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Evaluation of Comparative Effect of Feed Additive of *Allium Sativum* and *Zingiber Officinale* on Bird Growth and Histomorphometric Characteristics of Small Intestine in Broilers

ABSTRACT

The present study was conducted to evaluate the effects of supplementation of garlic, ginger in the diets of broiler chickens and assessment in terms of feed intake, growth performance and economics of feeding. The results showed that groups supplemented with 0.5% garlic powder and 0.5% ginger powder has shown significant effects on body weight as compared to the control group at day 28. Groups supplemented with 0.5% garlic powder and 0.5% ginger powder show significant increase in body weight than the groups supplemented with 0.25% garlic powder and 0.25% ginger powder. Between different supplemented groups, villus length and width of duodenum and jejunum of birds served with 0.5% garlic powder and 0.5% ginger powder is significantly higher than the villus length and width of birds supplemented with 0.25% garlic and 0.25% ginger powder. Between different supplemented groups, villus length of ileum of the group supplemented with 0.5% garlic powder is significantly ($p < 0.05$) lower than the villus length of the groups supplemented with 0.25% garlic powder and 0.25% ginger powder.

INTRODUCTION

Feed additives are a group of nutrient compounds which helps in improving the efficiency of feed utilization and making it cost effective. Feed is the major component of total costs of poultry venture as 80% of the total expenditure is on procurement of feed (Asghar *et al.*, 2000). *Allium sativum* is commonly known as garlic, belongs to the family Amaryllidaceae and subfamily Alliioideae. Its other common names are garlic, shallot, leek chive and rakkyo. It grows prominently in sub-continent. It is a well-known spice. It is also used as traditional medicine for therapeutic response and prevention of various health problems (Adimoradi *et al.* 2006). Furthermore, it has antimicrobial, antihypertensive and antioxidant properties (Konjufca *et al.* 1997; Sivam, 2001). Hypocholesterolemic effect was observed in chickens due to the use of garlic powder (Konjufca *et al.* 1997; Qureshi *et al.* 1983). Tollba & Hassan (2003) stated that garlic is a well-known natural supplement which has decreased mortality rate, brought positive changes in feed conversion ratio and improved the growth of broiler chicken. The exact mode of action is hard to understand. The moderate dose of the garlic can increase the villus height linearly in duodenum, jejunum and ileum. However garlic supplementation at higher doses 0.5 to 2.0% increases ($p < 0.05$) the crypt depth, which resulted in lower crypt to villi ratio in duodenum and higher in jejunum and ileum (Konjufca *et al.* 1997). One of the purposes of this study is to investigate its effects on intestinal morphology as it has the ability to change the intestinal absorption.

Zingiber officinale (Ginger) is another spice. It belongs to the family Zingiberaceae. Other notable members of this plant family are



turmeric, cardamom, and galangal. It is well known for its medicinal and flavouring properties (Onyenekwe & Hashimoto, 1999). For centuries it has been used as an edible plant and it remained as an important constituent of Tib, Unani and Chinese medicine for the cure of diabetes, asthma, stroke, constipation, gingivitis, nervous diseases and catarrh (Tapsell *et al.* 2006, Wang and Wang, 2005, Awang, 1992). Nine compounds are found in ginger which may bind to serotonin receptors and may influence gastrointestinal function. Some *in-vitro* studies showed that ginger extract can have the ability to control the quantity of free radicals and the peroxidation of lipids (Al.amin *et al.* 2006)

Ginger is also used as a treatment of different diseases i.e. dyspepsia, flatulence, diarrhea and colic (Borrelli *et al.*, 2004) and works as an appetite stimulant (White, 2007). Moreover, it has an anti-inflammatory (Grzanna *et al.*; 2005) as well as cancer prevention properties (Shukla & Singh; 2007). Herbal extracts, organic acid, Prebiotics, enzymes and probiotics are widely used in feed of poultry as an alternative of sub-therapeutic antibiotics (Yahkeshi *et al.* 2011). Its mode of action includes the variation in gut histomorphometric parameters (Markovic *et al.* 2009), intestinal and growth performance (Patterson & Burkholder 2003), immune system and antibacterial activity (Thenmozhi *et al.* 2012, Schley & Field 2002). Reduction in gut pathogenic bacteria causes reduction of competition for microbial nutrients, increase availability of nutrients and decrease in size of muscularis mucosa resulting in increased absorption capacity (Yahkeshi *et al.* 2011). The absorptive surface area is increased due to the increase in width and length of the intestinal villi. Enterocytes undergo proliferation in the intestinal crypt and decreased crypt height can therefore be correlated with the increase in growth rate in broilers (Markovic *et al.* 2009). It was hypothesized that the use of dietary *Allium sativum* and *Zingiber officinale* in broilers will induce structural changes in favor of higher body functions in major body organs. The aim of the present research is to evaluate the comparative efficacy of *Allium sativum* and *Zingiber officinale* supplementation on bird performance and histomorphometry of small intestine in broilers.

MATERIALS AND METHODS

The present research trial was conducted at the experimental Animal House, Department of Biosciences, Faculty of Veterinary Sciences, Bahauddin

Zakariya University Multan for a period of 42 days (6 weeks). Samples were processed in the Histology lab. 80 day-old chicks (hybrid) were purchased from Rising Poultry and were randomly divided into five groups with 04 replicates in each group comprising of 04 birds in each replicate. The control group (A) was fed with corn based basal diet while Group B was given basal diet + 0.25% Garlic powder, Group C was given basal diet + 0.50% Garlic Powder, Group D was given basal diet + 0.25% Ginger Powder and Group E was given basal diet + 0.50% Ginger Powder. The young chicks were raised in environmental controlled conditions and up to 42 days, water and feed were provided *ad libitum*. Infectious bursal disease and Newcastle Disease vaccines were given to the chicks as stated by (Giambrone & Clay, 1986).

Sampling and Processing

On day 42, 8 birds were selected randomly from each group and then weighted for FCR. The birds were conventionally slaughtered and collection of intestinal samples was done. Furthermore, whole intestinal tract was removed. Whereas, Ileo-cecal junction served as the landmark for small intestine. Samples from the duodenum, the jejunum as well as the ileum were taken with length of five centimeter each, respectively, as stated by Murakami *et al.* (2007). The samples were washed using normal saline and preserved in 10% buffer formalin solution. Tissue samples were dehydrated, cleared, impregnated and embedded in paraffin wax. 4µm thin sections were obtained with the help of microtome. Prior to mounting on slides, sections were cleared of wrinkles by floating on 55- 60°C warm water (Bancroft *et al.* 2008). Staining of intestinal samples was done by using eosin and haematoxylin (Bancroft *et al.* 2008). The slides were observed under light compound microscope. ProgRes® (CapturePro - Jenoptik AG) software was used to perform histomorphometry.

Gut Histomorphometry

In gastrointestinal morphometric variables, villus length and width were measured. One section per slide was taken from the tissue. On the basis of intact lamina propria five villi were selected for length and width of villi (Ashraf 2011). Later, average of fifteen values was calculated. The measurements for villus width from the midpoint and for villus length, from the top of the villus to the lamina propria were taken. Depth of invagination between the adjacent villi was measured for crypt depth (Awad *et al.*, 2009).



Statistical Analysis

Data was analyzed with the statistical package for social science (SPSS) version 22 (Chicago IL, USA). Data was presented as mean \pm S.D. The group differences were compared by Duncan's Multiple Range Test (Panneerselvan 2004). Difference was considered significant at $p < 0.05$.

RESULTS

In the present study, body weight of the birds at different days is showed in table 01. All the supplemented groups showed significant ($p < 0.05$) body weight compared to the body weight of the control group at day 14. Between different supplemented groups, Group-C and Group-E showed significant ($p < 0.05$) body weight compared to the body weight of group-B, body weight of Group-C is also significantly higher ($p < 0.05$) than the body weight of group-D at day 14. Groups supplemented with 0.5% garlic powder and 0.5% Ginger powder has shown significant increased ($p < 0.05$) effect on body weight as compared to the control group at day 28. Between different supplemented groups, groups supplemented with 0.5% garlic powder and 0.5% ginger powder show significant ($p < 0.05$) increase body weight compared to the groups supplemented with 0.25% garlic powder. Furthermore, group supplemented with 0.5% garlic powder show significantly higher body weight compared to the body weight of the group supplemented with 0.25% ginger powder. At day 42, birds served with supplemented feed expressed significant response ($p < 0.05$) to body weight compared to the body weight of the control group. Between different supplemented groups, body weight of group supplemented with 0.5% garlic powder showed significant result ($p < 0.05$) compared to the body weight of the groups supplemented with 0.25% Garlic powder and 0.25% ginger powder.

Gut histomorphometric measurements of villus width and villus length for duodenum, jejunum & ileum are showed in table 2. Duodenal villus length and width of all supplemented groups are more significant

($p < 0.05$) than the villus length and width of group A (controlled group). Between different supplemented groups, villus length and width of duodenum of birds served with 0.5% garlic powder and 0.5% ginger powder is significantly higher ($p < 0.05$) than the villus length and width of the birds supplemented with 0.25% garlic and 0.25% ginger powder. The birds of the group served with 0.5% garlic powder showed higher ($p < 0.05$) values of villus length and width of duodenum than the birds served with 0.5% ginger powder. Duodenal crypt depth of all supplemented groups is significantly higher ($p < 0.05$) than the controlled group. Between different supplemented groups, the crypt depth of the groups supplemented with 0.5% garlic powder and 0.5% ginger powder is significantly more ($p < 0.05$) than the crypt depth of the groups supplemented with 0.25% garlic powder and 0.25% ginger powder.

Villus length of the jejunum of the birds of all supplemented groups is found more significant ($p < 0.05$) than the villus length of the control group. Between different supplemented groups, villus length of the jejunum of the birds served with 0.5% garlic powder and 0.5% ginger powder is more significant ($p < 0.05$) than the villus length of the birds fed 0.25% garlic powder and 0.25% ginger powder. Furthermore, jejunal villus length of the group supplemented with 0.5% garlic powder is significantly ($p < 0.05$) higher than the group supplemented with 0.5% ginger powder. Villus width of the jejunum of the birds of all supplemented groups is significantly lower ($p < 0.05$) than the villus width of the birds of the control group. Between different supplemented groups, the jejunal villus width of the group supplemented with 0.5% garlic powder is significantly ($p < 0.05$) decreased compared to the villus width of the groups supplemented with 0.25% garlic powder and 0.25% ginger powder. The jejunal crypt depth of all supplemented groups is significantly higher ($p < 0.05$) than the controlled group. Between different supplemented groups, the crypt depth of the groups supplemented with 0.25% garlic powder and 0.25% ginger powder is significantly lower ($p < 0.05$) than the crypt depth of the groups supplemented with

Table 01 – Weight of birds at day 01 to 42

Weight (gm)	Group-A Controlled	Group-B 0.25% Garlic	Group-C 0.50% Garlic	Group-D 0.25% Ginger	Group-E 0.50% Ginger
Weight at Day 01	44.50 ^a \pm 0.57	45.13 ^a \pm 0.35	44.13 ^a \pm 0.44	43.63 ^a \pm 0.60	44.0 ^a \pm 0.46
Weight at Day 14	320.63 ^a \pm 1.46	326.38 ^b \pm 1.35	333.88 ^d \pm 1.62	329.25 ^{bc} \pm 1.40	331.88 ^{cd} \pm 1.76
Weight at Day 28	1002.13 ^a \pm 4.35	1031.50 ^b \pm 5.63	1068.50 ^d \pm 5.54	1042.88 ^{bc} \pm 2.12	1052.63 ^c \pm 5.28
Weight at Day 42	2016.88 ^a \pm 10.98	2066.38 ^b \pm 13.53	2139.50 ^c \pm 12.77	2090.0 ^b \pm 9.21	2107.63 ^{bc} \pm 26.90

Different superscripts show values in a row differed significantly.



0.5% garlic powder and 0.5% ginger powder. The jejunal crypt depth of the group supplemented with 0.5% garlic powder is significantly higher ($p < 0.05$) than the crypt depth of the group supplemented with 0.5% ginger powder.

The villus length of the Ileum of the birds of all supplemented groups is significantly ($p < 0.05$) higher than the villus length of the controlled birds. Between different supplemented groups, the villus length of the ileum of the group supplemented with 0.5% garlic powder is significantly ($p < 0.05$) lower than the villus length of the groups supplemented with 0.25% garlic powder and 0.25% ginger powder. The villus width of the Ileum of all supplemented groups is significantly ($p < 0.05$) less than the villus width of the controlled birds. Between different supplemented groups, the villus width of the groups supplemented with 0.5% ginger powder and 0.5% garlic powder is significantly ($p < 0.05$) lower than the villus width of the groups supplemented with 0.25% ginger powder and 0.25% garlic powder. The crypt depth of the ileum of groups supplemented with 0.25% garlic, 0.5% garlic and 0.5% ginger powder is significantly higher ($p < 0.05$) than the controlled group. Between the different supplemented groups, the crypt depth of the group supplemented with 0.5% garlic powder is significantly higher ($p < 0.05$) than the groups supplemented with 0.25% garlic powder and 0.25% ginger powder.

DISCUSSION

Demir *et al.* (2003) and Lewis *et al.* (2003) stated that broilers fed with garlic supplementation showed increased body weight and feed conversion ratio. The birds served with the mixture of ginger and garlic had increased final body weight as stated by Ademola *et al.* (2009). Similarly, (Onu 2010) stated that the birds served with ginger and garlic mixture (combination of 0.25%) expressed increase in body weight as compared to the control group. In contrast, Omage *et al.* (2007) and Konjufca *et al.* (1997) stated that broiler birds fed with supplementations of ginger and garlic did not affect feed conversion ratio and body weight gain from the age of day 1 to 21. Similarly, Qureshi *et al.* (1983) reported that the birds fed the basal diet with different products of garlic added at the level of 50 gm/kg did not affect the daily feed consumption and final body weight of the birds. The increase in body weight is observed due to healthy micro flora as well as gut environment produced by supplementation of ginger and garlic powder. The use of ginger and garlic in the present study gives a clear speculation about the direct involvement of these supplements on production traits.

Gupta & Sandhu (1998) stated that the intestinal villus length increased due to cellular hyperplasia and hypertrophy when rats were offered garlic

Table 02 – Small Intestine Histomorphometric parameters.

Parameters (um)	Group-A Controlled	Group-B 0.25% Garlic	Group-C 0.50% Garlic	Group-D 0.25% Ginger	Group-E 0.50% Ginger
Duodenum Villus Length	981.0 ^a ± 13.69	1295.63 ^b ± 17.25	1533.63 ^d ± 21.44	1338.75 ^b ± 26.11	1418.25 ^c ± 26.91
Duodenum Villus Width	152.88 ^a ± 4.25	175.0 ^b ± 2.90	246.38 ^d ± 6.02	185.0 ^b ± 5.39	222.13 ^c ± 7.05
Duodenum Crypt Depth	139.63 ^a ± 1.56	145.63 ^b ± 0.98	152.75 ^c ± 0.67	144.0 ^b ± 1.16	149.88 ^c ± 1.11
Jejunum Villus Length	886.25 ^a ± 7.16	998.88 ^b ± 7.15	1154.0 ^d ± 23.52	980.13 ^b ± 16.55	1076.13 ^c ± 19.99
Jejunum Villus Width	165.0 ^d ± 6.90	134.0 ^{bc} ± 4.71	113.50 ^a ± 4.38	145.0 ^c ± 3.49	126.0 ^{ab} ± 3.93
Jejunum Crypt Depth	107.63 ^a ± 1.29	115.13 ^b ± 1.80	138.88 ^d ± 1.56	113.75 ^b ± 1.58	132.13 ^c ± 1.23
Ileum Villus Length	532.13 ^a ± 4.70	569.63 ^{cd} ± 13.14	548.50 ^b ± 3.54	577.75 ^d ± 5.56	559.0 ^{bc} ± 3.59
Ileum Villus Width	186.0 ^d ± 2.29	155.0 ^b ± 2.16	143.0 ^a ± 1.95	166.75 ^c ± 1.77	147.88 ^a ± 1.39
Ileum Crypt Depth	105.88 ^a ± 0.93	110.88 ^{bc} ± 1.33	115.50 ^d ± 1.74	108.88 ^{ab} ± 1.38	114.50 ^{cd} ± 0.98

Values showing different superscripts in a row differed significantly.

agglutinin diet. Furthermore, it was also observed that the thinning and lengthening of the jejunal villus, sloughing off jejunal mucosa and the increased number of goblet cells were caused in rats due to garlic agglutinin supplements in the diet. On the other hand, it has been studied that birds fed barley-based diet showed reduction in the villus width, length and surface area (Moharrery & Mohammadpour, 2005). They also noticed the gradual decrease in villi length and surface area from the duodenum to the ileum.

The inhibition of the volatile fatty acids as well as microbial production of the polyamines accounted for the thinning of the gastrointestinal wall which resulted in enhanced activity and rate of enterocyte. Ultimately the increase in net energy accounted for sustaining the luminal tissue due to more productive purposes such as muscle accretion (Bedford, 2000). Longer and thinner villi, especially in the duodenal section, noticed due to ginger and garlic supplementation were reported in the present study.



The findings of the present study clearly demonstrated that garlic and ginger supplementations improve the bird performance and histomorphometric parameters of small intestine in chickens.

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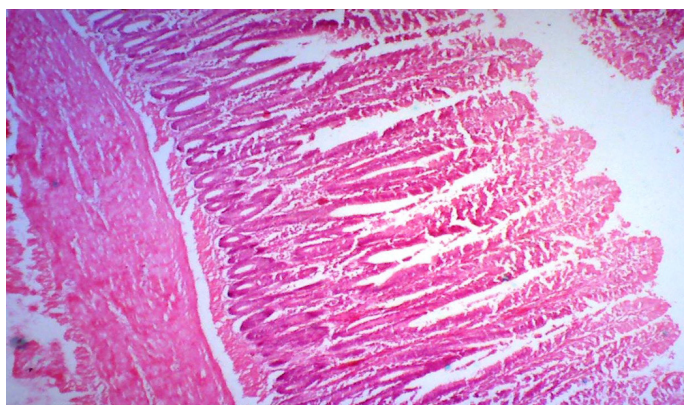


Figure 1 – Duodenum Villi of birds fed with 0.5% ginger powder.

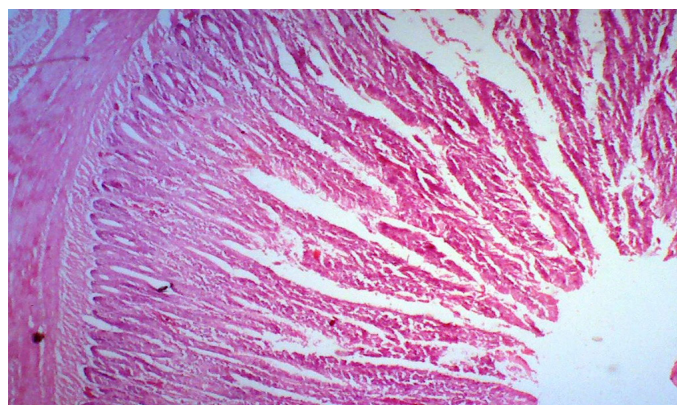


Figure 3 – Jejunum Villi of birds fed with 0.5% garlic powder.



Figure 2 – Duodenum Villi of birds fed with 0.5% garlic powder.

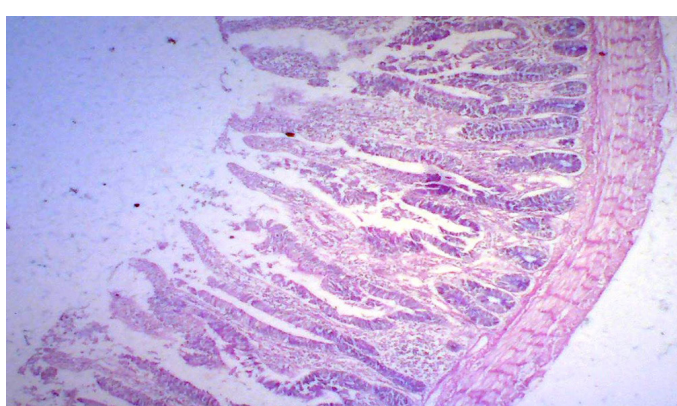


Figure 4 – Ilium Villi of birds fed with 0.25% ginger powder.