Effect of Dietary Garlic Meal on Histological Structure of Small Intestine in Broiler Chickens

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This study was conducted to evaluate the effect of garlic (*Allium sativum*) meal on the intestinal mucosal morphology of broiler chickens between 21 and 42 days of age. A total of 1800, 21 days old Ross 308 broiler chicks were weighed and randomly allotted into 0, 0.125, 0.25, 0.5, 1 and 2% dietary garlic meal groups of each 100 birds. Experimental diets were formulated by addition of each level of garlic meal to commercial finisher mash diet (CP : 20.45%, ME : 3,130 kcal/kg), and fed *ad libitum* for 21 d. At day 42, 15 chicks from each treatment were randomly killed to obtain intestinal samples.

Villus height, epithelial thickness, goblet cell numbers, crypt depth and the ratio of crypt depth to villus height in each intestinal segment were compared using a light microscope. Garlic meal as a feed additive significantly enhanced villus height and crypt depth and decreased epithelial thickness and goblet cell numbers in duodenum, jejunum and ileum of birds. Fundamentally, jejunum and ileum revealed an almost similar morphological alteration to that in the duodenum except that dietary garlic meal supplement resulted in an increase in the ratio of crypt depth to villus height in duodenum but a decrease in jejunum and ileum. In present study, small intestinal morphological changes in chickens due to dietary garlic meal supplement demonstrate that absorptive process could be activated by garlic meal as an antibiotics alternative growth promoter.

Key words : broiler chickens, garlic meal, intestinal morphology

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Introduction

Antibiotics at subtherapeutic doses are widely used in animal production to decrease susceptibility to pathogens and disease and to improve meat and egg production. It has been hypothesized that gut microflora decrease nutrient absorption by increasing gastrointestinal tract (GIT) thickness, the rate of digesta passage, and also increased nutrient requirements of the host by increasing turnover of the gut mucous and by competing with the host for a portion of the dietary energy and protein (Ravindran *et al.*, 1984 ; Apajalahti *et al.*, 2004). Solomon and

Tullett (1988) noted taller, narrower, and regularly shaped villi in the ileum of antibiotic treated birds. They concluded that these villi provided greater surface area for absorption.

At the present time, there is increasing pressure to reduce or eliminate the use of antibiotics in poultry feed due to the negative human health issue of antibiotic resistance. On the other hand, the proposed ban of antimicrobial growth promoters in the European union in 2006 and the voluntary reduction in the use of them in other countries have forced producers to look for alternative growth promoters to be used in poultry feed. Non-conventional growth

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enhances are utilized in broiler nutrition to improve nutrient digestibility, control of pathogenic microorganisms, facilitate a favorable intestinal microbial balance, and enhancing absorption of calorigenic nutrients across the gut wall through increasing its absorption capacity (Al-Harthi, 2002 ; El-Deek *et al.*, 2003).

Garlic (*Allium sativum*) is widely distributed and used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases. Garlic is thought to have various pharmacologic properties. For example, it has been found to lower serum and liver cholesterol (Qureshi *et al.*, 1983), inhibit platelet aggregation (Apitz-Castro *et al.*, 1983), inhibit bacterial growth (Cavallito and Bailey 1944) and reduce oxidative stress (Horie *et al.*, 1992). Tollba and Hassan (2003) found that garlic as a natural feed additive, improved broilers growth, feed conversion ratio (FCR), and decreased mortality rate. Demir *et al* (2003) indicated that there was a reduction in crypt depth in the ileum of broilers given dietary natural growth promoters such as garlic.

The aim of this work was to evaluate the effect of garlic meal used as growth promoters on morphology

of duodenum, jejunum and ileum of broiler chickens during finishing period.

Materials and Methods

In this experiment one hundred one day old Ross 308 mixed sex broiler chicks were assigned randomly to each of 18 floor pens, creating replicates of 6 experimental treatments. Chickens had *ad libitum* access to feed during the entire experiment. Birds were managed in accordance with the guidelines of the Ross manual. Tylosin tartrate used prophylactically at 5 to 8 days of age (100 g per 200 liter drinking water) as the sole antibiotic treatment according to usual veterinary recommendation. Diets (Table 1) were formulated to meet all the nutrient requirements of the birds for starter (0 to 21 d) and finisher (22 to 42 d), phases (National Research Council, 1994). Experiment had a completely randomized design with six treatments and 3 replicates pens of 100 birds each (total of 1800 birds). Treatments were consisted of a common corn-soybean meal based finisher diet supplemented by 0, 0.125%, 0.25%, 0.5%, 1% or 2% garlic meal and experiment carried out at 21 to 42 days of age. The ingredients of garlic meal used in experiment were 70% dried garlic powder and 30% wheat bran as a carrier.

Table 1. Ingredients and chemical composition of experimental diets

Ingredients %	Starter	Finisher					
		Dietary garlic meal ¹ %					
		0	0.125	0.25	0.5	1	2
Corn	56.24	60	60	60	60	60	60
Soybean Meal	34.71	30	30	30	30	30	30
Fish Meal	4	3.5	3.5	3.5	3.5	3.5	3.5
Soybean Oil	2.13	3.25	3.25	3.25	3.25	3.25	3.25
Dicalcium phosphate	1.19	1.21	1.21	1.21	1.21	1.21	1.21
Oyster shell	1	1	1	1	1	1	1
Salt	0.2	0.23	0.23	0.23	0.23	0.23	0.23
Met	0.125	0.09	0.09	0.09	0.09	0.09	0.09
Minerals & vitamins premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition							
Apparent metabolizable energy (kcal/kg)	2948	3130	3130	3130	3130	3130	3130
Crude Protein	22.25	20.45	20.45	20.45	20.45	20.45	20.45
Calcium	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Phosphorous	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Metionine	0.4	0.39	0.39	0.39	0.39	0.39	0.39
Metionine + Cysteine	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Lysin	1.28	1.2	1.2	1.2	1.2	1.2	1.2
Ether extract	4.13	6.46	6.46	6.46	6.46	6.46	6.46
Crude Fiber	3.71	3.4	3.4	3.4	3.4	3.4	3.4

¹ different levels of garlic meal added to a balanced finisher diet.

At 42 days of age, 5 mixed sex chicks per replicate pen (3 male and 2 female) were randomly sampled for morphometric analysis, and then killed. The intestinal tract was removed immediately and severed from the gizzard and the pancreas was removed. Three 1-centimeter tissue segments were taken from the proximal, middle and distal parts of duodenum, jejunum and ileum sections. All samples from each of those birds were taken from the same area of each section of the tract. Samples stored in 10% buffered neutral formalin for fixation, where they were gently shaken to remove any adhering intestinal contents. Cross sections ($5\mu\text{m}$ thick) of each intestinal segment were processed in low-melt paraffin and stained with hematoxylin and eosin. This procedure causes a longitudinal section of villi. Using a Zeiss light microscope, 15 measurements per intestinal section were made for each parameter and averaged into one value per bird. As a matter of fact, each histological data obtained from the mean of 45 records (3 sections and 15 villi per section).

Data on villus height, epithelial thickness, goblet cell number, crypt depth and ratio of crypt depth to villus height, were analyzed with the general linear model procedure and differences among treatments means were classified by Duncan's multiple range test (Version 6.12, SAS Institute, Inc.)

Results

The most pronounced differences observed between 2% dietary garlic meal and the control group. Figure 1 illustrates the duodenum villus and crypt

condition of these two groups.

The effect of garlic meal on villus height, epithelial thickness, goblet cell number, crypt depth and the ratio of crypt depth to villus height of different sections of the small intestine of experimental birds are shown in Table 2. Villi in the duodenum, jejunum and ileum of birds fed the diets supplemented with increasing levels of garlic meal were found to be correspondingly longer ($P < 0.05$). Specially, the villus height tended to be higher ($P < 0.05$) in 1 and 2% dietary garlic meal groups than that of other groups. Feeding garlic meal significantly decreased epithelial thickness in different sections of the small intestine ($P < 0.05$) and this reduction was more obvious in 1 and 2% dietary levels of garlic meal. The goblet cell numbers in the villi of duodenum, jejunum and ileum of birds fed the diets supplemented with more than 0.5% garlic meal were lower ($P < 0.05$) than other treatments including control.

In all sections of the small intestine, garlic meal inclusion increased ($P < 0.05$) crypt depth in a dose related manner. The only difference found in the small intestine sections resulting from dietary garlic meal supplement was an increase ($P < 0.05$) in the ratio of crypt depth to villus height in duodenum but a decrease ($P < 0.05$) in jejunum and ileum.

Discussion

The GIT development and health is the key to productivity in all farm animals and poultry. The digestive functions could be considered the most limiting factors in performances. Overall gut sur-

Fig. 1. Sections of a duodenal tissue of broilers chickens. A : Control bird with no dietary garlic meal intake. B : Bird treated group with 2% dietary garlic meal. In control, the villus structures are short and thickened compared to the birds fed 2% garlic meal. CD=Crypt depth, VH=Villi height. Hematoxylin and eosin staining. Scale bars represent $500\mu\text{m}$.

Table 2. Effects of garlic meal on morphological parameters of small intestine¹

DGM% ²	VH ³	ET ³	GN ³	CD ³	C/V ³
Duodenum					
0	1741.1c	49.1a	10.8a	147.1d	0.084a
0.125	1754.9c	48.1ab	10.2ab	147.4d	0.084a
0.25	1761.2c	46.3b	9.4bc	148.6cd	0.084a
0.5	1805.9b	39.7c	9c	150.9c	0.083a
1	2028.6a	38.3c	5.3d	156.6b	0.077c
2	2033.8a	35.9d	5.3d	162.7a	0.08b
S.E.M	9.9	0.67	0.38	0.93	0.0006
Jejunum					
0	849.6c	39.2a	10.1a	109.2d	0.128d
0.125	846.8c	38.1ab	10.9a	112.8c	0.133c
0.25	849.9c	38.4ab	10.1a	119.6b	0.141b
0.5	843c	39.2a	9.9a	122.1b	0.145b
1	881.1b	36.5b	7.8b	134.2a	0.152a
2	908.9a	32.2c	6.4c	135.5a	0.149a
S.E.M	3.3	0.71	0.44	0.13	0.00015
Ileum					
0	783.2c	36.7a	11.1a	101.7c	0.130b
0.125	797.7c	36.5a	10.5a	104.2c	0.131b
0.25	801.9c	34.5a	10.1a	106.1c	0.132b
0.5	877.9b	26.7b	8.9b	118.1b	0.135b
1	920.5a	21c	7.6c	131.9a	0.143a
2	924.1a	21.3c	7.1c	132.2a	0.143a
S.E.M	9.2	1.15	0.4	2.07	0.003

a-d Values in the same column, with no common superscript differ significantly ($P < 0.05$). ¹Values are means of 45 records from 15 birds from each treatment and their standard errors. ²DGM=Dietary garlic meal percent. ³VH=Villus height (μm); ET=Epithelial thickness (μm); GN=Goblet cell number (per 100 μm villus height); CD=Crypt depth (μm); and C/V=Ratio of crypt depth to villus height.

face area affect net utilization of dietary nutrients in chicken and will be determined by gross morphological features such as length and cross-sectional area of the duodenal, jejunal and ileal segments, and by finer morphological features such as villus height and surface area of the epithelium in each of those segments (Jin *et al.*, 1998 ; Iji, 1999).

Several researchers have studied intestinal morphology in poultry during the last decade but predominantly from the standpoint of normal development and not with regard to effects of antibiotics and other growth promoters (Uni *et al.*, 1999 ; Geyra *et al.*, 2001). No comprehensive published data are available on the effects of garlic meal on the gut morphology of broiler chickens.

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 and protein (Ravindran *et al.*, 1984 ; Apajalahti *et al.*, 2004).

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 (Vissek, 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). The minimization of gastrointestinal bacteria may also ease the competition for vital nutrients between the bird and the microbes (Ferket, 1991).

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 fast tissue turnover and a high demand for new tissue (Yason *et al.*, 1987). A decrease in either villus height or crypt depth may lead to a reduction in nutrient absorption. The increased crypt depth observed in this study is contrary to the report of Demir *et al.* (2003) that used female chickens. The villus : crypt 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, 2003).

Parker and Armstrong (1987) showed that inclusion of an antibiotic in the diet of pigs can result in a elongated villi and a lower crypt : villus ratio, which is indicative of a lower rate of enterocyte-cell migration from the crypt to the villus. The latter conclusion corresponds with the findings of the present study in duodenum section as the inclusion of garlic meal in the diet increased villi height and decreased crypt : villus ratio. The crypt : villus ratio were, however reduced by garlic meal addition in the villi of the jejunum and ileum sections. This is important with respect to axial role of duodenum in absorption. It has suggested that reduced microbial activity in digesta or microbial activity at the level of the brush border would reduce both the damage to enterocytes and the need for cell renewal in the gut (Hughes, 2003).

Goblet cells are responsible for the secretion of mucin that is used for the mucinous lining of the intestinal epithelium (Schneeman, 1982). Thus, a higher density of goblet cells may result in an increase in the secretion of mucin. Intestinal microbes might influence goblet cell dynamics via release of bioactive compounds or indirect activation of the immune system (Bienenstock and Befus, 1980). Dietary garlic meal resulted in a decreased proliferation of goblet cells into the surface of the villus membrane. This is indicative of a decreased host reliance on mucus secretion for protection. In the study of Ferket *et al.* (2002), contrary to expectations, antibiotic therapy increased goblet cell numbers. Decreasing numbers of viable Gram-positive bacteria, such as Lactobacilli and Bifidobacteria, may increase the presence of Gram-negative species. An increase in these types of microbes may actually necessitate the need for more mucus production and hence more goblet cells (Edens *et al.*, 1997). In present study,

garlic meal apparently had a more pronounced effect in this respect.

The results of present study show a dose related differences in gut morphology of birds fed dietary garlic meal which can influence the digestive capacity of the chickens and are indicative of digestive improvement potential of garlic meal as a feed additive.

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