved feed conversion compared to birds fed with control diet. However, Dono, (2012) reported that when given in combination at the rate of 10 g/kg each, supplementation of Garlic Meal and Turmeric Meal improved the final weight, body weight gain, and gain to feed ratio relative to the control diet. From this study the performance which did not show significant variation could be attributed to the sex of pigs
The pigs on T0 (control) which had the best weight gain and had the highest number of male animal in the treatment. Kamel, 2001 and Galib *et al*., 2010 reported that plant extracts inhibit the growth of harmful bacteria including *E.coli* and increase the growth of beneficial bacterial in the intestinal tract due to antimicrobial activity. When the number of harmful bacteria in the intestinal is low, nutrients are absorbed by the animals. As a result, the animals gain higher weight with lower feed consumption, this will be used to discuss the result obtained for the microbial counts of both beneficial and harmful bacteria namely Lactobacillus, *E. coli*, enterobacter and the total count of bacteria found in the microbial loads and gut morphology of weaned pigs used for the research work.

Bacterial counts in ileal digesta of are shown in Table 3. The population of *lactobacilli* in the ileum was numerically higher in pigs fed diets supplemented with plant extracts than in those fed the control diet. The *lactobacilli* counts in the ileum were increased in pigs fed combination of T2, T3, and also in T4 extracts diet compared T0 and pigs fed T1 which is an indication of increase in the beneficial microbe. However, findings of this study have indicated that when given in combination with turmeric meal, combined action of the active substances in turmeric meal and garlic meal likely exerted greater antimicrobial properties to create a better milieu in the digestive tract. As reported in some studies, phytochemicals in garlic have strong antibacterial properties to combat *E. coli*, *Salmonella*, (Johnson and Vaughn, 1969), *Clostridium botulinum* (De Wit *et al.*, 1979), and other pathogenic species. Kim *et al*. (2011) proved that lower numbers of certain gut pathogens such as *E. coli* may improve broiler performance. Furthermore, the morphology of the gut wall is altered by bacterial activity in the gastrointestinal tract (Rebolé *et al*., 2010). Indeed, an overgrowth of some microorganisms in the intestine has been reported to result in mucosal impairment and villus erosion, thus reducing its absorptive potential of nutrients (Pelicano *et al*., 2005). According to Bourlioux *et al*. (2003), enterobacteria can cause damage to the intestinal cells, and Fonseca *et al*. (2010) linked a decrease in the quantity of cecal enterobacteria with increased ileal villus height. However, in this study, the steady range of *E. coli*, and Enterobacteria could be as a result in a drop pH which is below that which these microbes grows and therefore inhibited the growth of these harmful bacteria thereby increasing the growth of beneficial bacteria as seen in the case of *lactobacillus* in this study.

Intestinal histomorphological parameters are markedly affected by dietary feed supplement components and changes in intestinal morphology correlates with the intestinal function in pigs and chickens. It is reported that an increase in the villus to crypt ratio corresponds to an increase in digestion and absorption, and the maximum absorption occurs at an increasing distance along the crypt villus axis in weaned pigs (Shakouri *et al.*, 2001; Xia *et al.*, 2004). 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. The histomorphological changes in the intestine of pigs reported in this study as shown in table 4 provide new information regarding the potential for using plant extracts in combination. Increase in the villus height suggests an increased surface area capable of greater absorption of available nutrients which is due to the synergistic effect of the combination of this extracts. A shortening of the villi and deeper crypts may lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, and lower performance (Xu *et al*., 2003). In contrast, increases in the villus height and villus height: crypt depth ratio is directly correlated with increased epithelial cell turnover (Fan *et al*., 1997), and longer villi are associated with activated cell mitosis (Samanya and Yamauchi, 2002). It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes, and nutrient transport systems (Pluske *et al*., 1996). It is understood that greater villus height is an indicator that the function of intestinal villi is activated (Langhout *et al*., 1999; Shamoto and Yamauchi, 2000).

CONCLUSION

Although, the control and the extracts treatments show no difference in the performance and microbial load of the experimental animal. It has been hypothesized that gut microflora decrease nutrient absorption by increasing GIT thickness, the rate of digesta passage, and also increase nutrient requirements of the host by increasing turnover of the gut mucosae and by competing with the host for a portion of the dietary energy. This experimental study can be concluded that the dietary supplementations used resulted in an increase in the villus height, villus width and crypt depth of intestinal mucosa of pigs. Therefore, these plant extract of turmeric, ginger and garlic might be promising alternatives for antibiotic growth promoters as pressure to eliminate antibiotic growth promoters in animal feed increases.

RECOMMENDATION

It is recommended that the use of combination of turmeric and ginger extracts may be more effective and reduce pathogenic microbes if the inclusion rate is increased as well as extends the period of the experiment.

CONFLICT OF INTEREST

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

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