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Duodenum Histomorphology and Performance as Influenced by Dietary Supplementation of Turmeric (*Curcuma longa*), Garlic (*Allium sativum*) and its Combinations as a Feed Additive in Broilers

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Abstract: This research was conducted to evaluate the effect of turmeric water extract, garlic and combination of turmeric and garlic as a feed additive in the broiler diet on performance and duodenum histomorphology. Affectivity of treatments was assessed by addition of phytobiotic (control, 015% zinc bacitracin, 2.5% TE, 2.0% GE, 2.5% TGE) which were arranged in a Completely Randomized Design with 4 replications. The variables measured were duodenum histomorphology (villi height, villi surface area, crypt depth, ratio villi height to crypt depth) and performances (body weight gain, feed intake, FCR). Results showed that diet with 2.5% of TE, 2.0% of GE and 2.5% of TGE significantly increased ($p<0.01$) the duodenum histomorphology as compared to positive control and negative control. The highest villi height was attained by feeding 2.5% TGE (1726.67 μm) and villi surface area (5054.17 μm^2) and the lowest was attained by feeding positive control 1005 μm and 1395.66 μm^2.No significant differences ($P > 0.05$), were observed in body weight gain and FCR beetwen different treatments, but the negative control feed intake increased significantly ($P < 0.05$), compared to the positive control. The research concluded that the incorporation of 2.5% TE, 2% GE and combined 2.5% TGE as feed additive enhanced duodenum histomorphology without no side effects on performances in broiler chicks.

Key words: Duodenum, performance, phytobiotic, turmeric, garlic

INTRODUCTION

Phytobiotic known for pharmacological effect and is widely used in traditional medicine. Leaves, roots, flowers and whole plants are used for production of phytobiotic products. Products may comprise the dried form of whole plants or their parts or extracts of some valuable ingredients (Grashorn, 2010).

Utilization of phytobiotic as Natural Growth Promoters (NGPs) has been identified as an effective alternative to antibiotics. Phytobiotic as NGPs highly developed as a feed additive, immunity, improves the performance and is highly effective in improving the health of the digestive tract (Panda *et al*., 2009) and stimulate livestock nutrition, antimicrobial and antihelmintik coccidiostatic (Panda *et al*., 2006).

Sarica *et al*. (2005) reported that garlic meal supplementation reduced the population of pathogenic coliform in the gut of broiler chickens. In addition, Gorinstein *et al*. (2005) and Kim *et al*. (2009) reported that garlic products have antioxidative properties in broiler chickens and layer hens. Other beneficial effects of garlic is lowering cholesterol and triglyceride levels, prevent atherosclerosis by the ability of anti-thrombotic, anti-platelet, anti-hypertensive, anti-lipidemia (Ali *et al*., 2000; Tattelman, 2005; Amagase, 2006; Rahman and Lowe, 2006; Corzo-Martinez *et al*., 2007; Rahman, 2007).

Commercial curcumin contains three main components, namely curcumin (77%), demethoxykurkumin (17%) and bisdes methoxy curcumin (3%), all of which referred to the curcuminoids (Aggarwal *et al*., 2003). Curcumin is the main active compound that furnishes turmeric with its characteristic yellow colour and is recognized as being responsible for most of its therapeutic effects, including antibacterial, antifungal, antiprotozoal, antiviral, antioxidant, antiinflammatory and hypocholesterolemic activities (Chattopadhyay *et al*., 2004). Feeding diets containing phytobiotics may result in inhibition of the growth and colonization of entero-pathogenic microbes in the digestive tract, thus contributing to the balance of gut microflora (Harris *et al*., 2001) and promoting the growth performance and health of birds (Adibmoradi *et al*., 2006), *in vitro* and *in vivo* studies have confirmed that phytobiotic in animal nutrition can stimulate feed intake, antimicrobial, coccidiostatic, antihelmintik and immunostimulant (Panda *et al*., 2006).

Because of the benefits of using turmeric and garlic extract in broilers when supplemented individually, there is the likelihood that a combination of both phytobiotics will confer additional benefits than the use of each individually. However, there is no information on the use of combination of garlic and turmeric extract on enteric health growth performance and nutrient utilization

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responses in broiler chickens. Therefore, the objective of the current study is to investigate the response of broiler chickens to diets supplemented with extract turmeric, garlic and its combinations on duodenal histomorphological and growth performance. The possible associative effects and additivity between garlic and turmeric water extract individually and combination in the diet have also been investigated.

MATERIALS AND METHODS

Experimental birds: One hundred commercial broiler chicks strain Cobb unsex were used in this study. They keep on the cages and randomly allotted into five treatments with four replicates of five chicks each.

Experimental diet: The experimental diet was formulated according to the standards prescribed in National Research Council (1994). Group I was offered a basal diet served as a negative control, group 2 was offered a basal diet with 0.015% *Zinc bacitracin* served as positive control, group 3, 4 and 5 were offered a basal diet with 0.25% turmeric extract (TE), 0.20% garlic extract (GE) and 0.25% turmeric and garlic extract (TGE), respectively during a six-weeks experimental period. Feed and water were provided as *ad libitum* to the birds through out the experimental period. The ingredients and nutrients composition of the experimental diet are represented in Table 1. Ration was formulated every week and turmeric and garlic extract was added to the formulated ration and was properly hand-mixed to ensure homogeneous distribution of the extract.

Collection of data: Feed intake and body weight gain were recorded at weekly to determined growth performance. Feed conversion ratio was calculated as the ratio between total feed consumption to final body weight. At 42 days of age, 20 birds (4 birds/treatment) were randomly selected and blend, then slaughtered following the normal procedure described by Merkley *et al*. (1980) to sample the duodenum. Duodenum samples were obtained for histomorphology measurement. Duodenum samples were washed with alcohol 70%, then they were stored in a chiller at 4°C and then samples were washed with buffer phosphate pH 7.4.

Sample preparation duodenum of the small intestine into preparations for SEM. Samples of the small intestine of broiler chickens are soaked in buffer phosphate was replaced by soaking in a solution of 1% osmic acid post-fixation fixed for 1-2 h at 4°C. Samples were washed with buffer phosphate 3 times 5 minutes each temperature 4°C. Then performed stratified dehydrated with 30, 50 and 70% alcohol, respectively for 15-20 min at 4°C. Dehydration followed by 80 and 90% alcohol and absolute alcohol each 15-20 min at room temperature, amyl acetate is replaced by absolute during the time dried with a dryer Critical Point Drying (CPD). Small bowel preparations placed on the stub (holder) using a special glue, then coated with gold or copper. Mixture of duodenum broilers observed and photographed by SEM. Parameters recorded villus height (from tip of villus to the crypt opening), villi surface area (μm^2/villi) is [(villi basal width+villi apical width)/apical width] x villi height (Iji *et al*., 2001), crypt depth (from the base of the crypt to the level of crypt opening) and villus height: crypt depth (calculated by dividing villus height with crypt depth).

Statistical analysis: Data for all parameters were subjected to an analysis of variance. The treatments means with significant differences at $p<0.05$ were compared using orthogonal contrast procedure (Gomez and Gomez, 1995).

RESULTS AND DISCUSSION

Duodenum Histomorphology: Duodenum histomorphology broilers indicated from the results of villi height, villi surface area, crypt depth and villi length to crypt depth were treated basal diet (negative control), 0.015% *Zinc basitracin* (positive control), 2.5% TE, 2.0% GE and 2.5% TGE presented in Table 2.

Structure of duodenum showed that all treatments were significantly different ($p<0.01$) from the villi height, villi surface area, crypt depth and villi height to crypt depth. The villi height and villi surface area of highest significantly different ($p<0.01$) on addition of 2.5% TGE were 1726.67 μm and 5504.17 μm^2, then the addition of 2.5% TE were 1340 μm and 3481.91 μm^2 and the lowest in the positive control 1005 μm and 1395.66 μm^2. Depth crypt highly significant ($p<0.01$) among all treatments and the addition of 2.5 GE lowest significantly ($p<0.01$). Addition of 2.5% TE, 2.0% GE and 2.5% TGE was significantly higher ($p<0.01$) on villi height to crypt depth when compared to the positive control and negative control. The villus: crypt depth ratio is an indicator of the likely digestive capacity of the small intestine. An increase in this ratio corresponds to an increase in digestion and absorption (Montagne *et al*., 2003).

Increased villi height be an indicator of increasing enzyme digestion are becoming more breadth surface area for absorption of nutrients (Miles *et al*., 2006). Increased intestinal villi height and the ratio villi height to crypt depth is an indication of the vast area for nutrient absorption and higher absorption function (Sieo *et al*., 2005).

Dietary supplementation of 2.5% TGE in this study stimulated the growth of absorptive cells in the small intestinal wall, as seen the increase in the length and width of the villus as well as the depth and width of the crypts of duodenum. The increase in the absorptive surface in current study was in line with the findings of Paul *et al*. (2007), Viola and Vieira (2007) and Senkoylu *et al*. (2007). In a recent study, Rajput *et al*. (2013) showed that dietary supplementation of 0.2 g/kg pure

Table 1: Ingredients and nutrient composition of broilers ration

	Percentage (%)				
Ingredients	Negative control	Positive control	2.5% TE	2.0% GE	2.5% TGE
Corn	63.00	63.00	63.00	63.00	63.00
Meat and bone meal	4.50	4.50	4.50	4.50	4.50
Poultry meat meal	6.00	6.00	6.00	6.00	6.00
Oil	2.00	2.00	2.00	2.00	2.00
Soybean	20.00	20.00	20.00	20.00	20.00
$CaCO_3$	1.00	1.00	1.00	1.00	1.00
DCP	0.30	0.30	0.30	0.30	0.30
Vitamins and minerals*	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
L-Lysin-HCl	0.10	0.10	0.10	0.10	0.10
DL-Methionin	0.10	0.10	0.10	0.10	0.10
Zinc Bacitracin	0	0.015	0	0	0
TE	0	0	2.50	0	0
GE	0	0	0	2.00	0
TGE	0	0	0	0	2.50
Filler	2.50	2.485	0	0.50	0
Total	100	100	100	100	100
Calculated composition					
Metabolizable energy, kcal/kg					3003.09
Crude protein (%)					20.03
Crude fibre (%)					3.00
Ether extract (%)					3.78
Calcium (%)					1.11
p-available (%)					0.56
L-Lysin-HCl (%)					1.06
DL-Methionin (%)					0.402

*Calculated value: one kg of vitamins and minerals contain:

270 g of calcium	189 g of phosphor	12 g of magnesium
300.00 IU of vitamin A	50.00 IU of vitamin D_3	Vitamin E of 100 mg
100 mg of vitamin K	20 g of trace mineral contains	(Zn, Mn, Fe, Mo, Cu, I, Co, Se)

Table 2: Villi height, villi surface area, crypt depth, villi height to crypt depth of broilers fed diet with turmeric extract and garlic and its combinations

	Treatments						
Variables	Negative control	Positive control	2.5% TE	2.0% GE	2.5% TGE	SEM	P-value
Villi height (μm)	1005^{a}	1320^{c}	1340^{d}	1210^{b}	1726.67^{e}	64.47	<0.00
Villi surface area (μm^2/villi)	1395.66^{a}	3355.78^{c}	3481.91^{d}	3115.5^{bd}	5054.17^{e}	340.86	<0.00
Crypt depth (μm)	370^{d}	466.6^{e}	273.33^{b}	210^{a}	320^{c}	24.24	<0.00
Villi height to crypt depth	2.78^{a}	2.83^{a}	4.94^{b}	5.79^{b}	5.41^{b}	0.36	<0.00

^{abcde}Different superscript at the same raw indicate significantly different ($p<0.01$)

curcumin derived from turmeric in a corn-soybean based diet increased the villus height and width of duodenum, jejunum and ileum of 42 days old Arbor Acre broiler chickens. The villus height to crypt depth ratio in the duodenum and ileum was also significantly increased in curcumin supplemented birds. Adibmoradi *et al.* (2006) reported addition 1 and 2% dietary garlic meal inclusion increased in the ratio of crypt depth to villi height in duodenum but decrease in jejunum and ileum. Krinke and Jamroz (1996) reported reduced duodenal cell proliferation and a thinner epithelial thickness in chicks fed antibiotic, which is in agreement with the observed effect of garlic meal in present research. Thinner intestinal epitheliums enhance nutrient absorption and reduce the metabolic demands of the gastrointestinal system (Visek, 1978). Thinning of the gastrointestinal walls tract may be due to the inhibition of the microbial production of polyamines and volatile fatty acids, known to increase enterocyte turnover rate and activity. This increased net energy committed to maintaining the luminal tissue comes at the expense of more productive purposes such as muscle accretion (Bedford, 2000). In the current study, it was clear that garlic administration maintained longer and more slender villi, especially in the duodenum section. Feeding diets containing TE and GE mixture likely to produce double inhibition of the growth of entero-pathogenic bacteria, thus contributing to maintain a healthy balance of microbial populations in the gut (Harris *et al*., 2001) and resulting in a greater growth performance improvements than obtained using only one additive (Lewis *et al*., 2003; Adibmoradi *et al*., 2006).

Performance: Observation of the performance include weight gain, feed intake and conversion ratio can be seen in Table 3. All treatments did not provide significant differences ($p>0.05$) on body weight gain and conversion ratio, except feed intake of positive control were significantly different ($p<0.05$) compare to negative control. Addition of 2.5% TE, 2.0% GE and 2.5% TE and

Table 3: Body weight gain (g/bird), feed intake (g/bird) and feed conversion ratio of broilers fed diet with turmeric extract and garlic and its combinations

	Treatments						
Variables	Negative control	Positive control	2.5% TE	2.0% GE	2.5% TGE	SEM	P-value
Body wight gain (g/bird)^{ns}	1312.69	1310.71	1339.96	1299.21	1314.26	7.88	0.89
Feed intake (g/bird)	2218.10^{b}	2044.52^{a}	2113.21^{ab}	2077.75^{ab}	2079.69^{ab}	23.15	0.16
Feed conversion ratio^{ns}	1.69	1.57	1.59	1.60	1.58	0.03	0.58

^{ab}Different superscript at the same raw indicate significantly different ($p<0.05$). ^{ns}Non significant

GE provide relatively equal feed intake consecutive 2113.21, 2077.75 and 2079.69 g/bird and still lower than positive control feed intake is 2218.10 g/bird. The results of this study indicate that the addition of 2.5% TE numerically increase the body weight gain. Some authors have not found any beneficial effects of adding Turmeric to the feed of poultry birds, that is similar with the results of this study. For example, use of the feed as much as 0.2% (El-Hakim *et al*., 2009), 0.25 to 0.75% (Emadi and Kermanshahi, 2006), 0.25 to 1% (Al-Sultan, 2003), 0.8% (Hosseini-Vashan *et al*., 2012), 1.5% (Purwanti *et al*., 2008), 10 g/kg (Dono, 2012), 0.25 and 0.5% (Tirupathi-Reddy *et al*., 2012), 1000 g/ton (Rahmatnejad *et al*., 2009), 0.4% of 2-6 weeks (Swathi *et al*., 2012), 0.1 and 0.2% except FCR in the first week (Mehala and Moorthy, 2008). Okada *et al*. (2001) showed that turmeric and curcumin have no negative effects on body weight and feed intake of broiler chickens. On the other hand, some authors reported beneficial effects of adding Turmeric as in the study of Al-Kassie *et al*. (2011) who used a mixture of cumin and turmeric at the level of 0.75 and 1% in the diet to increase weight gain and FCR, 0.5% (Durrani *et al*., 2006). Kumari *et al*. (2001) observed that the addition of *C. longa* as feed additive showed better results in the growth, feed intake and FCR in broilers. Ahmadi (2010) reported that FCR of broiler chickens was better when feed was supplemented with 0.9% Turmeric powder. Several studies showed that garlic supplementation in broiler feed had no significant effect on the performance with the addition of 1000 g/ton (Rahmatnejad *et al*., 2009), 0.5 and 1% (Abdullah *et al*., 2010), 10 g/kg (Dono, 2012), 4 g/kg (Toghyani *et al*., 2011), 5 g/kg (Onibi *et al*., 2009), 10 g/kg (Pourali *et al*., 2010), 3% (Elagib *et al*., 2013). Significant results were reported with the addition of 0.1 g/kg (Aji *et al*., 2011), 8.23 g/kg (Lewis *et al*., 2003), or 10 g/kg of GM (Mahmood *et al*., 2009). Different response is possible because of differences in basal rations used, breed, long maintenance (Dono, 2012), maintenance management, environmental conditions (Rahmatnejad *et al*., 2009) also used phytobiotic form.

Curcumin in the diet increased the activities of pancreatic lipase, amylase, trypsin and chymotrypsin. Dietary feeding of essential oils extracted from herbs can improve the secretion of digestive enzymes and so improve digestibility of the feeds and enhanced the performance of broilers (Al-Kassie, 2011; Radwan *et al*., 2008) this improvement could be attributed to its contents of essential oils that have active components which possess antibacterial, antioxidant and antifungal activities; and accordingly may improve the bird utilization of dietary nutrient. The variety of garlic preparation and administration methods limits the possibility of comparing results of the current study with the previous findings.

Conclusion: It is concluded that 2.5% TGE might be of beneficial effect on duodenum morphology as well as on performance parameters. It can be recommended that 2.5% TGE can be used as Natural Growth Promoters as an effective feed additives alternative to antibiotics.

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