Effects of *Cymbopogon citratus* leaf and *Zingiber officinale* rhizome supplementation on growth performance, ileal morphology and lactic acid concentration in broilers

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Abstract

The aim of this experiment was to investigate the effect of feeding *Cymbopogon citratus* leaf and *Zingiber officinale* rhizome on growth performance, digestibility, ileum morphology and ileal lactic acid concentration in broilers. A total of 108- day-old male Cobb broiler chicks were randomly assigned to three treatment groups with six replicates in each treatment. Dietary treatments were no supplementation (control) and supplementations with 2% dried *Zingiber officinale* rhizomes and 2% dried *Cymbopogon citratus* leaves, respectively. Growth performance, feed consumption, ileal lactic acid concentration and villus height and crypt depth of the ileum were measured at the end of study period of six weeks. The results obtained showed that the final body weight and body weight gain of the birds fed *Zingiber officinale* were similar to the corresponding figures of the birds fed the control diet. Overall, broilers fed with *Cymbopogon citratus*, showed poorer performance as compared to birds in the other two treatments. Digestibility and villus height: crypt depth ratio were not significantly different between treatments. It can be concluded that supplementing broiler feed with either *Zingiber officinale* or *Cymbopogon citratus* did not improve bird performance despite the higher lactic acid content observed with the bird fed the *Zingiber officinale* supplemented feed.

Key words: *Cymbopogon citratus*, *Zingiber officinale,* broilers, growth performance, villi, ileal lactic acid

Introduction

Medicinal plants have been traditionally sourced for the treatment of various diseases, in both humans and animals. A number of dietary herbs, plant extracts and essential oils have been studied for their antimicrobial and growth promoting abilities in poultry (Cross *et al*., 2007). When incorporated into broiler diets, some herbal supplements have improved growth performance, feed conversion efficiency and carcass and meat quality in broilers, with reduced feed cost (Huang *et al*., 1992). Lemon grass or *Cymbopogon citratus* is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root (Barbosa *et al*., 2008; Oloyede *et al*., 2009). Besides being used for fragrance and flavoring, it is also widely used in traditional medicine, especially in Brazilian folk medicine, as it has no toxic properties (Lucia *et al*., 1986). Among the chemical compounds in lemon grass include neral, geranial, limonene, citronellal, myrcene and geraniol (Barbosa *et al*., 2008; Loumouamou *et al*., 2010). Phytochemicals such as flavonoids and phenolic compounds like luteolon and quercetin can also be found in this plant (Shah *et al*., 2011). In *in-vitro* studies, both ethanol and aqueous extracts of *Cymbopogon citratus* leaves have been shown to inhibit the growth of some gram-positive and gram-negative bacteria like *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus aureus* and *Escherichia coli* (Oloyede *et al*., 2009).

Ginger or *Zingiber officinale* on the other hand is a perennial herb with thick tuberous rhizomes (Malu *et al*., 2008), widely used as a spice. It is also a medicinal plant which is widely used all over the world. Among the chemical compounds in ginger include zingiberine, gingerol and shagoal. The pungent taste of ginger is caused by gingerol (Jolad *et al.,* 2004; Shariq *et al*., 2010) which contains an enzyme called zingibain that aids digestion (Adulyatham and Owusu-Apenten, 2005). Immuno-modulatory, antitumorigenic, antiinflammatory, antiapoptotic, anti-hyperglycemic, anti-lipidemic and antiemetic properties are among the other therapeutic effects of ginger observed (Badreldin *et al*., 2008). Ginger extracts have shown to exhibit antibacterial activity in *in-vitro* studies (Indu and Nirmala, 2010; Malu *et al*., 2008) in this study.

The microflora of the small intestine is made up mostly of lactic-acid producing bacteria (Engberg *et al*., 2000). Lactic acid is the fermentation byproduct of lactic-acid producing bacteria and the increase in lactic acid concentrations in the poultry gastrointestinal tract causes the pH to drop, thus preventing the colonization of certain pathogens (Zhang *et al*., 2003). Some studies have shown that plant extracts can increase the number of lactic acid bacteria in the ileal and ceacal contents of broilers (Rahimi *et al*., 2011).

In this study, oven dried *Cymbopogon citratus* leaf and *Zingiber officinale* rhizome were incorporated into broiler diets to evaluate the effects *Cymbopogon citratus* and *Zingiber officinale* on growth performance, digestibility, ileum morphology and ileal lactic acid concentration in broilers, as feed supplements to improve growth performance.

Materials and Methods

One hundred and eight male Cobb day-old broiler chicks were used in this study. They were fed with a corn-soy based diet and randomly assigned to three groups each containing six replicates with six chicks each. The birds were fed with a starter diet from days 1 to 21 followed by a grower diet from days 22 to 42. The compositions of the basal diets are shown in Table 1. On days 7 and 21, all the chicks were vaccinated against Newcastle disease and Infectious bronchitis disease, while on day 14 they were vaccinated against Infectious Bursal disease.

Table 1. Ingredients and chemical composition of the experimental diet

Ingredient	Starter, %	Grower, %
Maize	59.75	59.92
Soybean meal	29.15	26.00
Crude palm oil	3.00	3.00
Salt (NaCl)	0.30	0.30
Lysine	0.11	0.11
DL-Methionine	0.25	0.25
Limestone powder	1.00	1.28
Dicalcium phosphate	1.60	1.40
Fish meal	3.00	1.00
Wheat pollard	1.60	6.50
Vitamin premix[1]	0.04	0.04
Mineral[2]	0.10	0.10
Choline chloride	0.10	0.10

[1]Vitamin premix (per kg): vitamin A 50 IU; vitamin D 10 IU; vitamin E 75g; vitamin K3 20g; vitamin B1 10g; vitamin B2 30g; vitamin B6 20g; vitamin B12 0.1g; D-calcium pantothenate 60g; nicotinic acid 200g; folic acid 5g; biotin 235g

[2]Gladron Poultry Min (per kg): selenium 0.2g; iron 80g; manganese 100g; zinc 80g; copper 15g; potassium chloride 4g; magnesium oxide 0.6g; sodium bicarbonate 1.5g; iodine 1g; cobalt 0.25g.

Two treatment groups were supplemented with 2% dried *Zingiber officinale* rhizome and 2% *Cymbopogon citratus* leaf, respectively while the control group was not given any supplements. The herbs used in this study were dried in a hot air oven at 55-60˚C to a constant weight and then ground to the size of 1mm. All the birds were raised in cages for a period of 42 days with *ad libitum* access to water and feed. Body weight and feed consumption were recorded at the beginning and at the end of the study. Feed conversion ratio (FCR) was calculated as feed intake consumed per unit of body weight gain. Mortality was recorded as it occurred.

Feed digestibility was measured by total collection method from days 40 until 42. The total feed and faeces excreted by all the birds in each replicate for the two-day period were weighed and recorded. The total excreta collected were then dried in a hot air oven at 60˚C to a constant weight and the dry weight of the excreta was recorded. For dry matter analysis, samples of feed and faeces were taken and oven dried at 105˚C to a constant weight. Digestibility was measured based on the amount of feed intake minus the faeces excreted divided by the feed intake, based on dry matter basis.

Lactic acid concentration was measured at the end of the study. One bird from each replicate was randomly selected and killed by severing the jugular vein. From each bird 1g of intestinal content was taken from the ileal section of the gut, defined as the section from the Meckel's diverticulum to the illea-caecal junction. The inoculum was then transferred into a test tube containing 1 ml of 24% meta-phosphoric acid in 2.5 mol sulphuric acid. The mixture was left overnight and the supernatant analyzed for non-VFA (lactic acid) concentration using a gas chromatograph (GC) fitted with a flame ionization detector (FID), and nitrogen as carrier gas with a flow rate of 1.0 ml/min, after centrifugation. A fused-silica capillary column (30 m x 0.25 µm; inside diameter) was used to analyse the lactic acid and the running conditions were maintained at 180ºC with FID at 150ºC and the injector temperature was programmed at 110ºC. The internal standard was 20 mM fumaric acid, while 10 mM each of fumaric and succinic acids, and lithium lactate (Sigma) were used as standards to identify the peaks.

For the analysis of the ileum morphology, 2 cm of the midpoint of the ileum was taken from each bird. Segments were then fixed in 10% neutral buffered formalin solution for at least 48 hours and then embedded in paraffin wax. All histological studies were performed on 5 µm sections and stained by haematoxylin and eosin. Slides were then examined by a light microscope fitted with a digital video camera (Olympus XC50). Three intact and well-oriented villus and crypt units were selected from each sample. The criterion for villus selection was based on the presence of an intact lamina propria. The villus length was measured from the villus tip to the villus-crypt junction, while crypt depth was taken as the depth of the invagination between two villi. The measurements were done with a software called 'Analysis capture image software'. The mean villus height and crypt depth were then taken for each treatment. Data were analysed by SAS version 9.10 (SAS Institute Inc., 2002) and the statistical significance were considered at $p < 0.05$.

Results and Discussion

During the first three weeks of the study, there were no significant differences between dietary treatments in body weight gain or feed conversion, as summarised in Table 2. However, body weight gains during the last three weeks of the study and over the total study period was significantly different ($p < 0.05$) between treatments, with broilers fed the diet supplemented with *Cymbopogon citratus* leaf meal showing a significantly lower final body weight and body weight gain. These findings were contrary to those of Mmereole (2010) who observed that inclusion of *Cymbopogon citratus* leaf meal in poultry diet resulted in a significantly ($p < 0.05$) higher body weight and body weight gain when compared to the control diet. The final body weight, and total weight gain of the birds supplemented with *Zingiber officinale* on the other hand were similar to those fed the control diet. This was consistent with the findings of Zhang *et al.*, (2009) but contrary to that of Moorthy *et al.*, (2009) who observed that ginger increased the body weight when included in the poultry diet. Variations in the quality of herbs used could be attributed to the differences in the findings.

There were no significant differences in FCR between all treatments throughout the period of the study, similar to the findings of Mmereole (2010). Similarly, Zhang *et al*. (2009) did not observe differences in feed efficiency between broilers fed ginger and the control birds.

The ileal lactic acid concentration observed in this study was numerically higher in birds supplemented with *Zingiber officinale,* compared to those fed the control diet (Table 3). This observation can be related to the findings of Rahimi *et al*. (2011) who observed that there is an increase in lactic acid bacteria count in broilers fed with ginger, as lactic acid is the fermentation by product of lactic acid bacteria.

There were no statistical differences in villus height and villus height: crypt depth ratio between the dietary treatments (Table 4). An increase in the villus height and villus height: crypt depth ratio is hypothesized to improve digestion and absorption (Awad et *al.,* 2011; Montage *et al.,* 2003). The finding of this study is consistent with the observation of the lack of differences in digestibility between the treatments (Table 3). It appeared that supplementation of *Zingiber officinale* rhizome and *Cymbopogon citratus* did not alter the digestive capacity of the birds. However, it is noted that despite the similar results observed for digestibility and the lack of differences in ileum morphology between the treatments, the performance of birds supplemented with *Cymbopogon citratus* leaf meal was inferior to that of the control birds.

The variations in performance of broilers in the present study compared to those reported in earlier works may be due to possible differences in the nutrient and chemical composition of the herbs, broiler strain used and feed quality.

Conclusion

There was no improvement in the overall performance of birds fed either *Zingiber officinale rhizome* or Cymbopogon *citratus* leaf supplements when compared to the birds fed the control feed, although higher lactic acid content was observed with the supplementation of *Zingiber officinale.* The growth rate of the birds fed with *Cymbopogon citratus* leaf was lower than that of the control birds. There were also no differences in feed digestibility, villus height and villus height: crypt depth ratio between the treatments. These findings indicate the variability expected with the use of the two herbs as feed additives. It is therefore essential to conduct more extensive and in-depth research before the two herbs can be considered for incorporation into broiler diets.

Table 2. Growth performance of broilers fed diets with or without herbal supplements

Parameter	Dietary treatment			p value
	Z. officinale	*C. citratus*	Control	
Body weight (g/bird)				
Day-old	45.14±0.55	46.18±0.27	45.12±0.55	0.22
21-d	761.43±13.71	743.37±20.99	769.28±24.73	0.66
42-d	2228.38±29.64[a]	2072.20±49.77[b]	2201.40±23.33[a]	0.02
Weight gain (g/bird)				
1 to 21 d	716.30±13.60	697.18±21.13	724.16±24.66	0.64
22 to 42 d	1467.95±36.35[a]	1328.83±41.27[b]	1432.12±14.62[a]	0.02
1 to 42 d	2183.25±29.45[a]	2026.02±49.72[b]	2156.28±23.04[a]	0.02
Feed conversion ratio				
1 to 21 d	1.61±0.05	1.66±0.02	1.57±0.01	0.29
22 to 42 d	2.08±0.06	2.05±0.04	2.04±0.06	0.88
1 to 42 d	1.92±0.05	1.91±0.02	1.88±0.04	0.57
Mortality %	0.06	0.03	0.03	ND

[ab] Means with different superscripts within row differ significantly at $p<0.05$

Table 3. Apparent digestibility and ileal lactic acid concentration of broilers fed diets with or without herbal supplements

Parameter[1]	Dietary treatment			p value
	Z. officinale	*C. citratus*	Control	
Digestibility	0.72±0.01	0.68±0.03	0.70±0.01	0.23
Lactic acid	70.61±9.22[a]	19.85±2.81[b]	37.19±8.91[b]	0.01

[1]Digestibility (per unit Dry Matter Intake) and Lactic acid (μmole/g digesta)
[ab] Means with different superscripts within row differ significantly at $p<0.05$

Table 4: Villus morphology of broilers fed diets with or without herbal supplements

Parameter	Dietary treatment			p value
	Z. officinale	*C. citratus*	Control	
Villus height (μm)	577.79±46.20	690.61±94.13	690.43±28.97	0.40
Crypt depth (μm)	110.27±1.91[b]	116.53±12.00[ab]	140.19±6.02[a]	0.05
Villus:Crypt Ratio	5.24±0.38	6.00±0.64	4.94±0.15	0.20

[ab] Means with different superscripts within row differ significantly at $p<0.05$

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