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Carcass Yield and Histo-Anatomical Changes in Broilers Fed on Diet Supplemented with Different Levels of Phytobiotics

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ABSTRACT

The present study investigates the comparative effectiveness of different concentrations of garlic (*Allium Sativum*) and ginger (*Zingiber Officinale*) on carcass yield and selected histo-anatomical parameters of broilers. A total of 80 one day-old chicks were divided into five different groups with each group having 4 replicates in a trial of 35 days. The first group was kept as control whereas, the second, third, fourth and fifth groups diet were supplemented with garlic at 0.25 and 0.5% and ginger at 0.25 and 0.5% respectively in the diet. At the end of trial all birds were slaughtered for analysis. Results revealed that carcass weight and carcass yields were significantly ($P < 0.05$) improved by garlic supplementation at 0.5% compared to other groups. Histo-anatomy revealed that the strength of tibia bone increased significantly ($P < 0.05$) whereas, the number of intraepithelial lymphocytes decreased significantly ($P < 0.05$) by the dietary supplementation of garlic and ginger irrespective of level. It is concluded that 0.5% garlic can be used as a potential replacement for antibiotic growth promoters in the broiler diet.

Key words: Carcass yield, IELs, Phytobiotics, Robusticity index, Tibiotarsal index.

INTRODUCTION

Antibiotic growth promoters (AGPs) were used extensively in the last few decades to increase the production performance and profitability of broilers. Prolonged misuse and overuse of AGPs caused harmfulness to human health (Min *et al.*, 2016) as a result of which the European Union in 2006 has banned the use of AGPs (Tehseen *et al.*, 2016). This ban has negatively affected profitability and performance of broilers (Manafi *et al.*, 2016) which has triggered scientists to find alternative to AGPs for improving growth performance of broilers by optimizing their gut health (Junaid *et al.*, 2018). Poultry diet contains a variety of additives and supplements (Hashemi *et al.*, 2014) among which phytobiotics are being investigated by many scientists for being "*Natural Safe Additives*" (Boka *et al.*, 2014).

Garlic (*Allium Sativum*) belonging to family *Amaryllidacea* has been reported to alter the microarchitecture of intestine resulting in better growth performance and feed conversion ratio (FCR) (Oladele *et al.*, 2012). It is effective in reducing the number of pathogenic bacteria residing in the intestine of birds that are responsible for causing many pathological conditions (Peinado *et al.*, 2013). It is added in feed due to it's antifungal, antibacterial, antioxidative, immunomodulatory and antiparasitic properties. Garlic contains allin, diallylcysteine, allicin, dithiin, ajoene and S-allylcysteine (Rehman and Munir, 2015).

Ginger (*Zingiber Officinale*) belongs to family *Zingiberaceae* and is rich in trace minerals and essential oils (Khonyoung *et al.*, 2017). Gingerdiol, zingeronen zingibrene, shogaols and gingeroiols are present in ginger giving it anti-inflammatory, hepatoprotective, analgesic, antioxidant, cardioprotective, immunomodulatory, neuroprotective, antioxidant and antimicrobial properties (Herve *et al.*, 2018). The proximate composition of fresh ginger is 2.3% protein, 12.3% carbohydrates, 1% fat, 2.4% fiber, 80.8% water and 1.2% ash whereas, dried ginger has 10% moisture (Muhammad *et al.*, 2017).

To the best of our knowledge no data is present regarding comparative effects of different concentrations of garlic and ginger on carcass weight, carcass yield, small intestinal histomorphometry and anatomy of tibia bone. This study therefore aims at investigating the comparative effects of garlic and ginger, in different concentrations on histo-anatomical parameters of broilers.

MATERIALS AND METHODS

This study was carried out in environmentally-controlled broiler shed. The experiment lasted for 35 days and was conducted on 80 day-old Hubbard broiler chicks obtained from a commercial hatchery. Upon arrival birds were weighed and randomly assigned to five groups with each group having four replicates. The number of birds in each replicate were four (n=4). The first group (CONT) was

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kept as control and was fed basal diet (Table 1). The second (GAS 0.25%) and third groups (GAS 0.5%) were supplemented with garlic (Garlic - Fooding group Ltd, Shanghai, China) 0.25gm/kg and 0.5gm/kg of basal diet, respectively whereas, the fourth (GZO 0.25%) and fifth (GZO 0.5%) groups were given ginger (Garlic - Fooding group Ltd, Shanghai, China) 0.25gm/kg and 0.5gm/kg of basal diet, respectively. Birds were vaccinated intraocularly by live attenuated Newcastle disease virus (Ceva-Phylaxia, Budapest, Hungary) on day 1, with a booster on day 21 in drinking water. Similarly vaccination against infectious bursal disease (Lohman Animal Health GmbH, Cuxhaven, Germany) was done by intraocular route on day 8 and repeated on day 20 in drinking water.

Carcass weight and carcass yield: During the whole experiment feed and water to birds of each replicate were provided *ad-libitum*. At the end of the experiment all birds were exsanguinated by cutting carotid arteries and jugular vein. Birds were allowed to bleed for approximately two minutes, viscera were removed immediately. Carcass and organ weights were taken using a sensitive digital scale. Carcass yield was calculated as the percentage of live body weight.

Tibia bone characteristics: After exsanguination right tibia bone was removed as drumstick with intact flesh. Drumsticks were labeled and placed in boiling water for 10 minutes. After being cooled at room temperature bone was de-fleshed by hand. Bone was air dried for 24 hours at room temperature. Characteristics of tibia bone which included length, weight, diaphysis diameter, medullary canal diameter, thickness of lateral and medial wall were determined by digital vernier caliper. The following formulae were used for estimating the tibiotarsal and robusticity indices

Tibiotarsal index = diaphysis diameter – medullary canal diameter/ diaphysis diameter × 100

Robusticity index = bone length/ cube root of bone weight. (Saleem *et al.*, 2018a)

Intra epithelial lymphocyte count: About 3cm long small intestinal segments from midpoints of duodenum (segment encompassing the duodenal loop), jejunum (segment between duodenum and ileum) and ileum (distal segment before the ileo-cecal junction equaling the length of caecum) were taken and fixed in 10% neutral buffered formalin. Segments were then embedded in paraffin, stained by haematoxylin and eosin and observed under microscope (Labomed, USA). Counting of intra epithelial lymphocytes (IELs) was done at 40×. The IEL are identified as rounded cells with large central or eccentric nuclei and scant cytoplasm. Counts were made in triplicates on 5 well oriented villi which were selected on the basis of intact lamina propria and average of results was reported (Saleem *et al.*, 2018b).

Statistical analysis: Data were found to be normally distributed after checking with Kolmogrov Simirnov test (Evans *et al.*, 2016). Data for groups were analyzed with one way analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$ and were calculated by applying Duncan's multiple-range test (Steel *et al.*, 1997).

Table 1: Composition of experimental diet given to broilers (Saleem *et al.*, 2019).

	Starter Diet (1-10 d)	Grower diet (11-21 d)	Finisher Diet (21-35d)
Ingredients (%)			
Corn	56.2	59.9	63.34
Soybean oil	2.26	3.3	3.94
Soybean meal	37.11	32.55	28.71
Dicalcium phosphate	1.92	1.86	1.74
Oyster shell	1.16	1.12	1.06
Common salt	0.3	0.3	0.3
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
L-Lysine hydrochloride	0.24	0.21	0.18
DL-Methionine	0.31	0.26	0.23
Nutrient Composition			
ME, kcal/kg	3000	3105	3180
CP, %	21.23	19.46	18
AP, %	0.50	0.48	0.45
Ca, %	1	0.96	0.9
Lysine, %	1.32	1.19	1.06
Methionine + cysteine, %	0.98	0.89	0.82

ME, metabolizeable energy; AP, available phosphorus; CP, crude protein. [1]Vitamin premix supplied the following per kg of diet; vitamin A, 18000U; vitamin D_3, 4000U; vitamin E, 36mg; vitamin K_3, 4mg; vitamin B_{12}, 0.03mg; thiamine, 1.8mg; riboflavin, 13.2mg; pyridoxine, 6mg; niacin, 60mg; calcium pantothenate, 20mg; folic acid, 2mg; biotin, 0.2 mg; choline chloride, 5000mg. [2]Mineral premix supplied the following per kg of diet; Cu, 20mg; Fe, 100mg; Mn, 100mg; Se, 0.4mg; Zn, 169.4mg.

RESULTS AND DISCUSSION

Results illustrating the effects of different concentrations of garlic and ginger on carcass weight and carcass yield are given in Table 2. The foresaid parameters in the birds supplemented with different concentrations of dietary treatments under study were significantly ($P < 0.05$) higher than birds of CONT group. Moreover, birds of GAS 0.5% had significantly ($P < 0.05$) higher carcass weight and carcass yield than birds of other treatment groups.

Increase in carcass weight and carcass yield after supplementation of garlic is due to the presence of antibiotic like substance allicin which decreases the number of pathogenic bacteria and aflatoxin producing fungi in intestine of birds resulting in better absorption of nutrients and higher carcass weight (Kharde and Soujanya, 2014). In line to our results Fayed *et al.* (2011) and Lukanov *et al.* (2015) reported that supplementation of garlic to broilers increases carcass yield. Beneficial effects of ginger which include improvement of endogenous digestive enzyme secretion, activation of immune response, antibacterial, antiviral and antioxidant actions improves growth of birds (Rahimi *et al.*, 2011) which explains our results of increase in carcass weight and carcass yield after ginger supplementation. Similar results were reported by Mansoub and Myandoab (2011) regarding improvements in carcass weight and carcass yields after supplementation of ginger to broilers.

Supplementation of feed with different concentrations of phytobiotics used in this study significantly ($P < 0.05$) increased the weight of liver, thymus, bursa of fabricius, heart and gizzard empty in birds of all treatment groups compared to CONT. However, among all birds no significant difference was observed regarding the weight of spleen, pancreas and proventriculus as shown in Table 3.

Currently we lack sufficient literature to explain the effects of different levels of garlic and ginger supplementation on weight of visceral organs. Bursa of fabricius, thymus and spleen are parts of immune system and an increased weight of these organs is an indicator of immunological advances as they produce lymphocytes which protect birds from the harmful effects of invading pathogens (Saleem *et al.*, 2018a). Our study confirms earlier findings of Rahimi *et al.* (2011) who reported no difference in weight of pancreas and spleen after supplementation of birds with ginger. However, Mansoub and Myandoab (2011) found and increase in the weight of other visceral organs after supplementation of ginger which is in agreement with our results. Analogous to the results of current study Alagawany *et al.* (2016) found that feeding of garlic to broilers did not improve the weight of spleen and pancreas. Our results regarding improvement in weight of liver and heart are also in line with the findings of Eltazi, (2014).

Tibia bone characteristics displayed in Table 4 show that the length, weight, thickness of lateral wall, thickness of medial wall and tibiotarsal index significantly ($P < 0.05$) increased whereas, medullary canal diameter and robusticity index significantly ($P < 0.05$) decreased in birds of all

Table 2: Effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on carcass weight and yield of broilers.

Parameters	Treatment groups					
	CONT (n=16)	GAS 0.25 %(n=16)	GAS 0.5% (n=16)	GZO 0.25% (n=16)	GZO 0.5% (n=16)	SEM
Carcass weight (g)	1221^{a}	1307^{b}	1357^{c}	1304^{b}	1316^{b}	22.14
Carcass yield (%)	60.52^{c}	62.59^{b}	63.45^{c}	62.37^{b}	62.42^{b}	0.47

Superscripts $^{a-c}$ within a row indicates significant difference between groups ($p < 0.05$). CONT, control group; GAS 0.25%, group supplemented with garlic (*Allium sativum*) 0.25 g/kg of basal diet; GAS 0.5%, group supplemented with garlic (*Allium sativum*) 0.5 g/kg of basal diet; GZO 0.25%, group supplemented with ginger (*Zingiber officinale*) 0.25 g/kg of basal diet; GZO 0.5%, group supplemented with ginger (*Zingiber officinale*) 0.5 g/kg of basal diet; SEM, standard error of mean.

Table 3: Effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on weight of vital organs.

Parameters	Treatment groups					
	CONT (n=16)	GAS 0.25 %(n=16)	GAS 0.5% (n=16)	GZO 0.25% (n=16)	GZO 0.5% (n=16)	SEM
Liver (g)	41.27^{a}	44.78^{b}	45.03^{b}	44.83^{b}	44.97^{b}	0.72
Spleen (g)	2.27	2.39	2.42	2.36	2.38	0.12
Pancreas (g)	3.91	3.97	3.95	3.98	3.93	0.18
Thymus (g)	2.17^{a}	2.75^{b}	2.91^{b}	2.83^{b}	2.88^{b}	0.13
Bursa of fabricius (g)	2.01^{a}	2.91^{b}	3.12^{b}	2.98^{b}	3.01^{b}	0.21
Heart (g)	9.53^{a}	10.71 ^{b}	10.89^{b}	10.75^{b}	10.79^{b}	0.25
Gizzard empty (g)	27.14^{a}	28.95^{b}	29.19^{b}	29.03^{b}	28.97^{b}	0.38
Proventriculus empty (g)	3.75	3.73	3.79	3.76	3.74	0.15

For rest of the details please see footnote of Table 2.

Table 4: Effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on anatomical parameters of tibia bone.

Parameters	Treatment groups					SEM
	CONT (n=16)	GAS 0.25 %(n=16)	GAS 0.5% (n=16)	GZO 0.25% (n=16)	GZO 0.5% (n=16)	
Length (mm)	72.13[a]	72.97[b]	74.18[c]	73.06[b]	73.14[b]	0.33
Weight (g)	6.85[a]	7.14[b]	7.21[b]	7.12[b]	7.17[b]	0.06
Diaphysis diameter (mm)	9.48	9.25	9.21	9.24	9.27	0.12
Medullary canal diameter (mm)	5.41[b]	5.35[a]	5.31[a]	5.33[a]	5.37[a]	0.02
Thickness of lateral wall (mm)	2.17[a]	3.14[b]	3.21[b]	3.17[b]	3.22[b]	0.21
Thickness of medial wall (mm)	1.27[a]	1.65[b]	1.77[b]	1.72[b]	1.69[b]	0.09
Tibiotarsal index	47.58[a]	48.59[b]	48.44[b]	48.47[b]	48.56[b]	0.19
Robusticity index	3.83[a]	3.77[b]	3.81[b]	3.79[b]	3.8[b]	0.02

For details please see footnote of Table 2.

Table 5: Effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on intraepithelial lymphocytes of small intestine.

Intraepithelial lymphocytes	Treatment groups					SEM
	CONT (n=16)	GAS 0.25 %(n=16)	GAS 0.5% (n=16)	GZO 0.25% (n=16)	GZO 0.5% (n=16)	
Duodenum (per villus)	79.23[c]	72.21[b]	70.13[a]	72.14[b]	70.03[a]	1.68
Jejunum (per villus)	26.21[c]	23.19[b]	21.18[a]	22.98[b]	20.98[a]	0.94
Ileum (per villus)	27.22[c]	24.18[b]	22.13[a]	24.31[b]	22.41[a]	0.91

For details please see footnote of Table 2.

treatment groups compared to CONT. No significance was observed for diaphysis diameter by supplementation of different concentrations of phytobiotics used in this study.

Bone is composed of organic and inorganic substances which give elasticity and strength to bone, respectively (Saleem *et al.,* 2018a). Bone problems are among the major health issues of fast growing birds and can adversely affect production performance and profitability of birds if not taken care of properly (Vashan *et al.,* 2016). Higher value of tibiotarsal index is an indicator of higher mineralization and higher strength of bone, whereas, higher robusticity index indicates poor mineralization and bone strength (Saleem *et al.,* 2018a). In our study supplementation of garlic and ginger increased bone strength as both of these phytobiotics enhance the absorption of minerals in feed by improving the morphology and microbiology of intestine (Eltazi, 2014) that leads to better mineralization of bone giving it more strength.

In duodenum, jejunum and ileum compared to CONT birds from all the treatment groups had significantly ($P < 0.05$) lower number of IELs. Among the treatment groups, birds from GAS 0.5% and GZO 0.5% had significantly ($P < 0.05$) lower number of IELs than birds from GAS 0.25% and GZO 0.25% respectively as presented in Table 5.

A greater number of IELs in the epithelium is allied to better immune response in birds as these cells are related to detection of antigens and modulation of epithelial response to those antigens (Agostini *et al.,* 2012). However, when the infiltration of these IELs is in lamina propria it is an indication of elevated inflammatory response which retards growth performance indices (Jiang *et al.,* 2000). Increase in the number of useful bacteria whereas decrease in the number of pathogenic bacteria leads to decrease in the number of IELs in small intestinal mucosa (Saleem *et al.,* 2018b). Supplementation of garlic and ginger to broilers decrease the number of pathogenic bacteria and increase the number of useful bacteria (Okoleh *et al.,* 2014) which explains the reason of our results regarding decrease in IELs number in the mucosa of all segments of small intestine.

CONCLUSION

In conclusion, 0.25%, 0.5% dietary supplementation of ginger and garlic improves carcass weight and carcass yield in broilers by improving their histo-anatomical indices. Moreover, 0.5% garlic and ginger is recommended as a substitute for antibiotic growth promoters in commercial broiler farms.

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